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**Structure-activity relationship analysis of the
subtype-selective Sirt5 inhibitor balsalazide**

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Erklärung

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“Curiosity is the most powerful thing you own.”

JAMES CAMERON

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1. Introduction

Cell-active, potent and highly selective chemical reagents with a known mechanism of action,^[1] that is, in brief, the definition of chemical probes that are predominantly used in target-based drug discovery. One of the fastest growing areas in this field is epigenetic therapy, which is in this regard no exception. Pathological dysregulation of epigenetics can cause cardio-vascular diseases, neurological disorders, metabolic disorders, and cancer development.^[2] For a number of proteins involved in these pathways small molecule tools are urgently needed to investigate and answer mechanistic and phenotypic questions.^[1-2] The same chemical probes that influence these epigenetic changes could then further pave the way towards approvable drugs and successful treatment of various diseases.

1.1. Epigenetics

The term epigenetics was first coined in 1942 by CONRAD WADDINGTON. In a discussion about the mechanisms of cell differentiation he defined epigenetics as “the branch of biology that studies the causal interactions between genes and their products which bring the phenotype into being”^[3-4]. Since then, this field of research has gained more and more interest. In 2013 alone 17,000 articles related to epigenetics were published, thus 45 new publications a day.^[5] These publications address the heritable change occurring in the genome in response to endogenous and environmental stimuli leading to altered gene expression without affecting the underlying DNA sequence of the organism. All cells in an organism inherit the same genetic material; the ability of cells to maintain unique physical characteristics and biological functions of specific tissues and organs is due to heritable differences in the packaging of DNA and chromatin.^[6-8] As depicted in Figure 1, chromosomal DNA in eukaryotic cells is wrapped around octamers of histone proteins, thereby forming complexes referred to as nucleosomes (~147 base pairs of DNA wrapped around dimers of H3 and H4 along with dimers of H2A and H2B). Nucleosomes are further organised by additional protein factors to form chromatin.^[6-7, 9] Chromatin thereby efficiently carries the genetic information within the limited space of a nucleus.^[9]

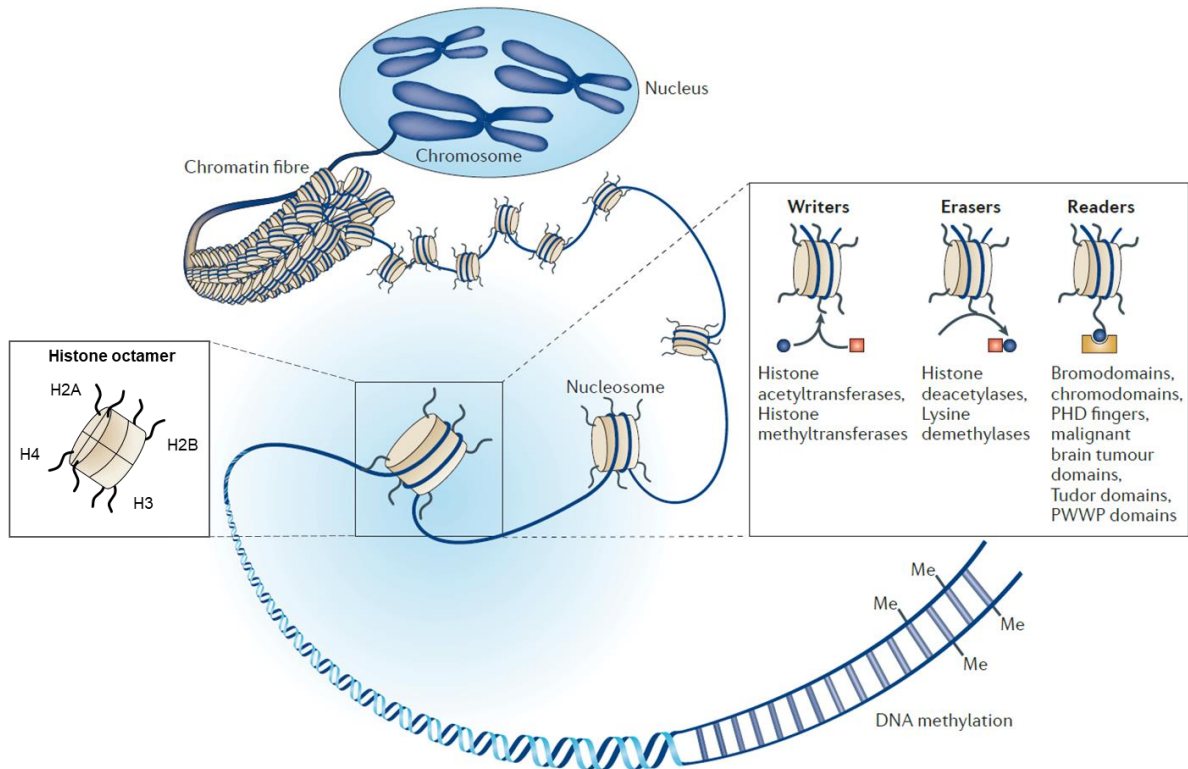


Figure 1: Packaging of chromosomal DNA with nucleosomes as the basic subunit in chromatin. Each nucleosome contains eight core histones, upon which post-translational modifications (PTMs) take place. Examples of protein families of writers, readers, and erasers that mediate methyl or acetyl histone PTMs are illustrated (cf. lit. [8-9]).

Post-translational covalent modifications of DNA and proteins that package DNA (predominantly histones) play a key role in the dynamic modulation of this complex hierarchical organisation of chromatin and therefore the accessibility of the compacted DNA; thus PTMs influence DNA-templated processes such as transcription, DNA replication, DNA repair, and recombination.^[9] Chromatin exists in two different states: closed chromatin (heterochromatin, e.g. through histone methylation), resulting in transcriptional repression, and open chromatin (euchromatin, e.g. due to histone acetylation or methylation) favourable towards transcription.^[6-7]

There are also several heritable mechanisms of post-transcriptional gene regulation such as the synthesis of non-coding microRNAs (miRNAs) that bind to corresponding messenger RNAs (mRNAs) and degrade them or inhibit translation.^[6-7] These mechanisms will not be discussed in further details in this thesis.

Apart from nuclear DNA, a mammalian cell contains multiple copies of mitochondrial circular DNA molecules (mtDNA). As with chromosomal DNA, mtDNA is organised into small protein-DNA complexes called nucleoids. The molecular composition of these nucleoids is still under debate. Mammalian mtDNA is replicated by proteins distinct from the nuclear DNA replication machinery. But similarly to DNA compaction by histone proteins in the nucleus, DNA compaction in mitochondria strongly influences transcription and DNA replication.^[10]

With increasing interest in epigenetics, the knowledge of proteins involved in post-translational modifications has grown tremendously.^[8] More than 100 distinct reversible, covalent modifications have been identified on chromatin, DNA and RNA with acetyl and methyl groups being the two most abundant and among the most widely studied histone PTMs.^[8, 11] The key players in PTMs are called epigenetic tools and constitute of: writers – enzymes capable of adding PTMs to histones and other proteins, readers – a diverse range of proteins that recognise and bind to specific epigenetic marks, and erasers – a group of enzymes removing these marks (Figure 1).^[7] By now, two inhibitors of DNA methyltransferases and five inhibitors of histone deacetylases (HDACs) have been approved for cancer treatment.^[2]

1.2. Metabolism meets epigenetics

The addition and removal of most of these post-translational modifications of histones and other proteins are catalysed by enzymes. The activity of these enzymes is dependent on the availability of substrate, cofactors and allosteric regulators that are derived from diverse metabolic pathways. Thus, they are directly responsive to particular cellular metabolic states. With this interplay it seems reasonable that there is a complex connection between metabolism and epigenetics.^[11-12]

Many metabolites are important as signalling molecules in the regulation of epigenetics including acetyl-CoA, which is required for the addition of acetyl groups to histones by histone acetyl transferases and nicotinamide adenine dinucleotide (NAD), which serves as a cofactor for class III histone deacetylases. Metabolites therefore are involved in regulating all essential steps in establishing, modulating and removing epigenetic marks on histones.^[13] Thus, metabolic changes due to nutrient and metabolite availability and thereby altered regulation of epigenetic tools can cause changes in chromatin and DNA state, subsequently affecting the epigenome to promote e.g. cancerogenic alterations.^[11, 13] Taken together, cell metabolism and epigenetics share an intricate relation, playing a key role in development, cancer, immune signalling and ageing. Targeting epigenetics and metabolism opens the possibility for novel therapeutic approaches.^[7, 13]

1.3. Bioreversible ϵ -*N*-acylation of lysine side chain amino groups

More than 300 types of post-translational modifications are known to occur physiologically.^[14] Methylation, phosphorylation, ubiquitination and acetylation are the most well-understood PTMs, but less studied modifications, including glycosylation, crotonylation, and succinylation, are also known to be functionally important and gain more and more interest in drug discovery. As aforementioned, PTMs bear enormous potential to have profound effects on gene expression and regulation of protein function, and therefore cellular processes. They affect the protein structure to modulate their stability, localisation, and activity. This thesis is based on

one of the most abundant PTMs: bioreversible ϵ -*N*-acylation of lysine side chain amino groups of histones and other proteins.^[11, 14-15] Proteins involved in this process of acylation and deacylation are lysine acyltransferases (KATs; writers), bromodomains (BRDs; readers), and lysine deacylases including sirtuins (KDACs, erasers) illustrated in Figure 2.

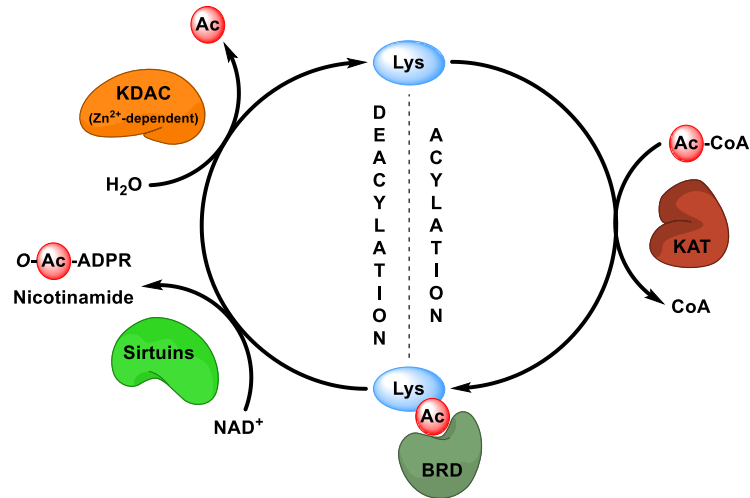


Figure 2: Enzymes and other proteins involved in bioreversible ϵ -*N*-acylation of lysine side chain amino groups of histones and other cellular proteins (cf. lit. ^[15]).

There are 18 human lysine deacylases (KDACs), which are divided into Zn^{2+} -dependent KDACs consisting of classes I, II, and IV, as well as NAD^{+} -dependent deacylases constituted by class III KDACs (Figure 3). The latter are also known as sirtuins due to their homology to the yeast silent information regulator 2 (SIR2), which was discovered in the late 1970s by AMAR KLAR.^[15-18]

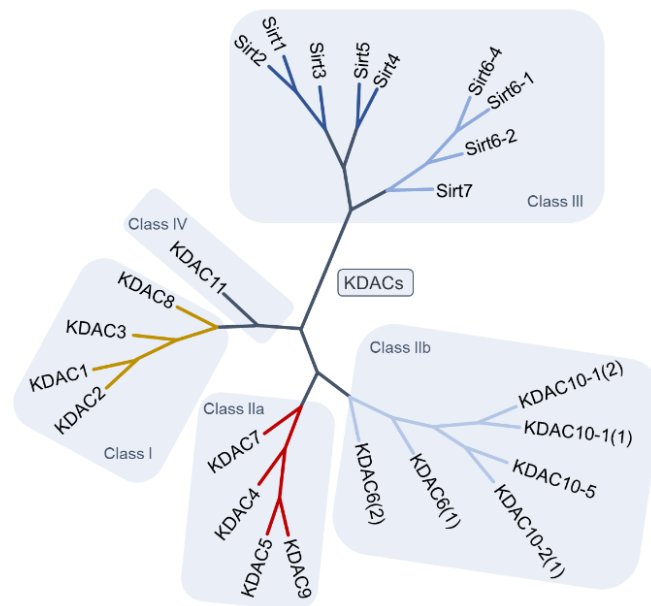


Figure 3: Phylogenetic tree of the protein family of the 18 lysine deacylases (KDACs; also known as HDACs; K: lysine, H: histone). Proteins are clustered on branches on the basis of the similarity of their amino acid sequence (structurally) and sometimes functions (cf. lit. ^[8]).

Sirtuins are highly conserved from bacteria to humans.^[19] On the basis of sequence similarities they are further classified into the five subclasses I – IV and U.^[20] While class-U sirtuins are found only in Gram-positive bacteria, classes I – IV include the mammalian sirtuins. They comprise seven isoforms (Sirt1 to Sirt7) with different cellular localisation.^[21] Figure 4 shows the predominant localisation of the sirtuins in a human cell.

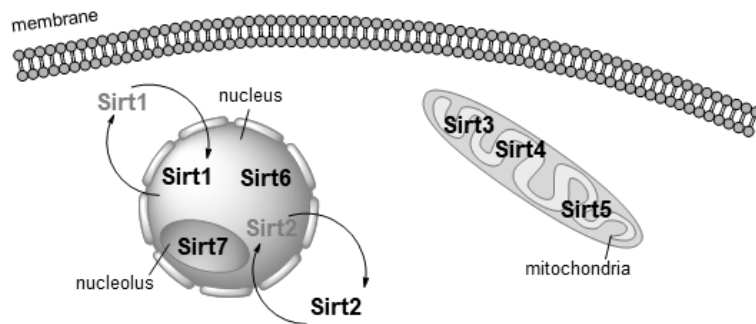


Figure 4: The seven mammalian isoforms Sirt1 – Sirt7 and their predominant cellular localisation (cf. lit. ^[18]).

Sirtuins have a catalytic core of ~275 amino acids flanked by N- and C-terminal extensions that contribute to cellular localisation and activity regulation. The catalytic core consists of an NAD⁺-binding Rossmann fold subdomain and a small Zn²⁺-binding module (not involved in catalysis), and the active site, which is located between those two subdomains and is in close proximity to the NAD⁺-binding site (Figure 5).^[18, 22]

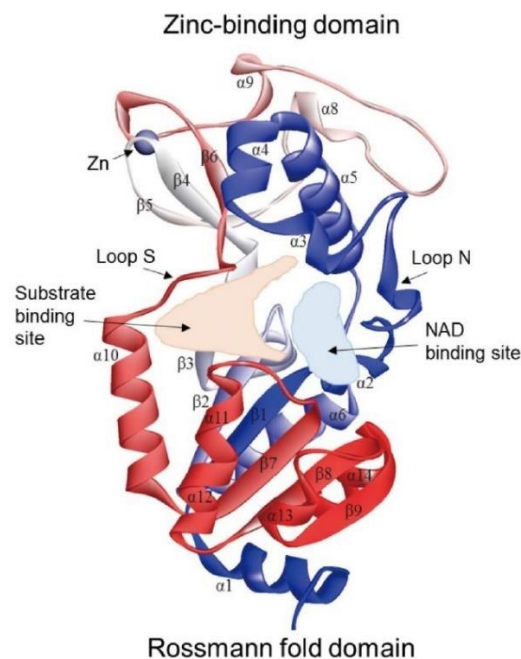


Figure 5: General structure of sirtuins exemplified with depicted Sirt5 (lit. ^[23]).

For their activity on histones, sirtuins have been categorised as class III KDACs, but in general, literature reveals that sirtuins are highly involved in deacylation of various non-histone

substrates.^[24] Furthermore, sirtuins catalyse the removal of several other PTMs beyond acetyl, including myristoyl and succinyl groups from lysine residues, as well as ADP-ribosylation.^[18] Because of their diverse activity and substrate spectrum, sirtuins are involved in metabolic or transcriptional regulation, DNA repair, genome stability, cell survival, aging processes, and stress response.^[18, 25-27] Therefore, it is not surprising that dysregulation of sirtuins can cause metabolic diseases,^[28-29] cancer,^[30-33] and neurodegeneration.^[34-37] In addition, the activity of sirtuins has been associated with life span extension^{[38-39].}^[40]

1.4. Sirt5

The focus of this thesis is on the isoform Sirt5 (class III sirtuins). As illustrated in Figure 4 (chapter 1.3), Sirt5 is, like Sirt3 and Sirt4, primarily located in the mitochondria, and subsequent research indicated that Sirt5 is also localised in the mitochondrial intermembrane and the cytosol.^[41-44] Many findings suggest that Sirt5 is involved in various biochemical pathways^[18] due to its numerous mitochondrial substrates, such as:

- The mitochondrial carbamoyl phosphate synthetase I (CPSI) – a ligase that is involved in the production of urea and is thereby important in ammonia detoxification.^[44-45]
- 3-Hydroxy-3-methylglutaryl-CoA synthetase 2 (HMGCS2) – a mitochondrial enzyme involved in ketogenesis.^[44, 46]

Moreover, through additional mitochondrial and cytosolic targets Sirt5 plays a role in:

- Reducing reactive oxygen species (ROS) by deacylating proteins like SOD1,^[47] IDH2, and G6PD.^[48-49]
- Glycolysis,^[43] the tricarboxylic acid (TCA) cycle,^[43, 50] fatty acid metabolism^[43, 50-51] and cellular respiration^[50].

This leads to the conclusion that not only dysregulation of sirtuins in general but in particular Sirt5 can cause metabolic disorders,^[52-53] cancer,^[54-55] Alzheimer's disease and Parkinson's disease^{[56-57].}^[40]

Among the sirtuin subtypes the activity for the removal of specific PTMs varies and Sirt5 is known to have only weak deacetylase activity, whereas it shows high activity regarding e.g. lysine demalonylation and desuccinylation *in vitro* and *in vivo*.^[41, 50, 58] Hence, deacylation catalysed by Sirt5 focuses on acyl residues derived from dicarboxylic acids. The potential mechanism is illustrated in Figure 6 and proceeds as follows: the first catalytic step results in the formation of an *O*-alkylamidate intermediate (I) *via* an S_N2-like reaction: NAD⁺ is attacked by the acyl-lysine at the 1'-carbon of the nicotinamide ribose whereby the glycosidic bond is cleaved resulting in nicotinamide and the *O*-alkylamidate species I. In the next step, the 2'-hydroxyl of the *O*-alkylamidate (I) is activated by a basic histidine residue in the active site and an intramolecular nucleophilic attack of the *O*-alkylamidate (I) leads to the bicyclic aminoacetal II. Finally, hydrolysis leads to formation of the deacylated peptide and 2'-OAADPr (III) and

nonenzymatic intramolecular transesterification results in a mixture of 2'-OAcADPr (III) and 3'-OAcADPr (IV).^[18]

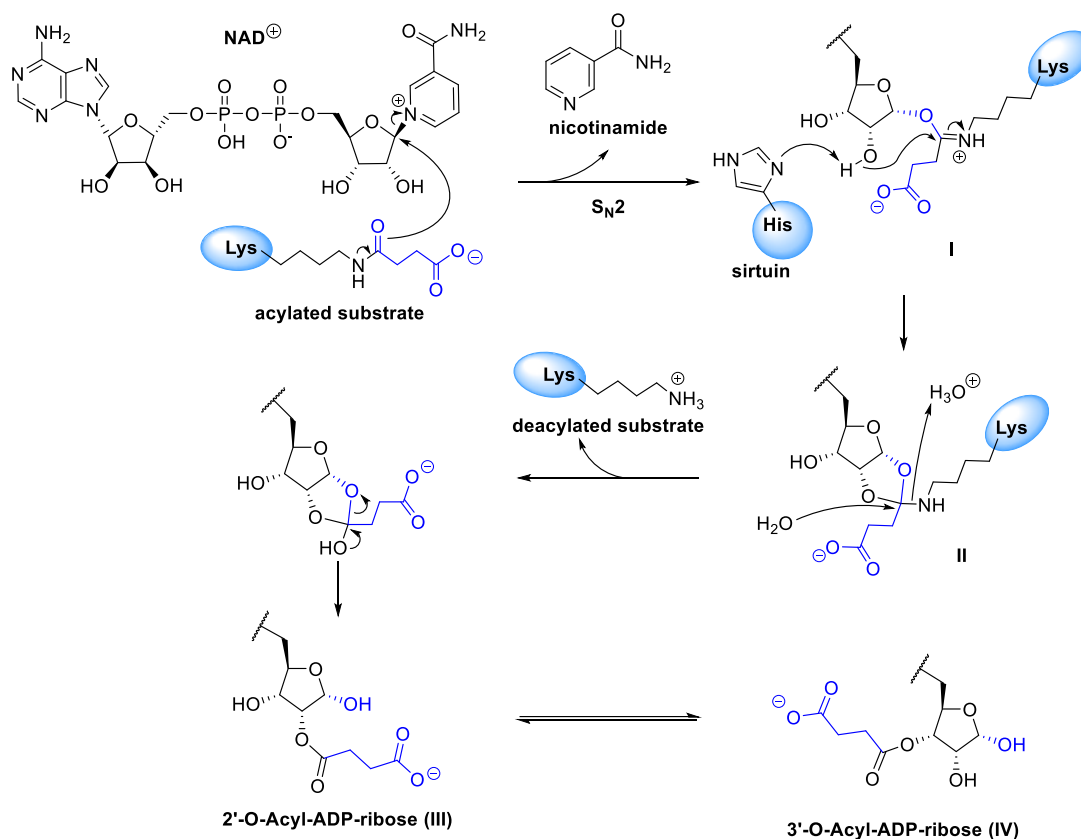


Figure 6: Potential deacylation mechanism of Sirt5 exemplified with a succinylated lysine residue (cf. lit. ^[18, 59-60]).

Lysine is an essential amino acid for humans and has a positively charged side chain at physiological pH. This makes lysine important for protein-protein interactions and the formation of protein complexes; thus the regulation of protein structure and function.^[14] In detail, the addition or removal of e.g. a succinyl group changes the charge of the lysine residue between -1 (succinylated) and +1 (desuccinylated) and introduces/removes a relatively large structural moiety compared to lysine methylation and acetylation. This may have significant effects on the function of a protein.^[14]

The preference of Sirt5 for deacylating proteins in contrast to deacetylation by KDACs in general can be explained by the active pocket of Sirt5. The substrate binding site of Sirt5 contains the presumably catalytically important and unique side chains Arg105 and Tyr102 (Figure 8, Chapter 2), with which a negatively charged carboxylate group of the substrate can interact.^[19, 58, 61-62] Apart from the physiological inhibitor nicotinamide, only a few small molecule Sirt5 inhibitors are known with good to moderate inhibitory activity as shown in Figure 7. Here it should be noted that further selectivity has not been determined comprehensively for all of them. In addition, highly reactive functional groups like enones or arylidene(thio)barbiturates remind of undesired pan-assay interference compounds (PAINS).^[40]

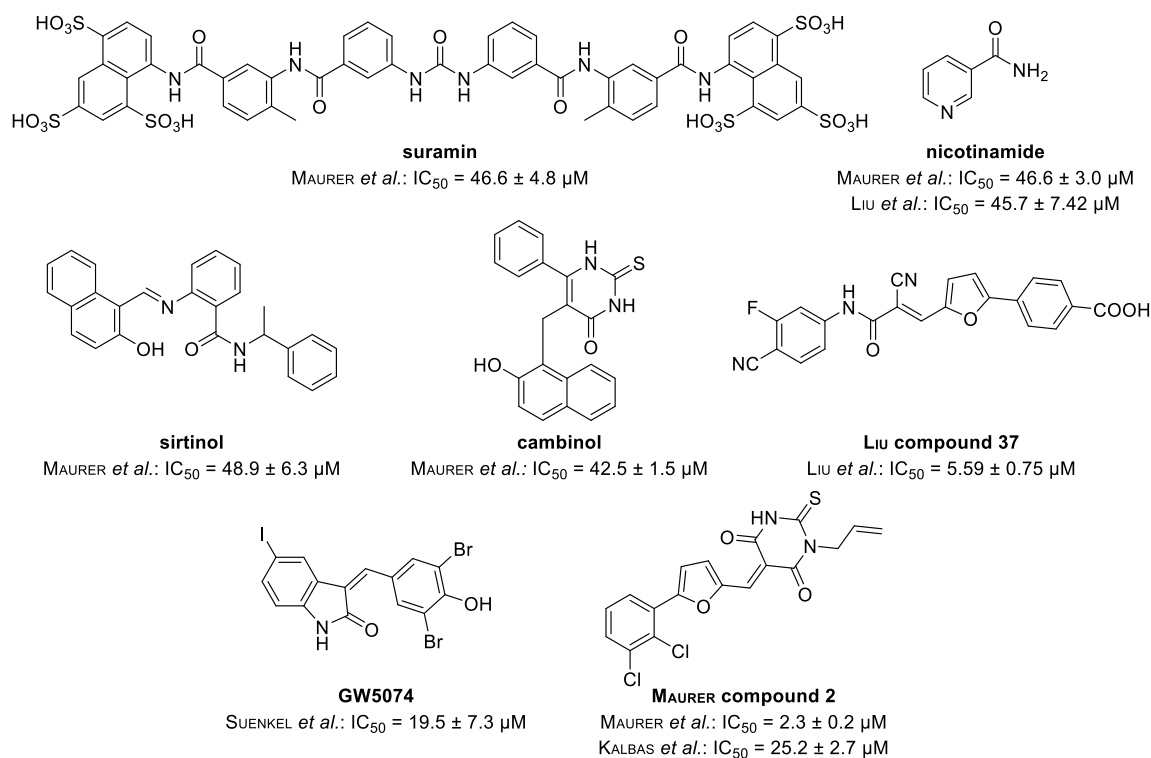


Figure 7: Reported IC_{50} values of known Sirt5 small molecule inhibitors: suramin,^[63] nicotinamide,^[19, 63] sirtinol,^[63] cambinol,^[63] LIU compound 37,^[19] GW5074^[64] and MAURER compound 2^[63, 65] (cf. lit. ^[40]).

There are also several peptidic/peptidomimetic inhibitors,^[15, 62, 65-66] which will not be discussed further in this thesis since they are not small and lipophilic; hence no useful membrane permeable chemical tools.

2. Objective

In continuation of previous work in the group of PROF. DR. FRANZ BRACHER on the development^[67-68] and biological characterisation^[69-72] of sirtuin inhibitors, the research conducted for this thesis aimed at the development of novel inhibitors of Sirt5. These should offer the opportunity for studying the affected cellular processes of Sirt5 (inhibitors as “chemical tools”). In addition, they could serve as innovative drugs (inhibitors as potential therapeutics) in the future. In general, inhibitors targeting Sirt5 are designed in a way to make use of the unique possibility of an interaction with the side chains Arg105 and Tyr102 of Sirt5. By incorporating a suitably located carboxylate group like in Sirt5’s natural substrates (e.g. succinylated lysine residues) this interaction is accomplished *via* electrostatic interactions and hydrogen bonds.^[23] For this thesis, a suitable lead structure had to be chosen where structure-activity relationship studies could lead to highly selective, potent, and particularly drug-like inhibitors. Parts of this thesis including the following description on the selection of the lead structure were published in the *EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY*.^[40]

Objective

A literature search revealed the results of a high-throughput screening of potential Sirt5 inhibitors published by GUETSCHOW *et al.* in 2016.^[73] As the method of choice the authors used microchip electrophoresis (MCE) and screened a library of 1280 drug-like compounds and found the eight promising Sirt5 inhibitors depicted in Table 1, which showed at least 50 % inhibition of Sirt5 activity at 10 μM (except antimycin A).^[73]

Table 1: Reported IC_{50} values of small molecule Sirt5 inhibitors identified by GUETSCHOW *et al.*^[73] in an HTS using MCE; *anthraline's IC_{50} was later determined at 453 μM by KALBAS *et al.*^[65]

Inhibitor name	Structural formula	IC_{50} against Sirt5	Undesired physiological effects
fulvestrant		2.6 μM	(anti)hormonal activity
antimycin A		90 μM	toxicity
balsalazide		3.9 μM	not absorbed from the intestine; upon release of 5-ASA: anti-inflammatory effect
probucol		1.6 μM	stability issues
closoantel		2.7 μM	antimicrobial activity
thyroxine		2.2 μM	hormonal activity
anthraline		0.1 μM *	toxicity, stability issues
methacycline		3.6 μM	antimicrobial activity

From those compounds balsalazide with its published IC_{50} of $3.9 \mu M$ ^[73] seemed most promising as a lead structure with its supposedly essential aliphatic carboxylic acid group. Other screening hits were not considered due to their undesired physiological effects, which seemed as if they could not easily be removed by minor structural modifications (Table 1). The selected lead structure balsalazide itself is an approved prodrug of 5-aminosalicylate (5-ASA), which is used for the treatment of inflammatory bowel disease^[74] in the form of balsalazide disodium. When balsalazide reaches the colon, coliform bacteria metabolise the azo-bond by reduction, liberating 5-ASA as the actual active form.^[75] Hence, as desired for its therapeutic indication, this compound has poor bioavailability from the gut. It is thereby not a suitable compound for simple “drug repurposing”,^[76-77] but this makes it a perfect candidate for this project, since modification of the core azo group removes its undesired original therapeutic indication.^[40]

Initial docking calculations, performed by EHAB GHAZY of the group of PROF. DR. WOLFGANG SIPPL at the Martin-Luther-University Halle-Wittenberg, gave first insights into why balsalazide is able to inhibit Sirt5. They analysed the interaction of a co-crystallised succinyl-lysine based peptide as a reported Sirt5 substrate (PDB 3RIY) (Figure 8a) and carried out docking calculations for the inhibitor balsalazide (Figure 8b) in the presence of NAD^+ .^[40]

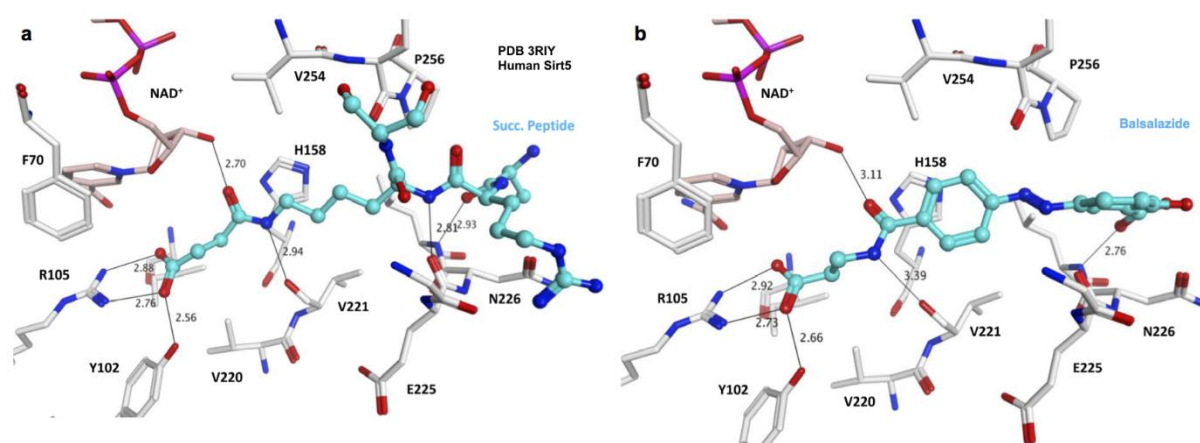


Figure 8: (a) Interaction of the co-crystallised succinyl-lysine based substrate peptide (carbon atoms in light blue) with Sirt5 (PDB 3RIY) and (b) docking results obtained for balsalazide (carbon atoms in light blue) in the presence of NAD^+ . Hydrogen bonds are shown as black lines and distances are given in Å.^[40]

First of all, the azo group in balsalazide, which must be replaced for removal of its original mechanism of action, showed no interactions with the active site of Sirt5. In contrast, the docking calculations proved the high relevance of a carboxylate group for utilisation of the side chains Arg105 (R105; guanidinium) and Tyr102 (Y102; phenol) in the active site, since Figure 8 shows that both the succinylated substrate peptide and balsalazide can interact with the protonated basic side chain of Arg105. Additional hydrogen bonds of balsalazide with a hydroxy group in the ribose subunit of NAD^+ as well as with the backbone carbonyl group of Val221 and Glu225 were also visible (Figure 8b). In conclusion, the side chain of balsalazide

significantly contributes to the binding affinity, the role of the salicylic acid part of balsalazide remained to be elucidated, and the azo group can potentially be modified without losing inhibitory activity.^[40]

With this information in hand, this study aimed at designing and synthesising analogues of balsalazide with comprehensive modifications on the functional groups of balsalazide as well as its core structural motif azobenzene to gain insights into the structure-activity relationships (Figure 9).

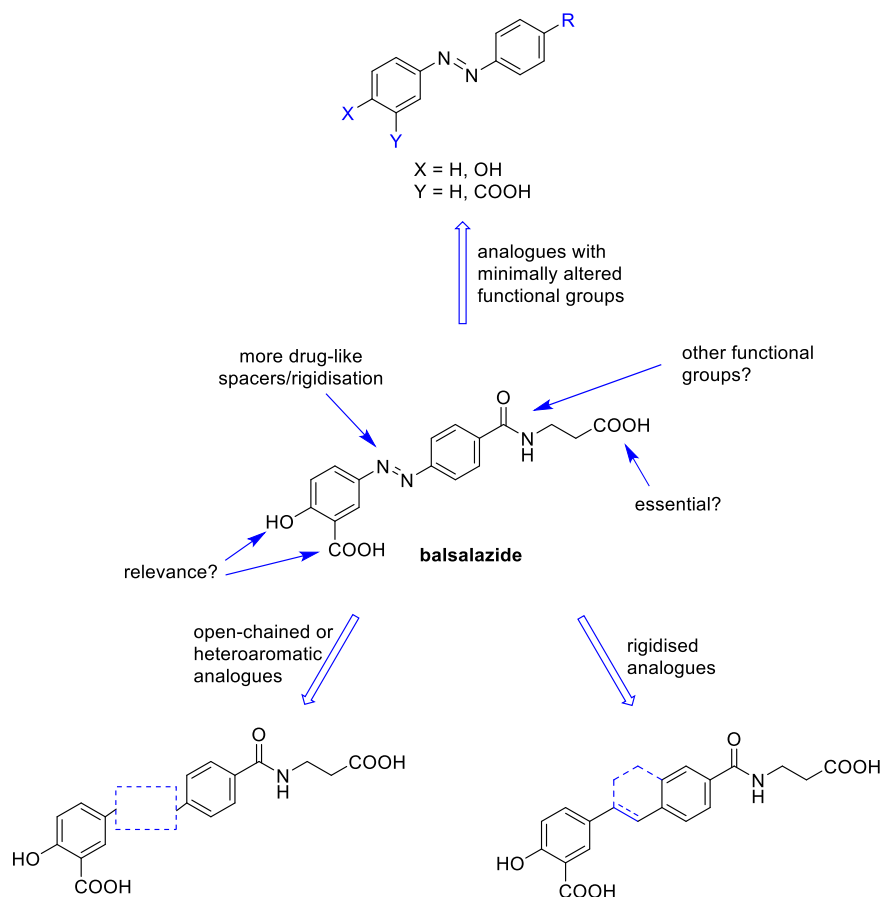


Figure 9: Structure of balsalazide and proposed structural modifications to exploit structure-activity relationships.

The purpose of the first set of analogues was to investigate which functional groups of balsalazide are crucial for high inhibitory activity. Therefore, single functional groups should either be deleted or minimally altered (Figure 9, top). To increase drug-likeness regarding metabolic properties of balsalazide, the modification of the central azo group was further considered as fundamentally important. Analogues addressing this structural element should follow two different structural principles as shown in Figure 9. On the one hand, the azo motif should be replaced by either open-chained spacers or heteroaromatic moieties, still retaining the flexibility of the molecule (Figure 9, bottom left). On the other hand, variations should not only replace the azo motif but at the same time rigidise the structure to reduce the loss of entropy that occurs during the binding process of the inhibitor to the enzyme (Figure 9, bottom

right). Their design and synthesis were limited by availability of starting materials and synthetic possibilities and will be discussed in the following chapters.

Consequently, this thesis should lead to the development and synthesis of highly selective and potent low-molecular inhibitors of Sirt5 that are urgently demanded for in-depth investigation of the physiological role of Sirt5 and could serve as innovative, potential therapeutics.

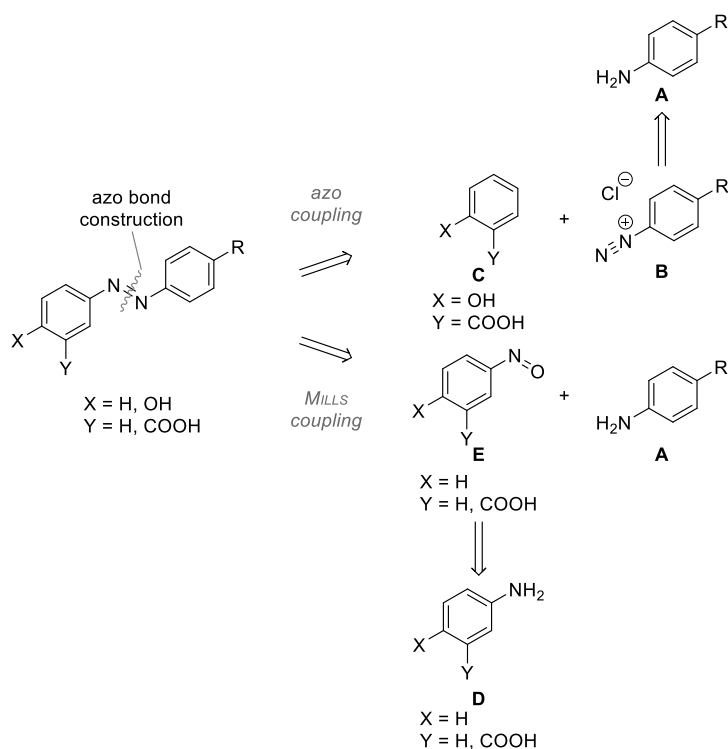
3. Synthesis and first biological evaluation

In this section all performed syntheses will be discussed and additional information such as changes in synthetic routes, essential reaction mechanisms and Sirt5 test results will be given and briefly explained. The first chapter (3.1) will include the preparation of balsalazide itself and all analogues with deleted or minimally altered functional groups, all of which still contain the central azo group. In the chapters that follow all variations of the spacer will be discussed (Chapter 3.2 and 3.4), including rigidised analogues (Chapter 3.2.1.3), as well as analogues of balsalazide carrying bioisosteric functional groups (Chapter 3.3).

3.1. Balsalazide and analogues with deleted or minimally altered functional groups

The results described in this chapter were published in the *EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY* in 2020.^[40]

Balsalazide consists of a central azo moiety and its synthesis is the basis for the preparation of all derivatives and analogues in this chapter. The syntheses of these, all of which still contain the central azo group, should be performed in the broadest sense following published syntheses of balsalazide^[78-79] with an azo coupling reaction (also known as diazo coupling) as illustrated in Scheme 1 of an *in-situ* prepared arenediazonium salt (**B**) and an electron-rich arene (**C**) as the central step (Scheme 1, top). Where this approach is not applicable due to electron poor coupling partners the azo unit should be constructed using a MILLS reaction with an aniline (**A**) and a nitrosoarene building block (**E**) (Scheme 1, bottom).

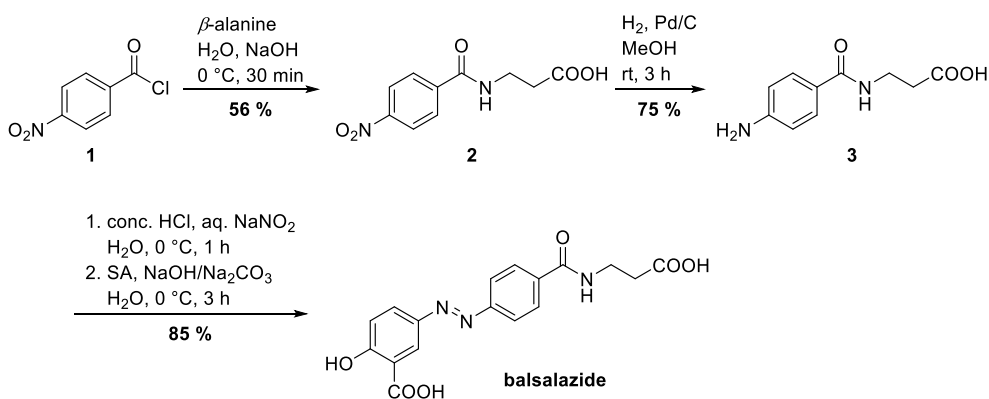


Scheme 1: Retrosynthesis of analogues still containing the central azo unit by azo coupling or MILLS coupling.

3.1.1. Synthesis

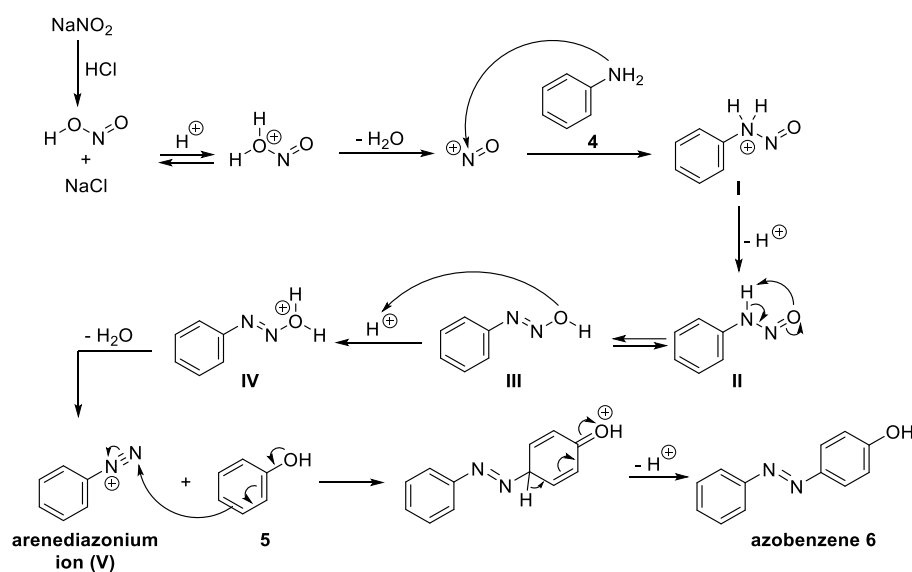
3.1.1.1. Balsalazide and analogues with a modified salicylic acid part of the molecule

For the preparation of balsalazide, a patent from 1983 by ROSALIND P. K. CHAN^[78] in combination with the slightly different published procedure from HOFMANN *et al.* from 2017^[79] was used as shown in Scheme 2. First, the β -alanine derived side chain was constructed by reaction of β -alanine and 4-nitrobenzoyl chloride (**1**) under cold and alkaline conditions to give amide **2** with 56 % yield. Subsequent reduction of the nitro group using palladium catalysed hydrogenation gave amine **3** in a good yield of 75 %. Balsalazide was then obtained in a regioselective azo coupling with salicylic acid (SA) in 85 % yield.



Scheme 2: Preparation of balsalazide according to published procedures.^[78-79] SA = salicylic acid.

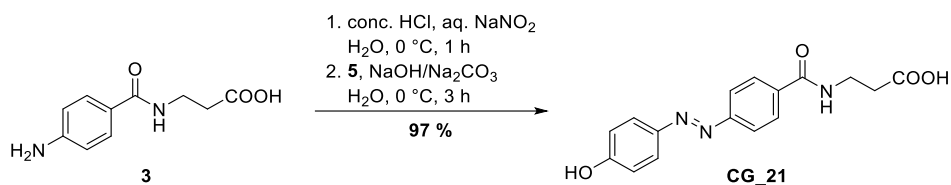
The mechanism of an azo coupling follows an electrophilic aromatic substitution reaction, where the arenediazonium salt is the electrophile and an electron rich activated arene is the nucleophile. In the following the mechanistic steps are exemplified with aniline (**4**) and phenol (**5**) (Scheme 3). The first step involves a diazotisation of an aromatic primary amine at low temperature: nitrous acid is generated *in situ* from NaNO_2 and acid (e.g. HCl) and is then protonated. Upon separation of water, nitrosonium is generated, which then is attacked by a primary aromatic amine (here: aniline (**4**)) to give the *N*-nitroso derivative **II**, a tautomer of diazohydroxide **III**. A second protonation and water elimination afford the relatively stable, isolable, although still reactive arenediazonium ion / salt **V**. Compared to alkanediazonium ions, arenediazonium salts are stabilised by resonance of the π -electrons in the diazo function with those of the aromatic ring. Thereby this species does not undergo immediate N_2 loss by the high energy of the resulting aryl cations. With an activated arene such as phenol (**5**) or aniline (**4**), carrying electron donating groups (EDGs), the weakly electrophilic arenediazonium ion can now form the desired azobenzene (**6**). The substitution reactions usually take place in *para* position to the EDG. When this position is already occupied, the substitutions occur in *ortho* position. Moreover, the reaction is very pH dependent. For the reaction to take place with a phenol it has to be present in the deprotonated form to increase its nucleophilicity. In addition, the diazonium salt evolves into a diazohydroxide in the presence of a base, thus inhibiting the coupling. Therefore, the pH must be adjusted to moderately basic (pH 8 – 10). For the coupling with aromatic amines the pH has to be mildly acidic (pH 4 – 5) to prevent *N*-coupling without reducing the nucleophilicity of the nitrogen that will be protonated under strong acidic conditions.^[80-81]



Scheme 3: Mechanism of diazotisation and subsequent azo coupling. Exemplified with aniline (**4**) and phenol (**5**).

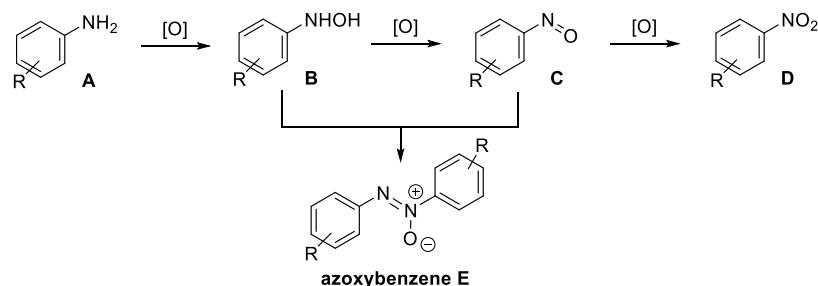
Employing this method again, the first analogue lacking the carboxylate group of the salicylic acid part was prepared (Scheme 4). Here, previously synthesised amine **3** (Scheme 2) was

used in an azo coupling reaction with phenol (**5**) to give azobenzene **CG_21** in almost quantitative yield (97 %).



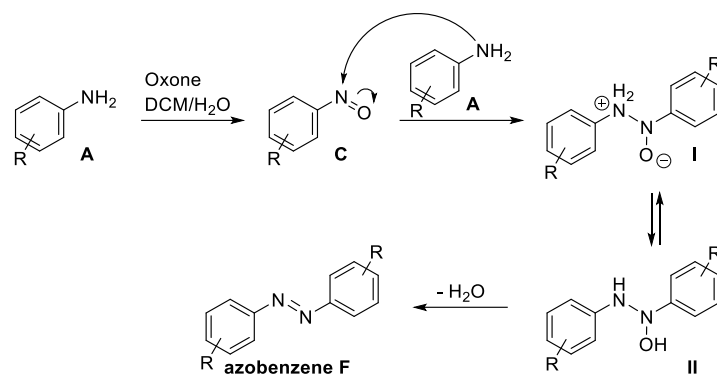
Scheme 4: Azo coupling for the preparation of phenol **CG_21**.

Two further analogues regarding variations of the salicylic acid part were then prepared. These analogues were missing the phenolic OH group, wherefore an azo coupling reaction could not be used for their synthesis. Consequently, MILLS coupling reactions with highly reactive aromatic nitroso derivatives and anilines were conducted. Some nitroso derivatives are stable and hence commercially available. Preparation of other nitroso compounds is commonly achieved by oxidation of primary amines with oxidants such as acetic acid/H₂O₂, KMnO₄, *m*-CPBA or from aromatic (methyl-)hydroxylamines using e.g. ferric chloride or sodium or potassium dichromate and sulfuric acid. These heterogeneous oxidation reactions are often slow and give low yields due to low stability of the hydroxylamine (**B**) or the nitroso product (**C**). Problems can also be over-oxidation, causing formation of nitro derivatives (**D**) or condensation reactions, yielding azoxybenzenes (**E**) from *N*-arylhydroxylamine intermediates (**B**) and nitroso intermediates (**C**) (Scheme 5).^[80, 82]



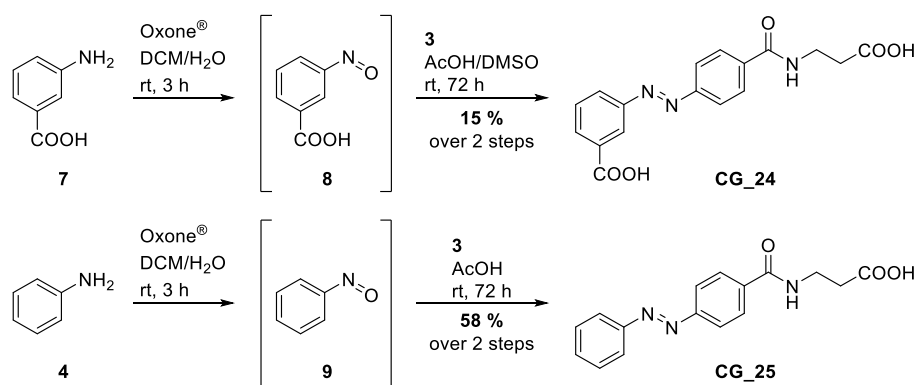
Scheme 5: Side reactions occurring under MILLS coupling standard oxidation conditions (cf. lit. ^[80]).

For this work a two-phase heterogeneous system consisting of Oxone[®] (2 KHSO₅ • KHSO₄ • K₂SO₄) in DCM/water was chosen to form the required nitrosoarenes. These intermediates can, if stable, be isolated or used without further purification to condense with the appropriate anilines. The biphasic system prevents undesired condensation reactions by separation of the generally less water-soluble nitroso compound (**C**) from the *N*-arylhydroxylamine intermediates (**B**) and aniline precursors (**A**). The mechanism of the desired MILLS reaction involves the attack of an aniline (**A**) onto the nitroso derivative **C** in acidic media (Scheme 5 and Scheme 6). Upon dehydration of intermediate **I** resp. **II**, azobenzenes (**F**) are formed.^[80]



Scheme 6: General mechanism of MILLS couplings to form azobenzenes (cf. lit. [80]).

Following the aforementioned conditions for MILLS coupling reactions, compounds **CG_24** and **CG_25** were prepared (Scheme 7). In a first step, literature known nitroso derivatives **8**^[83] and **9**^[84] were synthesised from the corresponding anilines **7** and **4**, respectively, by oxidation with Oxone[®] in DCM/water. Without purification, the nitroso compounds were coupled to amine **3** under acidic conditions^[83] for 72 h. Benzoic acid **CG_24** was obtained in only 15 % yield over two steps, whereas phenyl compound **CG_25** was obtained in 58 % yield over two steps.



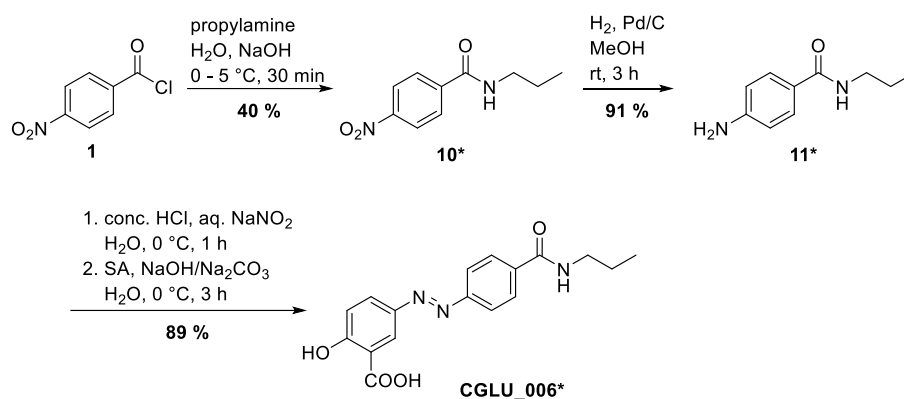
Scheme 7: Preparation of balsalazide derivatives **CG_24** and **CG_25** with truncated salicylic acid parts.

3.1.1.2. Analogues with variations affecting the *N*-aroyl- β -alanine side chain of balsalazide

The following analogues affected the *N*-aroyl- β -alanine side chain of balsalazide. Several of these analogues as well as precursors thereof were prepared in the context of an internship of LARS URBAN under my supervision (marked with *). In his report,^[85] successes and failures in synthesising these molecules were already comprehensively discussed, which is why in the present thesis only the syntheses will be mentioned that led to the desired results. If compounds were prepared in his internship and additionally in this work, they will be marked with **.

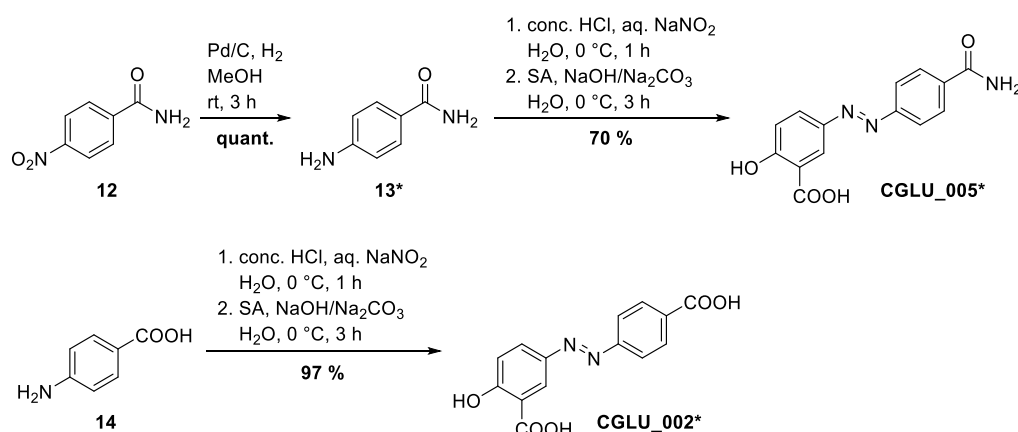
The first analogue should be missing the terminal carboxylic acid to determine, whether the interaction of a carboxylate group with the side chains of Arg105 and Tyr102 of Sirt5 observed

in the docking calculations is indeed essential. The reactions leading to the desired azo compound **CGLU_006*** could be performed according to the synthetic steps to balsalazide (Scheme 8 and cf. Scheme 2). In a first step, an amide coupling reaction was performed with 4-nitrobenzoyl chloride (**1**) and propylamine under cold and alkaline conditions to give amide **10***. Subsequent hydrogenation gave amine **11*** in a very good yield of 91 %. Azo coupling with salicylic acid resulted in azobenzene **CGLU_006*** with 89 % yield.



Scheme 8: Preparation of truncated (des-carboxy) derivative **CGLU_006***.

Two analogues with even shorter side chains lacking the β -alanine unit completely were prepared by azo coupling of salicylic acid with the precursors **13*** (prepared *via* hydrogenation of 4-nitrobenzamide (**12**)) and benzoic acid **14**, respectively, (Scheme 9). Primary benzamide **CGLU_005*** was obtained in 70 % yield. Benzoic acid **CGLU_002*** was obtained in almost quantitative yield (97 %).

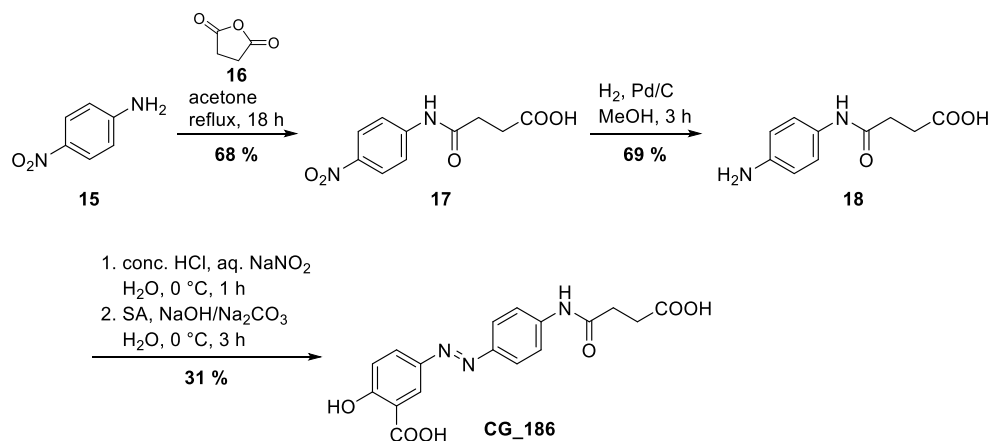


Scheme 9: Preparation of primary benzamide **CGLU_005*** and benzoic acid derivative **CGLU_002***.

Following these truncated analogues of balsalazide, the preparation of molecules was pursued where the role of the benzamide moiety could be investigated.

In comparison to natural *N*-succinylated substrates of Sirt5, the amide in balsalazide is inverted. Thus, an analogue of balsalazide was further synthesised carrying an inverted amide

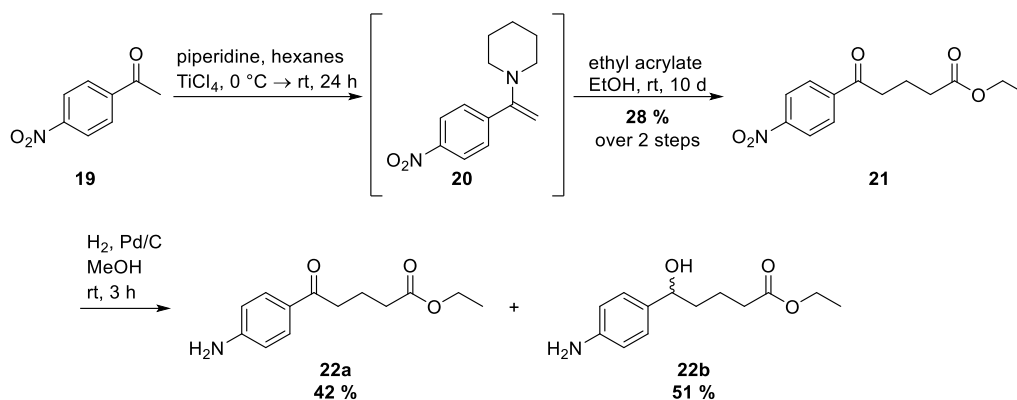
in the form of an *N*-succinylated aromatic amine (**CG_186**) (Scheme 10). Initially, precursor **17** should be synthesised according to a published procedure from CORREA-BASURTO *et al.*^[86] where the synthesis of **17** and amides in general was performed under solventless conditions by mixing an equimolar amount of monosubstituted anilines and succinic anhydride (**16**). In this case none of the starting materials were a liquid or at least an oil, which is possibly the reason why no reaction occurred even after stirring the solids overnight or using a mortar. To generate conditions where solids are more likely to react with each other, a solvent, in this case acetone, was added. The reaction mixture was vigorously stirred at room temperature overnight, but again unchanged starting materials remained. After another literature search, a procedure from BURDULENE *et al.*^[87] revealed the need for higher temperatures for the synthesis of *N*-substituted succinamic acids like **17**. Therefore, amine **15** was dissolved in acetone and succinic anhydride (**16**) was added and the mixture heated to reflux for 18 h. The precipitate was then collected by filtration and dried *in vacuo* to give **17** in 68 % yield. Subsequent hydrogenation (69 % yield) and azo coupling with salicylic acid generated *N*-succinylated aromatic amine **CG_186** (31 % yield).



Scheme 10: Preparation of **CG_186** containing an inverted amide.

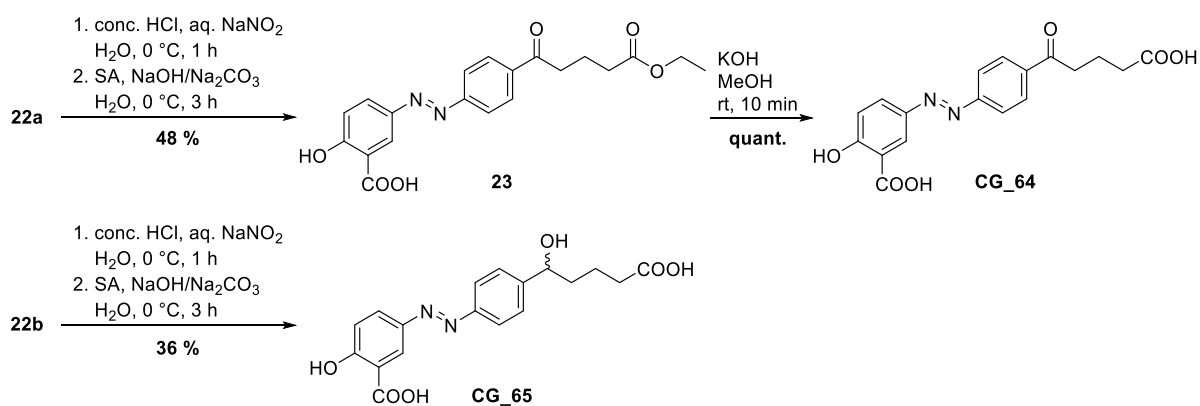
The benzamide unit of balsalazide was then further investigated by stepwise (molecule-wise) removing hydrogen bond accepting or donating parts until full replacement of the benzamide with an ethylene unit.

First, replacement of the amide nitrogen with a methylene led to aromatic ketone **CG_64** and secondary alcohol **CG_65**. For these, precursor **21** was prepared according to DÍAZ-MOSCOSO *et al.*^[88] Starting from 4-nitroacetophenone (**19**) and piperidine, a condensation reaction gave enamine **20**, and subsequent MICHAEL addition with ethyl acrylate gave the desired ketone **21** in 28 % yield over two steps.^[88] Hydrogenation using standard conditions then formed the desired δ -ketoester **22a** (42 %) as well as racemic hydroxy ester **22b** (51 %) as shown in Scheme 11.



Scheme 11: Preparation of azo coupling precursors **22a** and **22b**.

Both products were used in an azo coupling reaction with salicylic acid (Scheme 12). Ketone **22a** underwent azo coupling generating azobenzene **23** in 48 % yield. This intermediate was then deprotected in quantitative yield by alkaline hydrolysis of the ethyl ester to give the free carboxylic acid **CG_64**. Surprisingly, alcohol **22b** did undergo the azo coupling reaction as well but in contrast to **22a** the ethyl ester was cleaved simultaneously whereby hydroxyacid **CG_65** was obtained in 36 % yield. Possibly the conditions were slightly more basic, which led to concerted hydrolysis of the ester. Important to note here is the observed instability of both amine **22b** and azobenzene **CG_65**. Both compounds experience dehydration reactions (presumably to a styrene or lactone) among other decomposing reactions (**22b** to some extent slower). Therefore, these molecules have to be used for following reactions or tested shortly after preparation and biological results have to be interpreted with care.

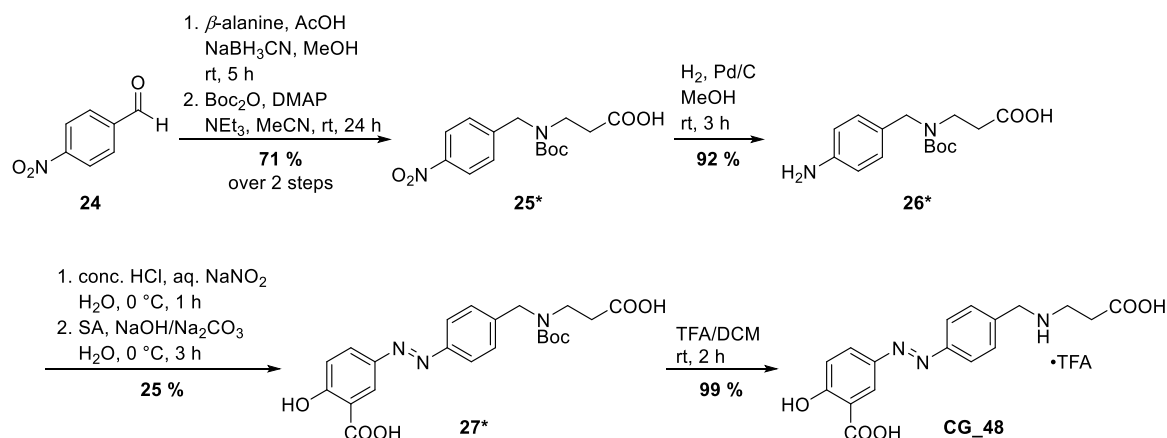


Scheme 12: Preparation of ketone **CG_64** and alcohol **CG_65**.

The interactions of the carbonyl oxygen of the benzamide moiety in balsalazide were further investigated by creating several molecules missing this carbonyl group.

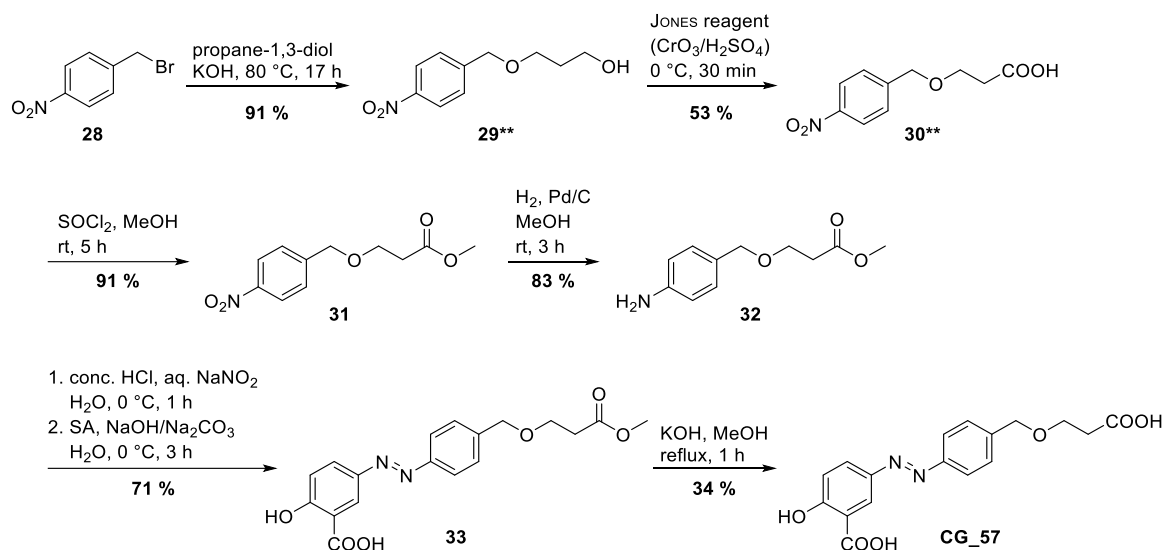
The reactions leading to secondary benzylamine **CG_48** depicted in Scheme 13 started with a published procedure from MENDOZA-SANCHEZ *et al.*^[89] comprising of a reductive amination of aldehyde **24** with β -alanine and NaBH_3CN , followed by Boc-protection of the benzylamine

to give **25*** in 71 % yield over two steps.^[89] Protection of the secondary amine was chosen to prevent the formation of toxic and cancerogenic nitrosamines in this position in the diazotisation/azo coupling step. As with previous analogues of balsalazide, hydrogenation of the nitro group (**26***; 92 %) followed by azo coupling with salicylic acid led to Boc-protected azobenzene **27*** (25 %). The low yield of the azo coupling here could be explained by undesired deprotection of the amine due to the strong acidic conditions used for the diazotisation. If higher yields were required, different protecting groups could be explored. Here this was not necessary and the obtained yield sufficient. For the final deprotection of the benzylamine, standard conditions with trifluoroacetic acid (TFA) and DCM at room temperature were used and the desired analogue **CG_48** was obtained as the TFA salt in 99 % yield.



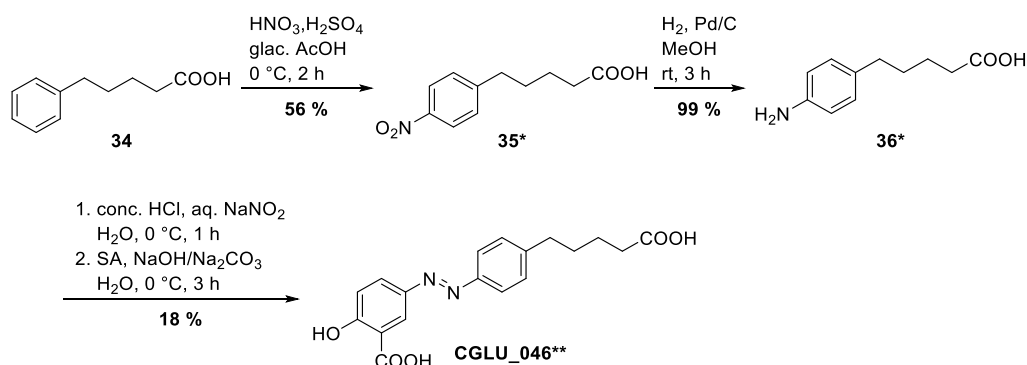
Scheme 13: Preparation of amine **CG_48**.

Another compound missing the carbonyl group was benzyl ether **CG_57** (Scheme 14). Compared to amine **CG_48** this structure is now also lacking hydrogen bond donating abilities. In a first step literature known benzyl ether **29**** was constructed from 4-nitrobenzyl bromide (**28**) and propane-1,3-diol in 91 % yield using WILLIAMSON ether synthesis conditions according to WANG *et al.*^[90] Oxidation^[91] of the terminal primary alcohol of **29**** was accomplished with freshly prepared JONES reagent in a moderate yield of 53 %. Because of the presumably high lability of the benzyl ether under hydrogenation conditions, carboxylic acid **30**** was protected as the methyl ester **31** (91 % yield) and the nitro group should be reduced using iron in acetic acid. Unfortunately, under these conditions the starting material (**31**) was never fully converted to the corresponding amine (**32**). Nevertheless, hydrogenation of **31** was then tried instead and fortunately, **32** was obtained in 83 % yield. Azo coupling with salicylic acid gave azobenzene **33** in a good yield of 71 %, which was deprotected in a final step upon alkaline hydrolysis to give the free carboxylic acid **CG_57** in 34 % yield.



Scheme 14: Preparation of benzyl ether **CG_57**.

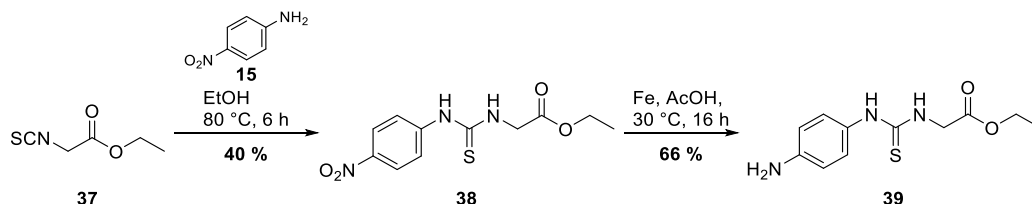
Replacement of the carboxamide with an ethylene unit provided an analogue missing both hydrogen accepting and donating abilities in the region of interest as shown in Scheme 15. For its (**CGLU_046****) preparation, 5-phenylpentanoic acid (**34**) was nitrated in *para* position in a procedure based on the work of FREEDMAN *et al.*^[92] and **35*** was obtained in 56 % yield. Reduction of the nitro group was again accomplished *via* palladium catalysed hydrogenation in almost quantitative yield (**36***; 99 %). Unfortunately, azo coupling then did not go as smoothly as before and gave **CGLU_046**** in a poor yield of 18 %.



Scheme 15: Preparation of ethylene analogue **CGLU_046****.

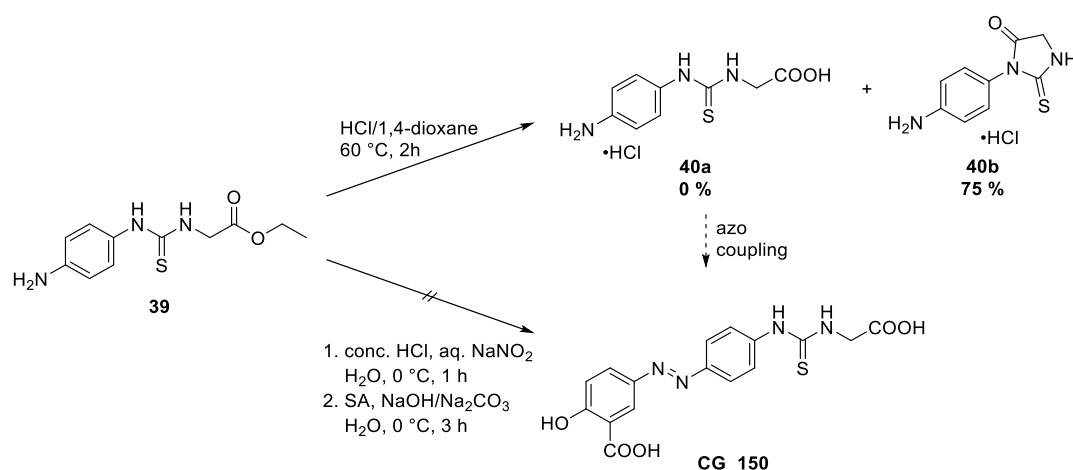
In this series of analogues with variations affecting the *N*-aroyl- β -alanine side chain of balsalazide another analogue was designed inspired by a publication of ZANG *et al.* from 2015,^[93] who postulated thiourea containing inhibitors to be highly effective “warheads” for Sirt5 inhibition (in this context see also a subsequent study by RAJABI *et al.*^[94]). Therefore, thiourea **CG_150** (Scheme 17 and Scheme 18) should be synthesised. In a first attempt, as with several preceding analogues, the modified *N*-aroyl- β -alanine side chain should be constructed before the final azo coupling reaction. As illustrated in Scheme 16, the thiourea motif was generated

by heating equimolar amounts of ethyl isothiocyanatoacetate (**37**) and 4-nitroaniline (**15**) to 80 °C for 6 h based on a procedure by KUZNETSOVA *et al.*^[95] Product **38** was obtained in 40 % yield. In contrast to previous syntheses, where hydrogenation with H₂ could be employed, subsequent reduction of the nitro group had to be performed using iron in acetic acid leading to **39** in 66 % yield. Hydrogenation was also tested but as expected led to unchanged starting material. Possibly this is due to catalyst poisoning by the thiourea itself.^[96]



Scheme 16: Preparation of thiourea intermediate **39**.

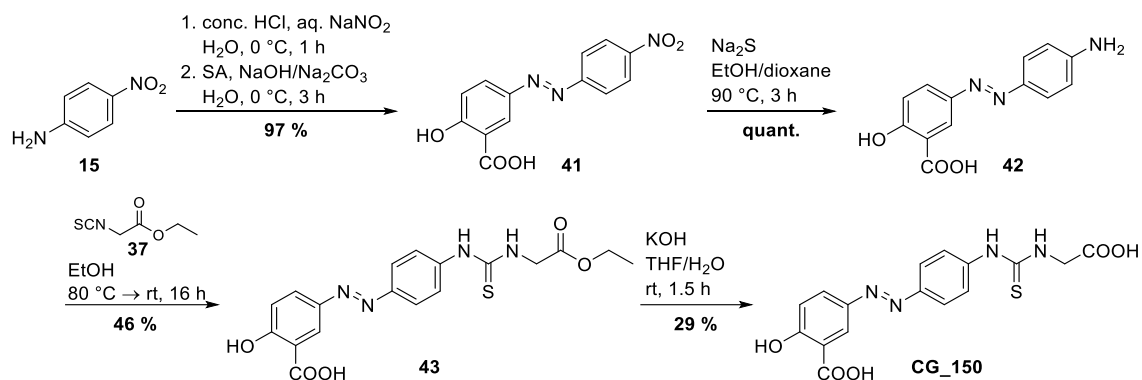
Before conducting the azo coupling, the ethyl ester of **39** should be cleaved to generate the free carboxylic acid. To avoid working with a zwitterionic product, the deprotection should be performed under standard conditions with HCl/1,4-dioxane at 60 °C (Scheme 17), generating the HCl-salt, which should be collected by filtration after precipitating. Unfortunately, **40a** was not obtained, but instead a cyclised side product (**40b**) was isolated in 75 % yield. A literature search revealed that this cyclisation indeed occurs under acidic conditions and was published by JÓZEF RYCZEK in 2003.^[97] In fact, 2-thiohydantoin **40b** was prepared and characterised in this publication as well (see experimental part Chapter 6.4.1 for further details). Another attempt to perform the azo coupling before deprotection of the thiourea derivative did not result in the desired product **CG_150**.



Scheme 17: Failed attempts to thiourea intermediates **40a** and azobenzene **CG_150**.

As a consequence of these outcomes, the thiourea motif should be constructed after azo coupling (Scheme 18). By coupling 4-nitroaniline (**15**) with salicylic acid, azobenzene **41** (also known as alizarin yellow R) was obtained in almost quantitative yield (97 %). Reduction of the nitro group was performed using Na₂S and heating to reflux for 3 h (ZININ reaction), following

a protocol published by RÜCK-BRAUN *et al.*^[98] Compound **42** was generated in quantitative yield as a dark purple solid. Now the thiourea unit was generated following the procedure described before (Scheme 16, preparation of thiourea **38**) but with prolonged stirring of the reaction mixture at room temperature for 16 h. Product **43** was obtained in 46 % yield as a yellow-greenish solid. Subsequent alkaline ester hydrolysis under standard conditions gave the desired final thiourea analogue **CG_150** (29 % yield) as an orange solid. The purification of **CG_150** by FCC was challenging since this product is barely UV active and streaks on TLC.



Scheme 18: Preparation of thiourea analogue **CG_150**.

In general, it should be noted that purifications using flash column chromatography (FCC) with molecules carrying several different polar functional groups, as it was always the case here with the final compounds, is not ideal. On the one side, separation of structurally very similar impurities was often not possible. On the other side, the desired compounds were usually not or only soluble in high amounts of polar solvents used for FCC (e.g. EtOAc or MeOH). This led to endless fractions of product until remaining product often had to be eluted with pure MeOH, which resulted in again impure material. Best results were usually obtained when the product could be precipitated from an aqueous solution by acidifying it to pH 1 – 2. Unfortunately, this was only possible when no structurally related impurities were present anymore and the scale of the reaction was big enough (~70 mg product). Detailed information on work-up procedures and purifications can be found in the experimental part (Chapter 6).

In summary, balsalazide itself and 13 analogues with deleted or minimally altered functional groups, all of which still contain the central azo group, were prepared as shown in an overview in Figure 10. These analogues were subsequently tested for Sirt5 inhibitory activity.

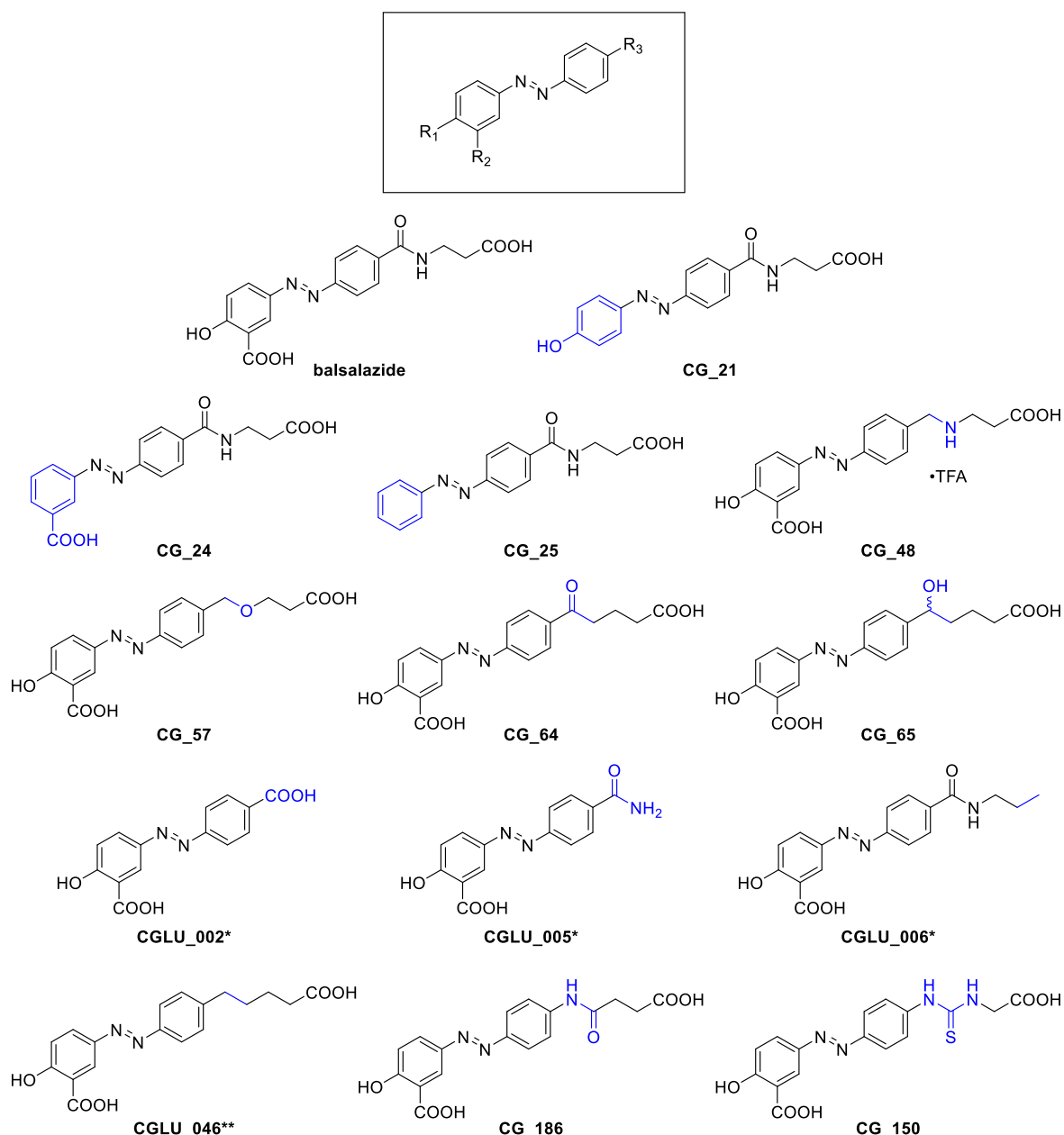


Figure 10: Balsalazide and the 13 synthesised analogues with deleted or minimally altered functional groups.

3.1.2. Inhibitory activity against Sirt5

The synthesised analogues should be compared in their inhibitory activity with the lead structure balsalazide in a first testing campaign. For this purpose, an enzyme-based fluorometric assay system from Enzo Life Sciences (Lörrach, Germany) was used according to manufacturer's instructions. In principle, the assay procedure comprises two steps as illustrated in Figure 11a and b: In the first step the FLUOR DE LYS[®]-Succinyl substrate is incubated with human recombinant Sirt5, the co-substrate NAD⁺, and the potential inhibitor. In the second step the desuccinylated FLUOR DE LYS[®]-Succinyl is treated with the FLUOR DE LYS[®] Developer to produce a fluorophore ($\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$; see Chapter 6.1.3 for detailed procedures).

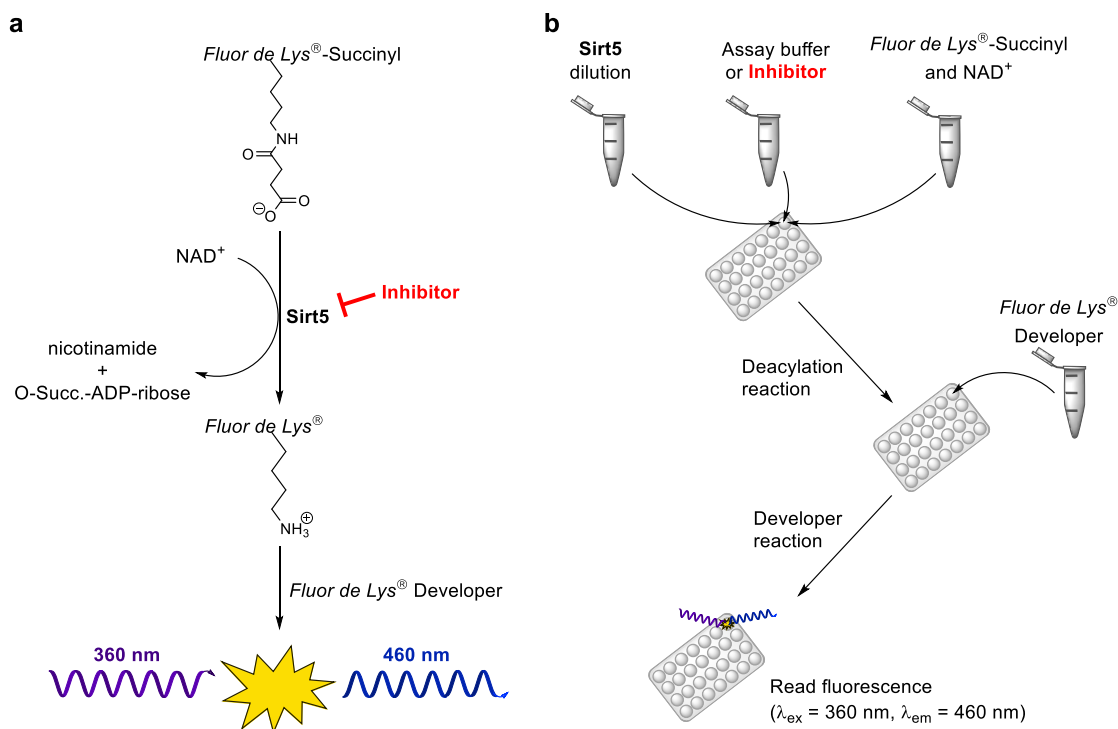


Figure 11: (a) The principle of the FLUOR DE LYS[®] SIRT5 fluorometric drug discovery assay kit and (b) the procedure to perform the assay (cf. [99]).

All compounds, including balsalazide and the known Sirt5 inhibitors nicotinamide and suramin, were then tested at a final assay concentration of 50 μM (Figure 12 and Table 2). A precise IC_{50} value should only be measured for analogues showing similar or higher inhibitory activity than balsalazide already at this concentration (see Chapter 4).

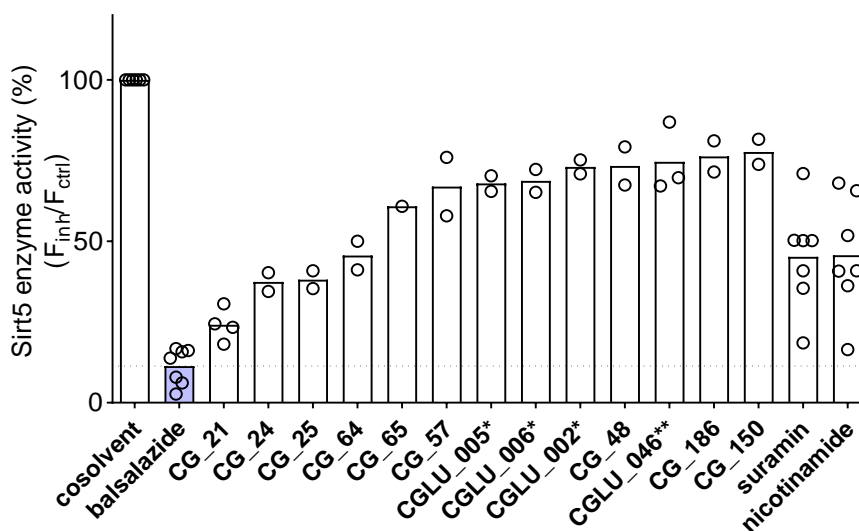


Figure 12: Residual Sirt5 enzyme activity after 1 h incubation with the inhibitors in % ($F_{\text{inh}}/F_{\text{ctrl}}$). The known non-selective inhibitors nicotinamide and suramin were included as positive controls. Fluorescence measured upon incubation with the cosolvent DMSO alone (no inhibitor) was set to 100 % enzyme activity. All inhibitors were tested at 50 μM final concentration. F = fluorescence units; $\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$; number of biological replicates stated with circles (\circ) for each bar (cf. lit. [40]).

Table 2: Sirt5 inhibition in %; values represent means of technical and biological replicates (cf. lit. [40]).

Compound name	Inhibition in %	Compound name	Inhibition in %
balsalazide	89	CG_65	39
CG_21	76	CG_150	28
CG_24	63	CG_186	22
CG_25	62	CGLU_002*	27
CG_48	27	CGLU_005*	32
CG_57	33	CGLU_006*	31
CG_64	54	CGLU_046**	25
nicotinamide	54	suramin	55

First of all, it could be verified that balsalazide is indeed an inhibitor of Sirt5 with a percentual Sirt5 inhibition of 89 % at 50 μM . The results shown in Figure 12 and Table 2 also reveal that some of the synthesised analogues (**CG_21**, **CG_24**, **CG_25**) showed comparable inhibitory activity to balsalazide with 62 – 76 % Sirt5 inhibition. The known Sirt5 inhibitors nicotinamide and suramin were less active than balsalazide and show more scattering (Figure 12).^[40]

In more detail, when taking a look at the poorly active analogues **CGLU_002*** (27 %), **CGLU_005*** (32 %), and **CGLU_006*** (31 %) missing the terminal carboxylate, the results showed that the carboxylic acid in the β -alanine-derived side chain has to be present and in an appropriate distance from the aromatic ring. This strongly supports the postulated binding mode *via* the crucial interaction of a carboxylate in the side chain with Arg105 and Tyr102 (see Figure 8, Chapter 2). For analogues **CGLU_046**** (25 %), **CG_48** (27 %), **CG_57** (33 %), and **CG_65** (39 %) it was presumed that the variations of the benzamide unit could also have a stronger impact on the activity since hydrogen bond donating and/or accepting abilities were modified. All these analogues showed significantly reduced inhibitory activity and no major differences could be identified between no heteroatoms (**CGLU_046****), an ether as a hydrogen bond acceptor (**CG_57**), a secondary amine as a hydrogen bond donor (HBD) and acceptor (HBA) (**CG_48**), or a secondary alcohol (**CG_65**). Interestingly, with keto analogue **CG_64** the weakest drop in activity was observed to 54 % Sirt5 inhibition in this subgroup of variations in the side chain. This is in accordance with the postulated hydrogen bond of the corresponding amide carbonyl group of balsalazide to NAD^+ (see Figure 8, Chapter 2), which seems important for high affinity. This finding is also undermined through the results obtained with the inverted amide in **CG_186**, where the inhibitory activity was also strongly reduced to only 22 %. Therefore, not only the carboxylic acid of the side chain must be in an appropriate

distance from the aromatic ring but also the carbonyl unit of the benzamide has to be retained. This hypothesis is also supported by the last variant with modifications in the β -alanine derived side chain: the thiourea-based analogue **CG_150**. This compound did in this study, in contrast to previous claims,^[93] not lead to higher affinity (only 28 % Sirt5 inhibition at 50 μ M). As mentioned before, this could be due to a change in position of the thiocarbonyl group compared to the benzamide carbonyl, leading to disruption of the hydrogen bond with a hydroxy group in the ribose subunit of NAD⁺.^[40]

Interesting results were further obtained among the compounds containing changes on the salicylic acid part of the lead structure (**CG_21**, **CG_24**, **CG_25**) with comparable inhibitory activity to balsalazide. Overall, the drop in potency of these inhibitors was not as significant as it was observed before for the analogues with modifications on the side chain. Benzoic acid **CG_24** (*E/Z* ratio at the azobenzene unit of 90:10) and phenyl analogue **CG_25** (*E/Z* ratio of 89:11) resulted in 63 % and 62 % Sirt5 inhibition, respectively. Surprisingly, with 76 % inhibition, phenol **CG_21** showed less significant loss of activity. The higher activity of phenol **CG_21** compared to **CG_24** and **CG_25** can, at least in part, be attributed to the fact that *para*-hydroxy azobenzenes show fast thermal *cis*-to-*trans* isomerisation in protic polar solvents.^[100] Therefore, *para*-hydroxy azobenzenes like balsalazide and **CG_21** appear configurationally stable in *E*-configuration. These results lead to the conclusion that changes on the salicylic acid part have a weaker impact on Sirt5 inhibition and are tolerated to some extent.^[40]

With these 13 analogues and balsalazide itself, it could be revealed that none of the investigated structural features of balsalazide should be lightheadedly changed in order to retain Sirt5 inhibitory activity. Changes on the salicylic acid part are marginally tolerated, whereas even minor modifications on the β -alanine-derived side chain lead to massive loss of potency.

3.2. Variations of the core azo group (1)

As pointed out in preceding chapters, balsalazide contains a central azo moiety. In contrast to all previously synthesised analogues, the following chapter focuses on the first set (1) of variants where this azo unit was replaced by different spacers to continue exploring structure-activity relationships with the goal of designing and synthesising an analogue of balsalazide with increased drug-likeness for systemic use. Among the structures that were synthesised are variations carrying an open chained spacer modification as well as structures with a rigidised central unit (Figure 13). A second set (2) of analogues that carry heteroaromatic spacer variations will be discussed in Chapter 3.4.

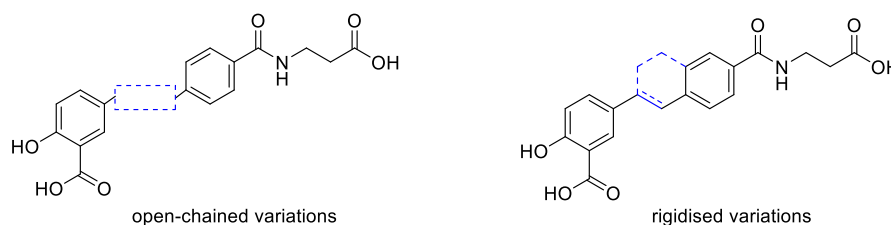


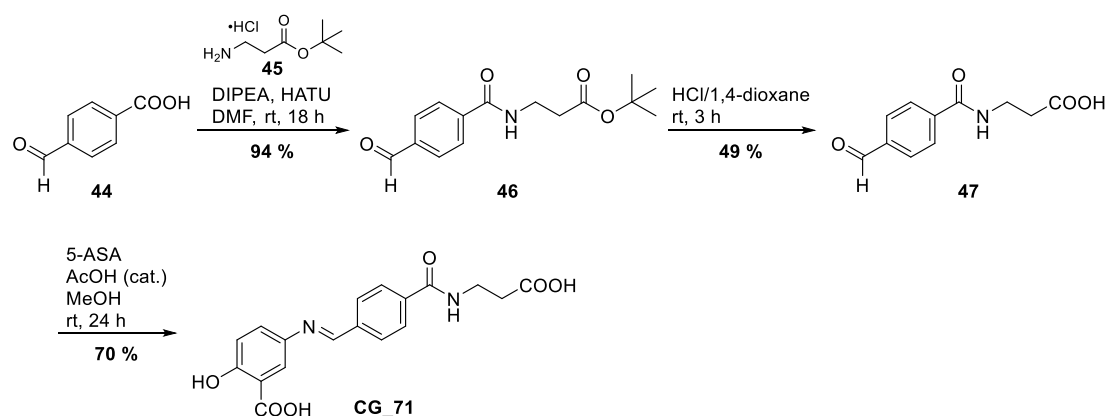
Figure 13: General structures of analogues with a modified azo group: open-chained and rigidised variations.

The syntheses of these analogues did not follow a general synthetic strategy. For each compound a synthetic route had to be designed individually.

3.2.1. Synthesis

3.2.1.1. Analogues with open-chained variations of the azo moiety

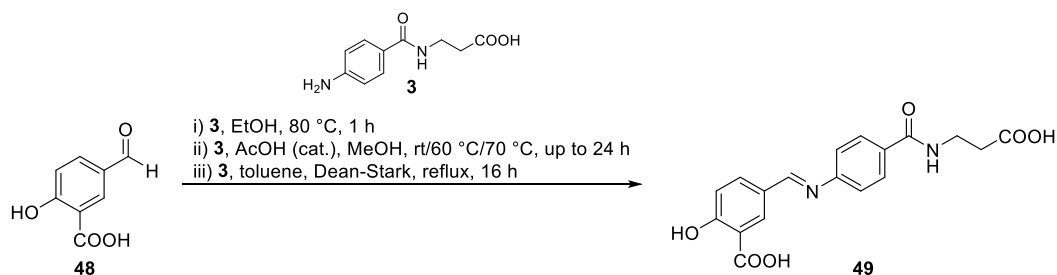
In order to change structural features step by step, in the first two analogues the azo group should be replaced by an imine resulting in one hydrogen bond acceptor less than in balsalazide. Imine **CG_71** was therefore prepared starting with an amide coupling according to a patent from CHIESI FARMACEUTICI S.P.A. from 2016^[101] between 4-formylbenzoic acid (**44**) and β -alanine *tert*-butylester hydrochloride (**45**) using DIPEA as the base and HATU as the coupling reagent.^[101] Aldehyde **46** was obtained in an almost quantitative yield of 94 %. Following the same patent, the ester group of **46** was cleaved using HCl/1,4-dioxane at room temperature to give the free carboxylic acid **47** in 49 % yield.^[101] To generate the final imine **CG_71**, a procedure was chosen published by Wu *et al.*,^[102] who prepared structurally related compounds based on 5-aminosalicylic acid (5-ASA) for the treatment of retinal degeneration. Condensation of **47** and 5-ASA gave imine **CG_71** in 70 % yield.



Scheme 19: Preparation of imine **CG_71**.

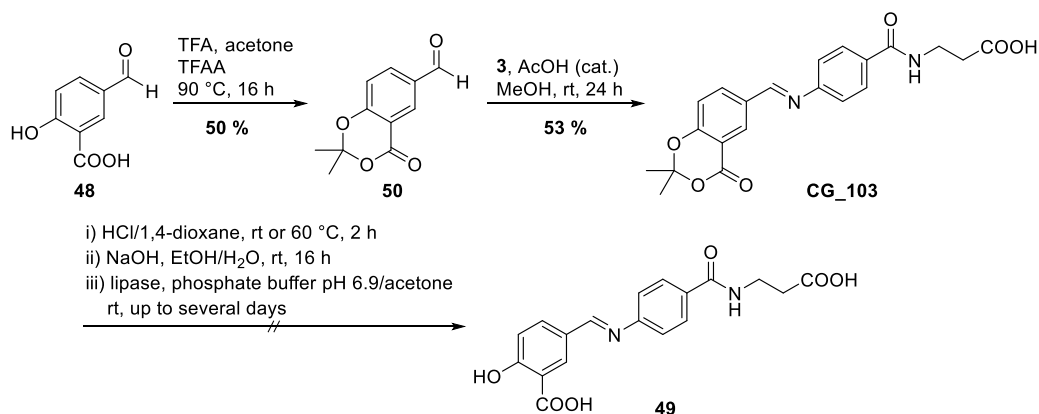
This imine as well as several following spacer variations should also be synthesised laterally reversed since possible interactions of the spacer could not be identified in the docking calculations yet. Therefore, it was intended to prepare isomeric imine **49** in the same manner as **CG_71** by condensation of 5-formylsalicylic acid (**48**) with previously prepared amine **3**

(Scheme 20). Three attempts were performed (i – iii), but unfortunately, a complete conversion of the starting materials could not be reached neither with varied equivalents of starting materials nor with prolonged reaction times, heating, or different solvent systems (Scheme 20). Starting material, impurities, and the desired imine **49** could not be separated, wherefore this approach was no longer pursued.



Scheme 20: Initial approaches for the synthesis of imine **49**.

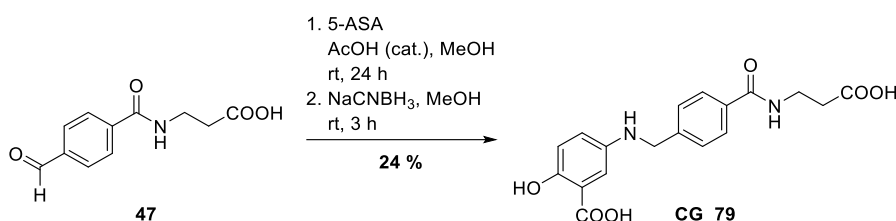
As the influence of the salicylic acid moiety on this reaction was not clear and to increase chances of isolating the desired product, the same reaction was performed now using acetonide protected 5-formylsalicylic acid (**50**) (Scheme 21). Aldehyde **50** was prepared following a patent from APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. from 2005^[103] using acetone, TFA and trifluoroacetic anhydride (TFAA). After heating the reaction mixture to 90 °C for 16 h, the acetonide protected aldehyde **50** was obtained in 50 % yield.^[103] Subsequently, the same condensation reaction as before was performed and this time acetonide protected imine **CG_103** could easily be collected by filtration in 53 % yield. Unfortunately, the following acetonide deprotection of **CG_103** under standard conditions led to decomposition of the whole molecule using both acidic (Scheme 21, i) and alkaline (Scheme 21, ii) conditions. A third attempt was an enzymatic cleavage^[104] of the protecting group using lipase (porcine pancreas lipase) (Scheme 21, iii). Only unchanged starting material was found even after prolonged reaction times up to several days, possibly due to the acetonide protecting group, which is not a regular ester, but a cyclic acylal that is in addition sterically hindered.



Scheme 21: Second approach for the preparation of **49**.

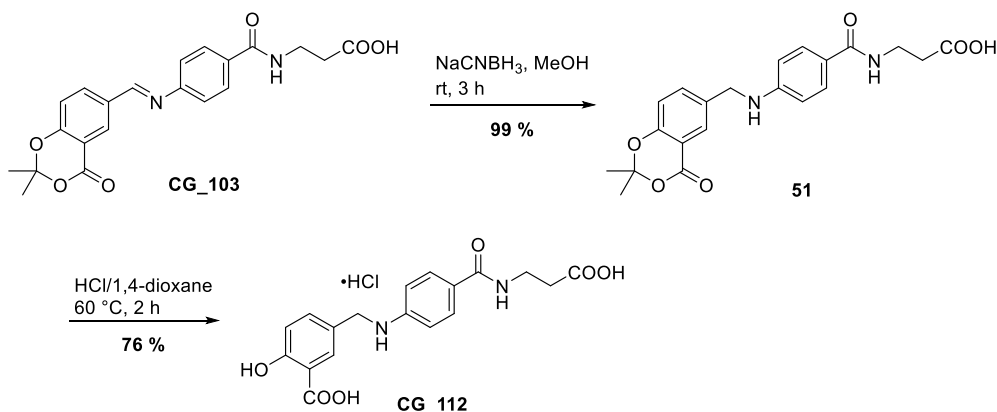
Other approaches for the synthesis of **49** were not pursued as imines are anyways considered as not lead- or drug-like^[105-106] and first insights in structure-activity relationships can also be obtained with the reversed imine **CG_71** and the following analogues.

Nevertheless, with synthetic procedures to **CG_71** and **CG_103** in hand, two further target compounds with additional hydrogen bond donating ability could easily be synthesised. This was accomplished by adding another reduction (e.g. reduction with NaCNBH₃ as in lit.^[107]) of the imines to the corresponding amines **CG_79** and **CG_112**, respectively. This way amine **CG_79** was synthesised starting from aldehyde **47** and 5-ASA in a low yield of 24 % (Scheme 22).



Scheme 22: Preparation of amine **CG_79**.

As depicted in Scheme 23, amine **51** was prepared starting from the corresponding isolated imine **CG_103** and was obtained in 99 % yield. Additional acidic hydrolysis of the acetonide protecting group gave HCl-salt **CG_112** with 76 % yield.

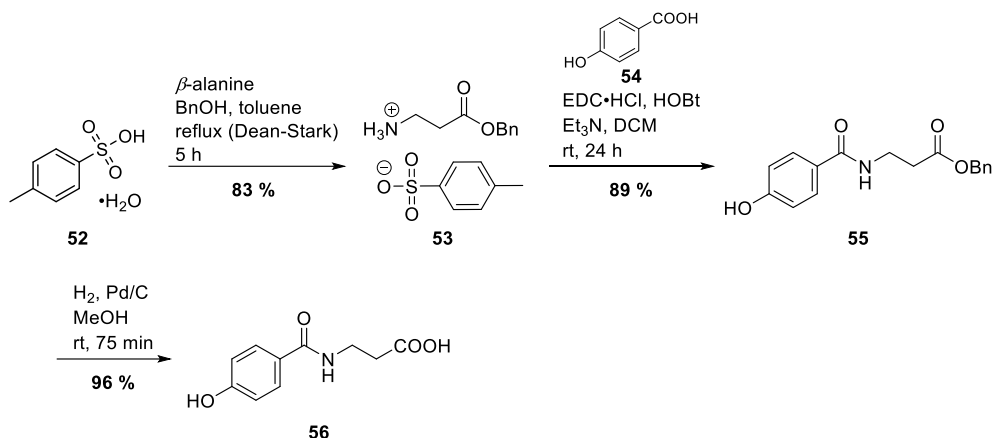


Scheme 23: Preparation of amine **CG_112**.

Replacing the amine with an ether should now generate variants with only hydrogen bond accepting abilities and, compared to an imine, a more stable modification.

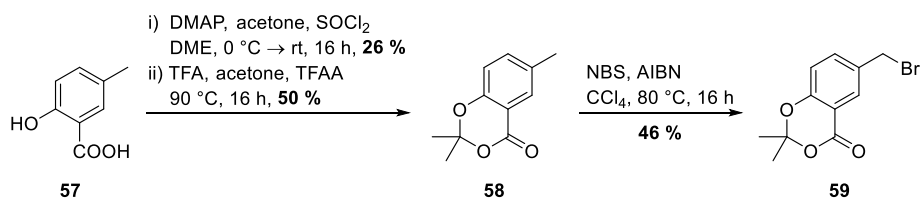
For the desired ether **60** (Scheme 26) the synthesis of several precursors was required. Hence, phenol **56** was prepared over three steps with very good yields following published protocols by KLOSS *et al.*^[108] Starting from β -alanine, *p*-toluenesulfonic acid hydrate (**52**), and benzyl alcohol in toluene, aminoester salt **53** was obtained in 83 % yield (Scheme 24). The following amide coupling with 4-hydroxybenzoic acid (**54**) with a combination of EDC•HCl and HOBT

yielded **55** in 89 %. Subsequent hydrogenolysis cleaved the benzyl ester and gave **56** in an excellent yield of 96 %.^[108]



Scheme 24: Synthesis of precursor phenol **56**.

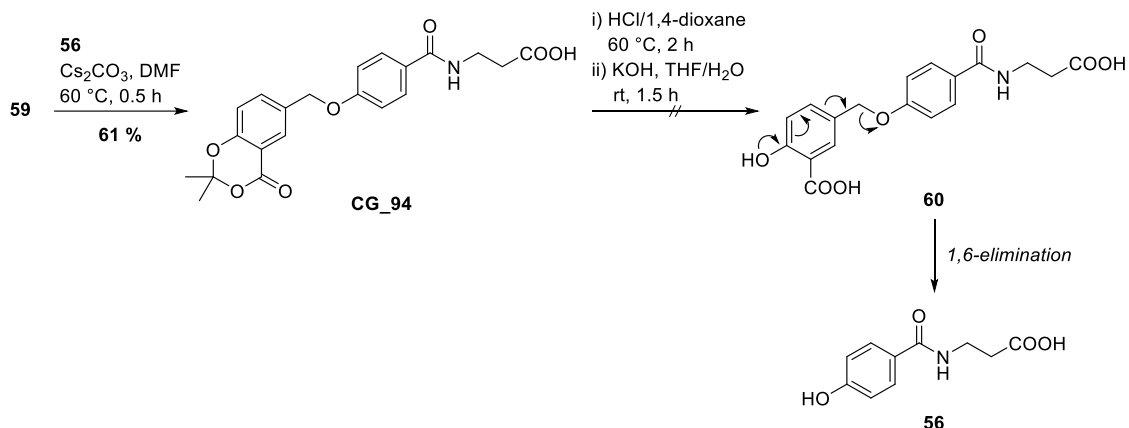
The second precursor, benzyl bromide **59** was prepared starting from 5-methylsalicylic acid (**57**) (Scheme 25). In the first instance, 5-methylsalicylic acid (**57**) was protected as an acetonide. Several publications such as MUKKAMALA *et al.*^[109] suggested to perform this protection using SOCl_2 , DMAP and acetone in DME (Scheme 25, i). Unfortunately, this approach gave **58** in a maximum yield of 26 % with high amounts of starting material remaining unchanged in the reaction mixture. Therefore, it was tested to apply the method used for the preparation of aldehyde **50** here as well to possibly increase the yields. Using TFA, acetone and TFAA, intermediate **58** was obtained in 50 % yield. Benzylic bromination was performed using MUKKAMALA's protocol^[109] with NBS and AIBN in carbon tetrachloride (CCl_4) under reflux for 16 h. Bromomethyl compound **59** was obtained in 46 % yield.^[109] It has to be noted, that the use of a pressure tube is highly recommended due to the use of highly toxic CCl_4 under reflux conditions. This solvent in turn is nowadays often replaced by alternative solvents such as MeCN, DCM or chloroform, which unfortunately often leads to lower yields and the resulting succinimide cannot easily be filtered off as it is the case with CCl_4 .



Scheme 25: Synthetic approaches to precursor bromide **59**.

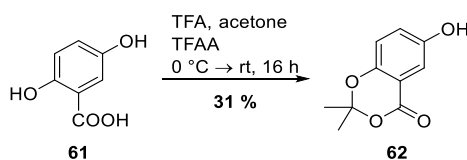
With precursors phenol **56** and bromide **59** a WILLIAMSON ether synthesis was then performed using cesium carbonate as the base, giving ether **CG_94** in 61 % yield (Scheme 26). For the

last step, the acetonide deprotection, several tests using acidic and alkaline conditions were performed. Here it could be verified *via* NMR and mass spectrometry that under deprotection conditions a 1,6-elimination occurs and **56** was recovered, but no product was obtained (1,6-elimination of **60** illustrated in Scheme 26). Thus, this spacer variant could not be synthesised.



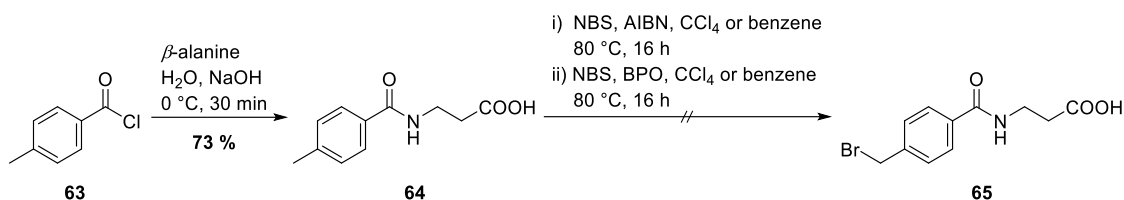
Scheme 26: Synthetic approach for the synthesis of ether **60** *via* compound **CG_94** and illustrated occurring 1,6-elimination.

Nevertheless, an isomer of **60** should also be prepared with the ether laterally reversed, where no such 1,6-elimination can occur. In a first attempt, a WILLAMSON ether synthesis was tried for constructing the desired ether bond. One precursor necessary for the preparation of ether **CG_129** (Scheme 33) was synthesised following a patent from BIOLIPOX AB from 2009.^[110] Here again, TFA, acetone and TFAA is used, but for this product the reaction mixture is stirred at 0°C to room temperature. Phenol **62** was obtained in a moderate yield of 31 % (Scheme 27).^[110]



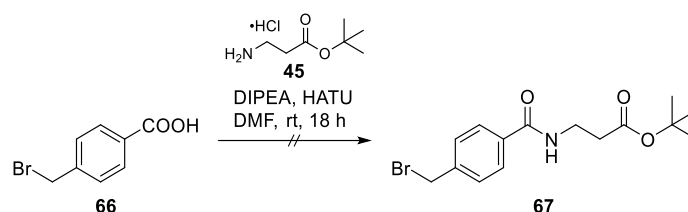
Scheme 27: Preparation of phenol **62**.

The second precursor (**65**) should be synthesised starting from 4-methylbenzoyl chloride (**63**) in an amide coupling according to the conditions used for amide **2** (Scheme 28 and cf. Scheme 2). Amide **64** was obtained in a good yield of 73 %. Subsequently, bromination of the methyl group should be performed. Unfortunately, the desired product **65** could not be formed using radical bromination conditions.



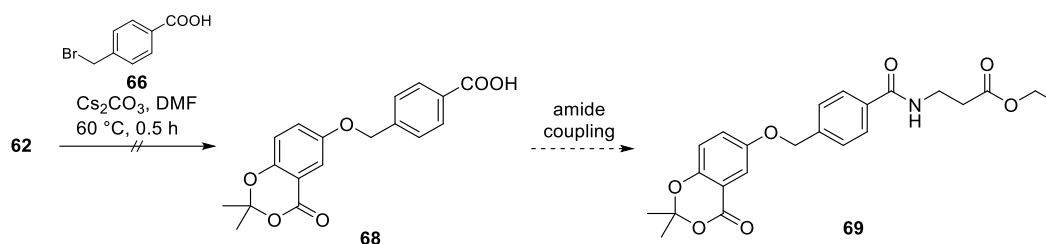
Scheme 28: Attempts for the synthesis of bromide **65**.

Therefore, it was decided to use commercially available 4-(bromomethyl)benzoic acid (**66**) instead, which can undergo amide coupling with β -alanine *tert*-butylester hydrochloride (**45**) to generate bromide **67**. Unfortunately, bromide **67** could not be generated this way (Scheme 29).



Scheme 29: Failed attempt for the synthesis of bromide **67**.

So, in a last attempt for the WILLIAMSON ether synthesis, it was decided to perform the ether synthesis with 4-(bromomethyl)benzoic acid (**66**) first and construct the side chain afterwards (Scheme 30). Unluckily, the conditions used for the ether synthesis of **CG_94** did in this case not result in the desired product **68**.



Scheme 30: Failed attempt for the synthesis of **68**.

An alternative approach should then be a Pd-catalysed C-O cross coupling. This method was developed by BUCHWALD and co-workers in 2018^[111] under the title “*Palladium-Catalyzed C–O Cross-Coupling of Primary Alcohols*” (synthesis of required precatalysts published before by BRUNO and BUCHWALD^[112]). The authors presented a precatalyst system, which supposedly provides mild and general conditions for the purpose of Pd-catalysed C-O cross coupling. The necessary precatalyst **70** (Figure 14) was already synthesised in the group of PROF. DR. FRANZ BRACHER by DR. DESIRÉE HEERDEGEN in the context of the “*Synthesis of steroid-like analogues of cholesterol biosynthesis inhibitors*”^[113]. As a ligand was used *t*-BuBrettPhos. With precatalyst **70** in hand, only the precursors **CG_100** and **CG_101** for the coupling had to be synthesised.

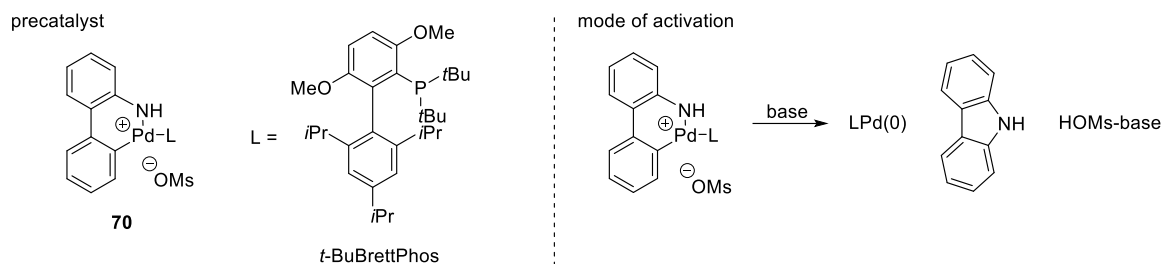
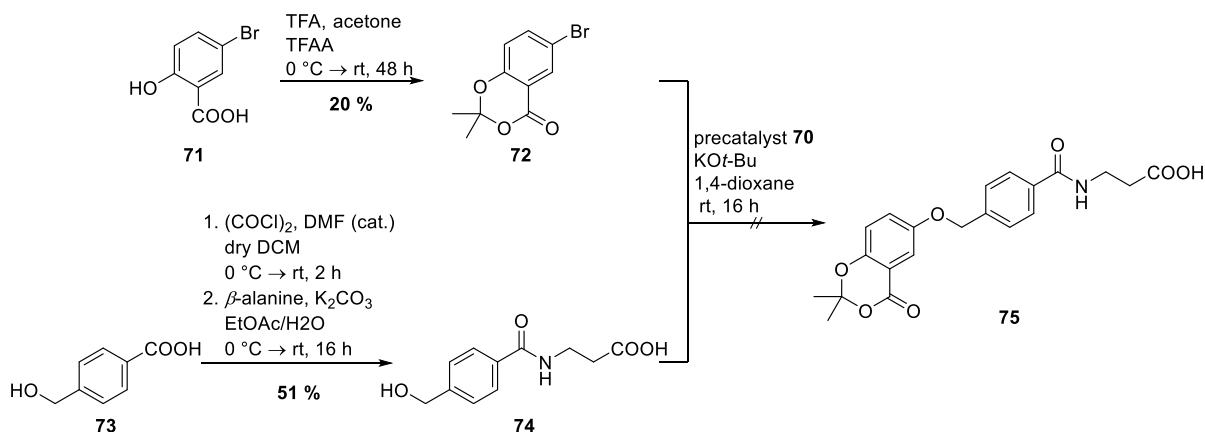


Figure 14: Illustration of precatalyst **70** with ligand t -BuBrettPhos and its mode of activation.

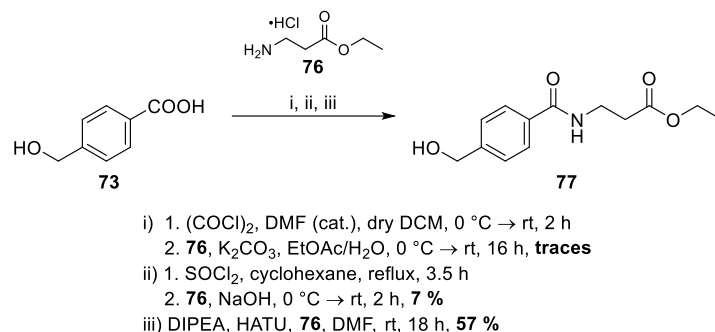
As illustrated in Scheme 31, aryl bromide **72** was prepared from 5-bromosalicylic acid (**71**) according to a published procedure by MANTEL *et al.*^[114] using TFA, acetone and TFAA. In this case, **72** was only obtained in a low yield of 20 % (lit. 44 %^[114]). Primary alcohol **74** was prepared under standard amidation conditions starting from 4-(hydroxymethyl)benzoic acid (**73**) using oxalyl dichloride and β -alanine, resulting in **74** in 51 % yield. With **72**, **74** and precatalyst **70** in hand, Pd-catalysed C-O cross coupling should then lead to ether **75**. Unfortunately, the desired product (**75**) was not formed even after prolonging the reaction time to several days or increasing the reaction temperature to 60 °C. Only unchanged starting materials remained.



Scheme 31: Attempted Pd-catalysed C-O cross coupling for the synthesis of ether **75**.

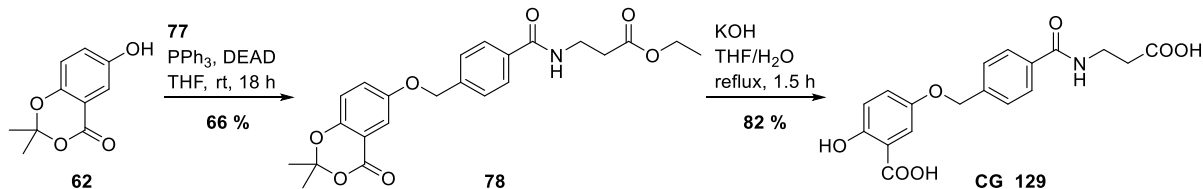
Since several approaches *via* a WILLAMSON ether synthesis and *via* Pd-catalysed C-O cross coupling were unsuccessful, it was decided to use a MITSUNOBU reaction instead. For this, first, alcohol **77** with the carboxylic acid protected as an ethyl ester had to be prepared by amide synthesis between 4-(hydroxymethyl)benzoic acid (**73**) and β -alanine ethyl ester hydrochloride (**76**) (Scheme 32). In an initial approach, the acid chloride derived from 4-(hydroxymethyl)benzoic acid (**73**) should be formed using standard conditions, and subsequently coupled with the amine (**76**). But both oxalyl dichloride (Scheme 32, i) and thionyl dichloride (Scheme 32, ii) did not lead to an isolable amount of the product (**77**) (traces for (COCl)₂ and 7 % yield for SOCl₂, respectively). Possibly the major reason for this is that both

reagents are known to convert alcohols to alkyl chlorides resulting in side reactions. Therefore, in another approach the use of the coupling reagent HATU in combination with the base DIPEA was used in the same way as for the synthesis of compound **46** (Scheme 32, iii and cf. Scheme 19). This approach gave hydroxymethyl benzamide **77** in 57 % yield.



Scheme 32: Attempts and final approach for the preparation of alcohol **77**.

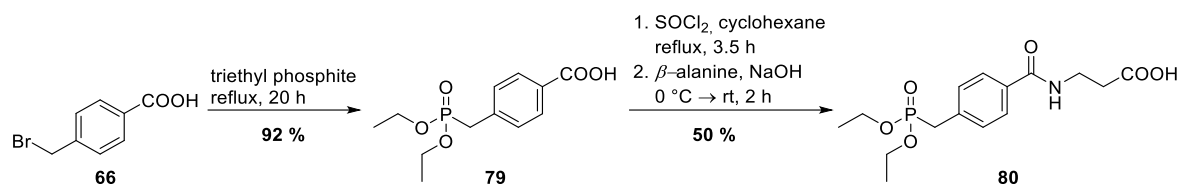
With previously prepared phenol **62** the MITSUNOBU reaction could now take place. Using standard MITSUNOBU conditions with triphenylphosphine and DEAD, **78** was obtained after 18 h at room temperature in 66 % yield. Simultaneous hydrolysis of the ethyl ester and the acetonide with potassium hydroxide in THF/water finally gave the target compound **CG_129** in a good yield of 82 % (Scheme 33).



Scheme 33: Preparation of ether **CG_129**.

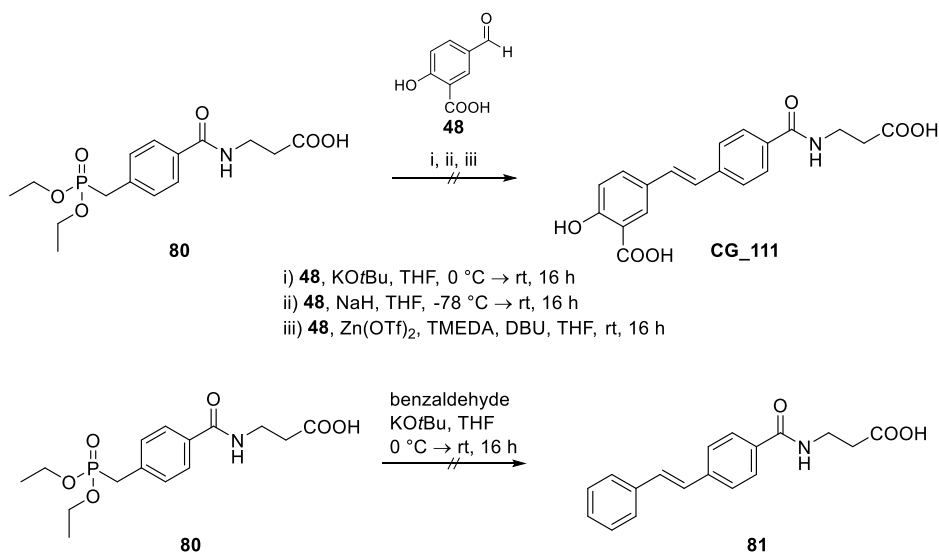
The next variation should then comprise a molecule lacking both hydrogen bond accepting and donating abilities in the spacer, resulting in stilbene **CG_111** (Scheme 35). From the docking experiments (Chapter 2, Figure 8) and subsequent first Sirt5 inhibitory test results (Figure 12) it was concluded that also the stilbene analogue had to be in *E*-configuration. Therefore, a HORNER-WADSWORTH-EMMONS reaction (HWE reaction) that produces stereoselectively (*E*)-alkenes should generate the desired *E*-configured stilbene **CG_111**.

For the preparation of the required phosphonate **79** depicted in Scheme 34 an ARBUZOV reaction was performed by heating 4-(bromomethyl)benzoic acid (**66**) in triethyl phosphite to reflux for 20 h according to a procedure published by KIM *et al.*^[115] Phosphonate **79** was obtained in a very good yield of 92 %.^[115] The side chain was then built by an amidation reaction using thionyl dichloride and β -alanine (preparation of intermediate benzoyl chloride derived from BAZIARD-MOUYSSET *et al.*^[116]) giving phosphonate intermediate **80** in 50 % yield.



Scheme 34: Preparation of phosphonate intermediate **80**.

With phosphonate **80** several attempts for an HWE reaction were made to generate stilbene **CG_111** (Scheme 35). Standard conditions derived from literature using $\text{KO}t\text{Bu}$ ^[117] (Scheme 35, i) or NaH ^[118] (Scheme 35, ii) as base both led to unchanged starting materials. The same observation was made when applying conditions of a mild zinc triflate-promoted HWE reaction^[119] (Scheme 35, iii). Unfortunately, all approaches (i – iii) did not result in the desired stilbene **CG_111**. Because of these results, it was tried adding benzaldehyde instead of 5-formylsalicylic acid (**48**) for the formation of stilbene **81** to see, whether the substitution pattern caused these problems (Scheme 35, bottom). The starting materials remained unchanged, even though in this as well as the previous attempts a change in colour indicated the formation of the phosphonate carbanion.



Scheme 35: Attempts for the synthesis of stilbene **CG_111** via a HORNER-WADSWORTH-EMMONS reaction and attempt for the synthesis of stilbene **81**.

In another literature search, a publication from MUKKAMALA *et al.*^[109] from 2017 was found, which was once before consulted for the synthesis of compound **59** (Scheme 25). Here the authors worked on a “Valuable building block for the synthesis of lunularic acid, hydrangeic acid and their analogues”.^[109] The natural products lunularic acid (total synthesis hereof published by the group of PROF. DR. FRANZ BRACHER in 2000^[120]) and hydrangeic acid, depicted in Figure 15, as well as some of their synthesised analogues thereof are quite similar to the core structure of the analogues desired in this thesis.

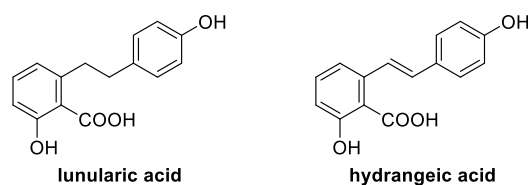
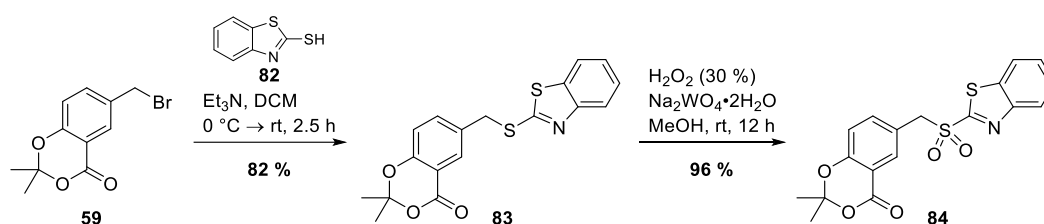


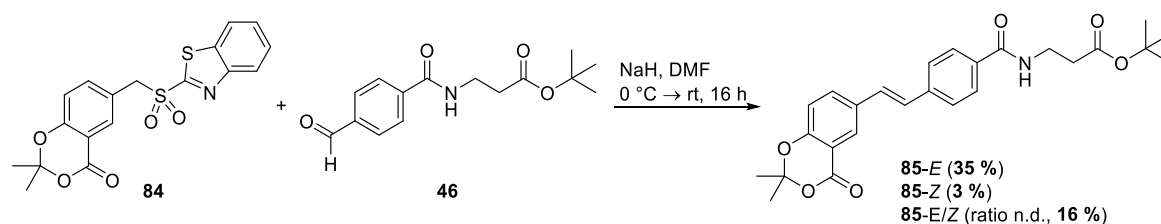
Figure 15: Molecular structures of lunularic acid and hydrangeic acid.

MUKKAMALA and co-workers designed a sulfone-based building block to perform C-C bond formations utilising a modified JULIA olefination (also known as one-pot JULIA olefination). As only the exact position of the substituents varied slightly, the concept of their building block and their synthetic protocol should be used (Scheme 36). Therefore, thio-alkylation of 2-mercaptobenzothiazole (**82**) with previously prepared **59** (Scheme 25) in DCM in the presence of triethylamine gave thioether **83** in good yield (82 %). Subsequently, **83** was oxidised using H_2O_2 (30 %) and $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ to generate sulfone **84** in a very good yield of 96 %.^[109]



Scheme 36: Synthesis of sulfone-based building block **84**.

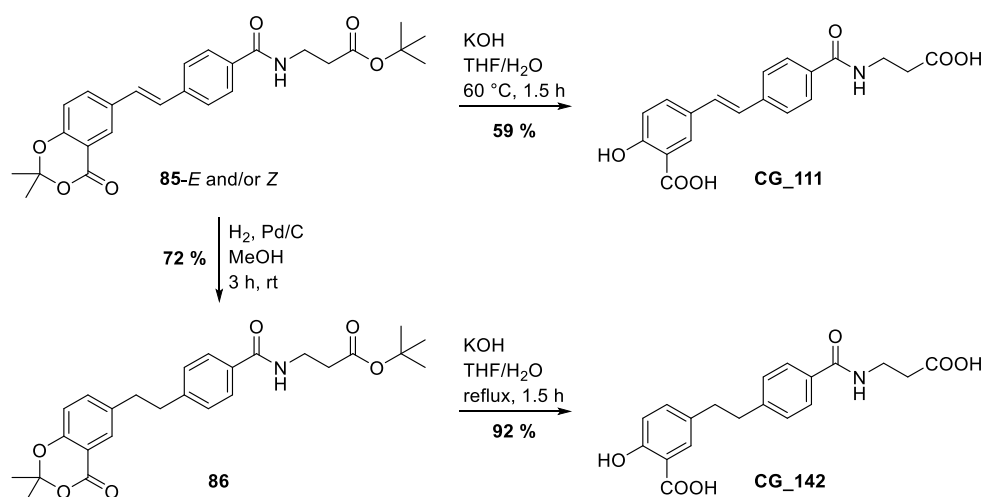
Still following the synthetic procedure by MUKKAMALA *et al.*,^[109] sulfone **84** was now reacted with aldehyde **46** using sodium hydride as the base and DMF as the solvent (Scheme 37). This one-pot JULIA olefination is known to generate a mixture of alkene stereoisomers, which was also the case here. After the removal of remaining aldehyde by shaking the crude product dissolved in methanol with saturated aqueous NaHSO_3 to produce the bisulfite adduct, the obtained product consisted of both isomers *E* and *Z*. Luckily, the isomers could be partially separated using FCC (*E*-Isomer: $R_f = 0.34$ (EtOAc/hexanes 40:60) and *Z*-Isomer: $R_f = 0.27$ (EtOAc/hexanes 40:60)). This way, the desired pure *E*-isomer **85-E** was obtained in a moderate yield of 35 %. In addition, **85-Z** could be isolated in 3 % yield and a mixture of *E*- and *Z*-isomer (**85-E/Z**) was obtained in 16 % yield (ratio not determined).



Scheme 37: Synthesis of *E*- and *Z*-isomers of stilbene **85**.

The modified JULIA olefination was also once tried using aldehyde **47**, which contains the free carboxylic acid instead of a *tert*-butyl ester as in **46**, but TLC showed, if at all, only moderate conversion to the desired stilbene. Therefore, and because stilbene **85** with no free carboxylic acids would be easier for FCC purification, this approach was not further pursued.

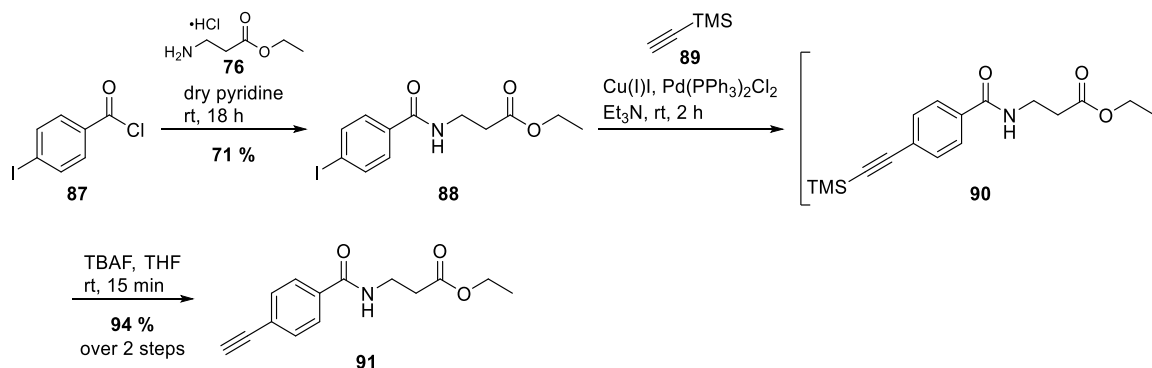
With **85-E** in hand, acetonide and *tert*-butyl ester hydrolysis using KOH in THF/water gave the desired stilbene **CG_111** in *E*-configuration in a moderate yield of 59 %. The mixture of **85-E** and **85-Z** was used for reduction of the double bond using standard hydrogenation conditions.^[109] Ethylene compound **86** was obtained in a good yield of 72 % and was then also deprotected under alkaline conditions to give the final product **CG_142** in a very good yield of 92 % (Scheme 38).



Scheme 38: Synthesis of stilbene **CG_111** and ethylene analogue **CG_142**.

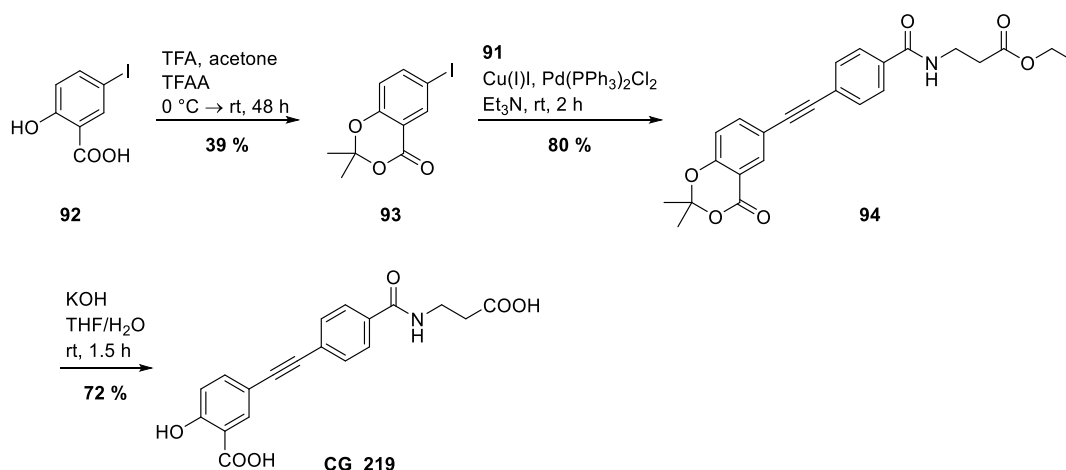
In accordance with stilbene **CG_111** and alkane **CG_142**, analogue **CG_219** (Scheme 40) with an alkyne spacer was synthesised. This modification reduces the compound's flexibility and changes its intramolecular geometric angle. For the synthesis of analogue **CG_219** several steps were required (Scheme 39). First, the β -alanine derived side chain as well as the necessary alkyne moiety had to be synthesised. Therefore, following the procedure for the synthesis of this compound in a patent from INDIANA UNIVERSITY FOUNDATION from 1997,^[121] **88** was prepared from 4-iodobenzoyl chloride (**87**) and β -alanine ethyl ester hydrochloride (**76**) in dry pyridine. The product (**88**) was obtained in 71 % yield.^[121] The alkyne was introduced *via* a SONOGASHIRA cross-coupling of iodide **88** and trimethylsilylacetylene (**89**). Hence, SONOGASHIRA reaction conditions using copper(I) iodide and Pd(PPh₃)₂Cl₂ in triethylamine generated TMS-protected intermediate **90**. Without purification this crude product was deprotected under standard conditions with TBAF in THF. Alkyne **91** was obtained after FCC in 94 % yield over two steps.

It is important to note that three different solvent systems were elaborated for the SONOGASHIRA cross-coupling, whereof 1,4-dioxane/triethylamine in a 1:1 mixture yielded **91** after deprotection in only 28 % yield over two steps. Adding only 2.5 equivalents of triethylamine instead of using it as a solvent gave **91** also in only 32 % yield over two steps after deprotection. Not until the solvent was changed into pure triethylamine,^[122] as it is depicted in Scheme 39, alkyne **91** was obtained after deprotection in a very good yield of 94 % over two steps.



Scheme 39: Preparation of alkyne **91**.

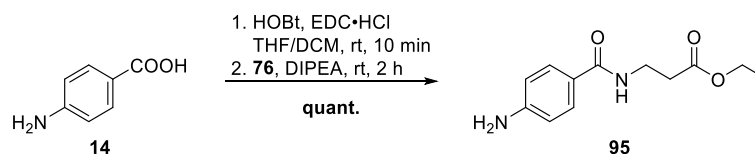
Further, acetal protected salicylic acid derivative **93** bearing an iodo substituent was synthesised following the synthetic procedure from HUYNH *et al.*^[123] giving **93** in a moderate yield of 39 % (Scheme 40).^[123] The following SONOGASHIRA cross-coupling was performed in the same way as intermediate **90** with only triethylamine as solvent. Alkyne **94** was obtained after FCC in 80 % yield. A final deprotection step under alkaline conditions at room temperature generated the desired alkyne **CG_219** in a good yield of 72 %.



Scheme 40: Preparation of alkyne **CG_219**.

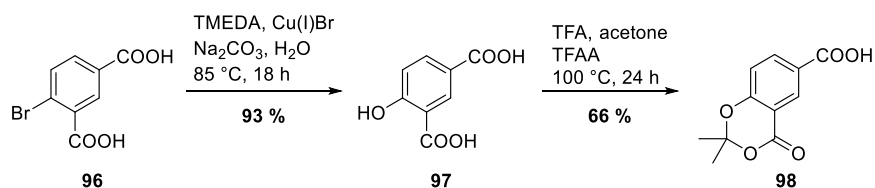
The spacer variations so far put the focus on reducing the number of heteroatoms and thereby reducing the number of hydrogen bond acceptors and donators. The following analogues were on the contrary designed to increase those factors.

The first two analogues of this set should contain an amide group. As for previous analogues, the salicylic acid motif and the β -alanine containing moiety should be prepared separately and then combined while constructing the appropriate spacer. For amide **CG_128** (Scheme 43), the required precursor amine **95** was prepared according to a patent from NOVO NORDISK A/S from 2004^[124] in an amide coupling between 4-aminobenzoic acid (**14**) and β -alanine ethyl ester hydrochloride (**76**) using HOBt and EDC•HCl as coupling reagents. The product (**95**) was obtained in quantitative yield (Scheme 41).^[124]



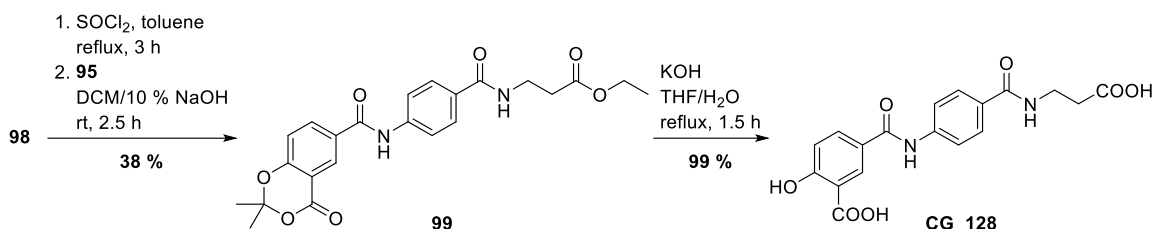
Scheme 41: Preparation of amine **95**.

The second precursor (**98**), consisting of a carbox-salicylic acid, was prepared in two steps (Scheme 42). In the first step, 4-bromoisophthalic acid (**96**) was converted into the corresponding salicylic acid derivative **97** in a copper catalysed reaction with copper(I) bromide and TMEDA as the ligand following the preparation of this compound in a patent from LEGO CHEM BIOSCIENCES, INC. from 2012.^[125] 4-Hydroxyisophthalic acid (**97**) was generated in a very good yield of 93 %. Protection of the salicylic acid functional groups was then performed following a patent from APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. from 2005^[103] again, yielding acetone **98** in 66 %.^[103]



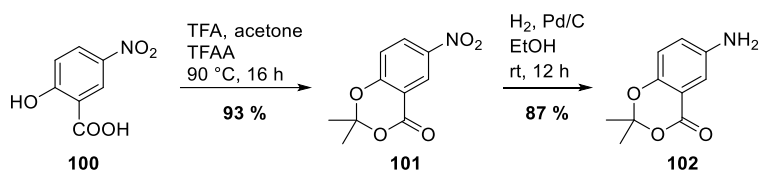
Scheme 42: Preparation of carboxylic acids **97** and **98**.

As illustrated in Scheme 43, the two precursors **95** and **98** then underwent amide coupling by combining procedures for preparing the aroyl chloride^[116] and performing the amide coupling^[126] with it. Product **99** was obtained in a moderate yield of 38 %. The yield could possibly be increased if alternative coupling reagents were used instead, but for the purpose of this thesis the obtained yield was sufficient. Deprotection with KOH in THF/water under reflux generated the desired amide **CG_128** in almost quantitative yield (99 %).



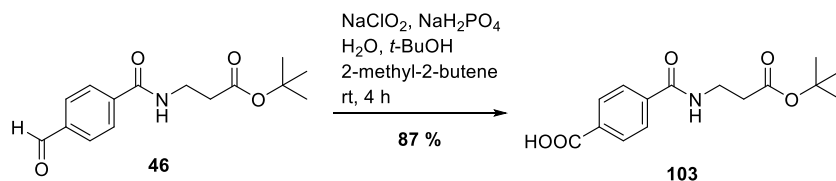
Scheme 43: Preparation of amide **CG_128**.

For the laterally reversed amide **CG_133** (Scheme 46), the same concept of preparing the two necessary precursors (Scheme 44 and Scheme 45) with subsequent amide coupling was applied. Here, the nitrogen of the amide was attached to the salicylic acid moiety and was introduced by protection of 5-nitrosalicylic acid (**100**) as an acetonide using the now established protocol (93 % yield) with subsequent hydrogenative reduction of **101** to the corresponding amine **102**^[127] (87 % yield).



Scheme 44: Preparation of amine **102**.

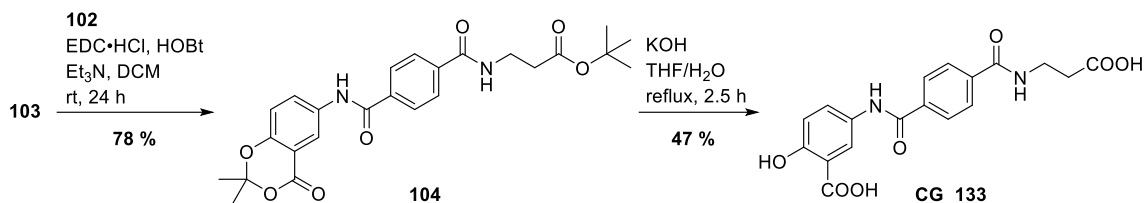
Benzoic acid **103** was then synthesised using a procedure derived from publications by HILLIS and RONALD from 1985^[128] and BAL *et al.*^[129] from 1981, respectively. With the method employed therein the oxidation using sodium chlorite is possible even for molecules where steric hindrance and/or sensitive functionalities are present. Although this is not the case here, this simple and inexpensive method was applied and oxidation of previously prepared aldehyde **46** (Scheme 19) by a $\text{NaClO}_2/\text{NaH}_2\text{PO}_4/2\text{-methyl-2-butene}$ solution gave benzoic acid **103** in a very good yield of 87 % (Scheme 45).



Scheme 45: Preparation of benzoic acid **103**.

The following amide coupling was then performed using coupling reagents according to the procedure for the synthesis of phenol **55** (Scheme 46 and cf. Scheme 24) in contrast to the conditions used for the synthesis of amide **99** (Scheme 43). As pointed out before, this change could lead to higher yields (**99** was obtained in only 38 % yield). Coupling of benzoic acid **103** and amine **102** afforded amide **104** in a good yield of 78 %. It should be noted that the doubled yield could be due to change in reaction conditions but also because of the slightly changed

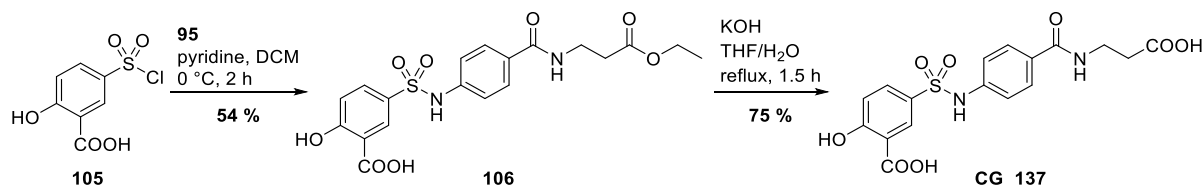
starting materials. Finally, alkaline deprotection gave the desired laterally reversed amide **CG_133** in 47 % yield.



Scheme 46: Preparation of laterally reversed amide **CG_133**.

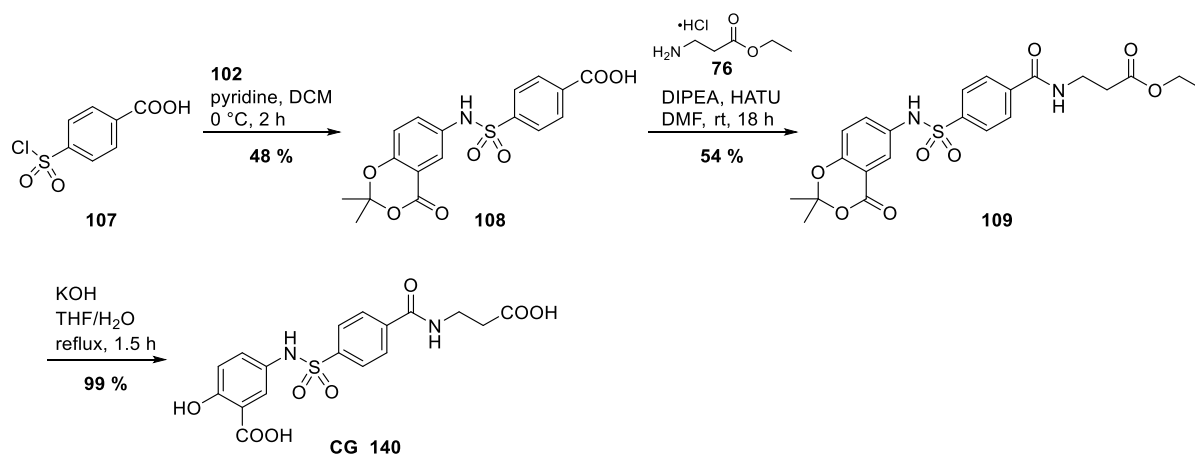
As mentioned before, these spacers should increase the number of heteroatoms. Hence, the following analogues consisted of sulfonamides, resulting in an additional heteroatom with the ability to form hydrogen bonds compared to amides **CG_128** and **CG_133**.

Beneficially, for the synthesis of the sulfonamide analogues the required starting materials were either commercially available or already prepared in the context of other analogues. The first analogue was synthesised following a modified general procedure published by ZHENG *et al.*^[130] The sulfonamide was built using commercially available 5-chlorosulfonyl-2-hydroxybenzoic acid (**105**) and previously prepared amine **95** (Scheme 41) in DCM and pyridine. After FCC, which proved more difficult than expected due to streaking of the product, **106** was obtained in 54 % yield. Subsequent alkaline deprotection afforded the desired sulfonamide **CG_137** in 75 % yield (Scheme 47).



Scheme 47: Preparation of sulfonamide analogue **CG_137**.

Here again, the laterally reversed analogue (**CG_140**) was prepared. Starting from commercially available 4-(chlorosulfonyl)benzoic acid (**107**) and previously prepared amine **102** the same conditions as for **106** were used. After recrystallisation from hot MeOH, sulfonamide **108** was obtained in 48 % yield. The β -alanine derived side chain was then constructed applying reaction conditions used before for compound **46** (Scheme 48 and cf. Scheme 19) leading to **109** in 54 % yield. The final deprotection was performed with KOH in THF/water, generating **CG_140** in almost quantitative yield (99 %).

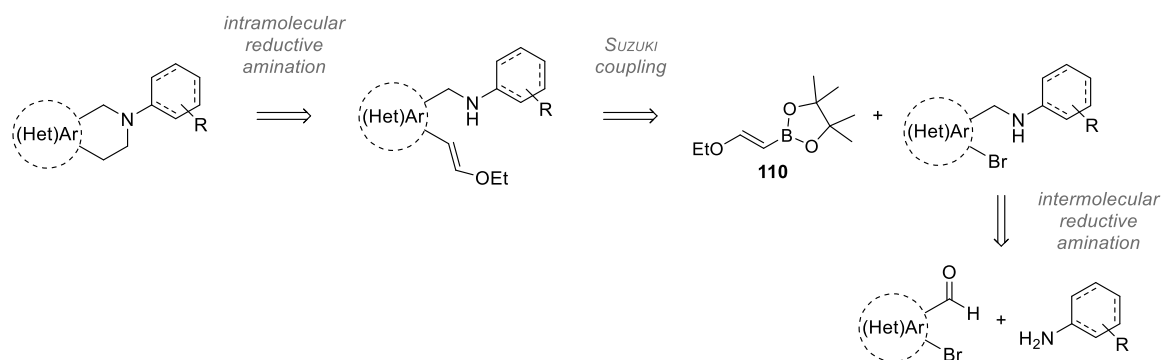


Scheme 48: Preparation of laterally reversed sulfonamide **CG₁₄₀**.

3.2.1.2. Excursus: New general method for the preparation of *N*-aryl-1,2,3,4-tetrahydroisoquinolines

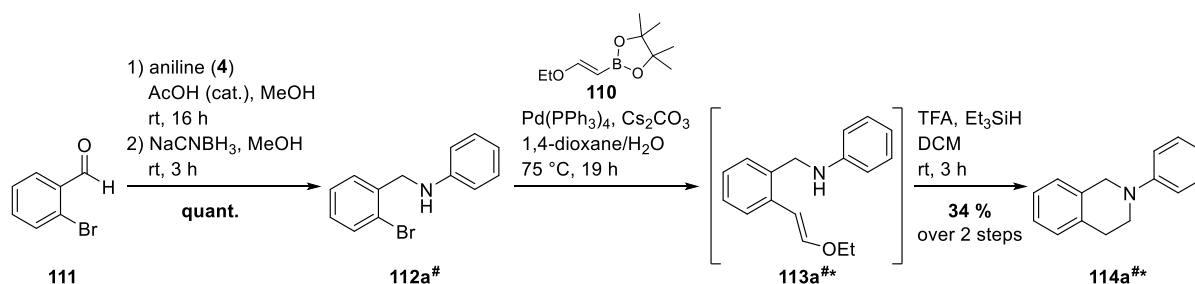
Before the synthesis of analogues with a rigidised central unit will be discussed, a short excursus will focus on the development of a new general method for the preparation of *N*-aryl-1,2,3,4-tetrahydroisoquinolines. This approach was substantial for one analogue within this thesis (see next Chapter 3.2.1.3) and was also the basis of a bachelor's thesis by RICKY WIRAWAN under my supervision (compounds prepared in this context are marked with #). In this chapter a short summary of this project will be given. For further details see the bachelor's thesis of RICKY WIRAWAN^[131] and the subsequent publication in *SYNTHESIS*.^[132] If compounds were prepared in the bachelor's thesis and additionally in this work, they will be marked with #*.

In order to get a short approach to *N*-aryl-1,2,3,4-tetrahydroisoquinolines with broad variability in both aromatic rings, a novel method starting from *ortho*-brominated aromatic aldehydes and primary aromatic amines was developed. Scheme 49 depicts the retrosynthesis. The target *N*-aryl-1,2,3,4-tetrahydroisoquinolines should be obtained *via* intramolecular reductive amination from *ortho*-ethoxyvinyl *N*-arylbenzylamines using the combination of triethylsilane (Et₃SiH)/trifluoroacetic acid (TFA) in DCM, an established solvent for silane/TFA reductions. The *ortho*-ethoxyvinyl *N*-arylbenzylamines in turn should be synthesised from commercially available 2-ethoxyvinyl pinacolboronate (**110**) and *N*-aryl 2-bromobenzylamines using SUZUKI cross-coupling. *N*-Aryl 2-bromobenzylamines should be prepared from *ortho*-brominated aromatic aldehydes and primary aromatic amines in an intermolecular reductive amination. Both aromatic building blocks are readily available with a variety of additional substituents.



Scheme 49: Retrosynthesis of the desired *N*-aryl-1,2,3,4-tetrahydroisoquinolines.

A pilot experiment was performed with 2-bromobenzaldehyde (**111**) and aniline (**4**) (Scheme 50; reactions depicted with optimised conditions). The first step involved a condensation under standard reductive conditions using NaCNBH_3 as reducing agent. *N*-Aryl 2-bromobenzylamine **112a[#]** was obtained in quantitative yield.^[132]



Scheme 50: Pilot experiment for the preparation of *N*-aryl-1,2,3,4-tetrahydroisoquinolines (cf. lit. ^[132]).

Then the SUZUKI reaction was performed according to protocols established in the group of PROF. DR. FRANZ BRACHER^[133-134] using 2-ethoxyvinyl pinacolboronate (**110**), $\text{Pd}(\text{PPh}_3)_4$ and cesium carbonate (Scheme 50). Formation of the intermediate *ortho*-ethoxyvinyl benzylamine **113a^{##}** could be monitored by TLC ($R_f = 0.48$ (hexanes/EtOAc 95:5)). Enol ether **113a^{##}** was isolated after work-up but not further purified. For the following *de novo* construction of the *N*-aryl-1,2,3,4-tetrahydroisoquinoline ring system, procedures derived from similar *N*-arylethylations of aromatic amines using enol ethers were explored. This type of reaction has been performed in PROF. DR. FRANZ BRACHER's group for the synthesis of arylethylated anilines and *N*-heterocycles.^[135-138] The examined conditions to optimise yields and reduce waste, purification efforts, and workload are shown in Table 3.^[132]

Table 3: Different conditions explored for the optimisation of the reductive cyclisation for the synthesis of *N*-aryl-1,2,3,4-tetrahydroisoquinolines (cf. lit. ^[132]).

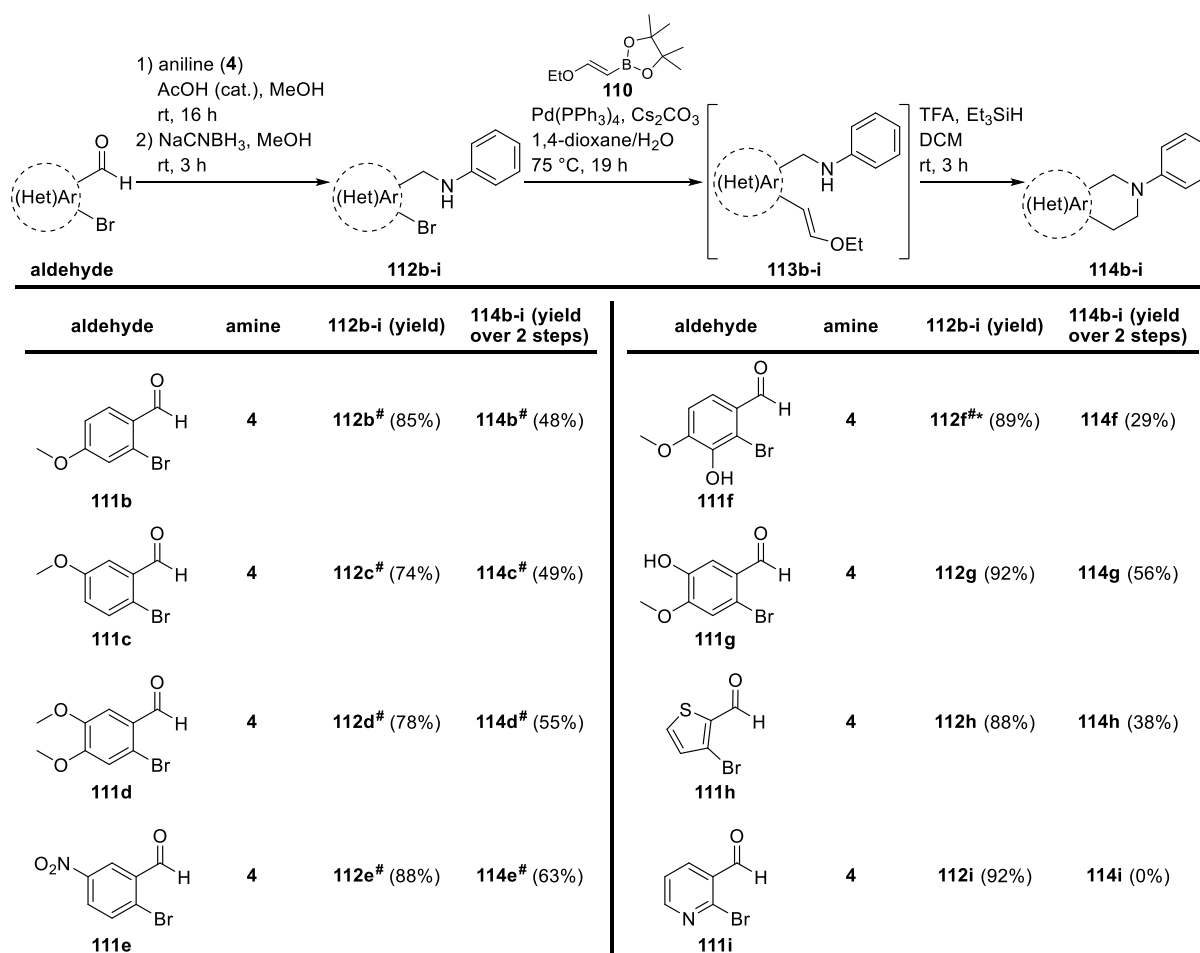
Entry	Comment	Et ₃ SiH (eq)	TFA (eq)	Solvent	Temp.	Time	Yield of 114a ^{**}
1	lit. ^[135]	10	10	dry DCM	0 °C	3.5 h	30 % over 2 steps
2	lit. ^[136]	2.5	13	dry DCM	rt	2.5 h	34 % over 2 steps
3	pure 113a ^{**}	2.5	13	dry DCM	rt	2.5 h	41 % over 1 step (21 % over 2 steps)
4	one-pot	10	13	-	0 °C → rt	72 h	6 % over 2 steps
5	lit. ^[133]	0	13	dry DCM	0 °C	3.5 h	6 % over 2 steps

As described in the publication in *SYNTHESIS*,^[132] the first experiment comprised of the synthesis of *N*-aryl-1,2,3,4-tetrahydroisoquinoline **114a**^{**} following a protocol by VÖGERL *et al.*^[135] using 10 equivalents of triethylsilane at 0 °C (Table 3, entry 1). The desired product **114a**^{**} was formed within 3.5 h in 30 % yield over two steps. Unfortunately, this approach required laborious purification by repeated FCC, due to the necessity to remove excess of triethylsilane. To minimise these purification efforts, it was decided to reduce the amount of triethylsilane. Therefore, conditions described by POPP *et al.*^[136] (Table 3, entry 2) with only 2.5 equivalents of triethylsilane were explored. In addition to the lower amount of triethylsilane, the reaction is performed at room temperature. After 2.5 h product **114a**^{**} was obtained in a slightly increased yield of 34 % after only one FCC. The next approach should aim at investigating a possible negative impact of residual reagents or impurities from the Suzuki reaction (Table 3, entry 3). Hence, enol ether **113a**^{**} was first purified by FCC (50 % yield), followed by the cyclisation reaction according to the conditions in entry 2. This time, product **114a**^{**} was obtained in 41 % yield (only 21 % over two steps). This observation leads to the conclusion that purification of **113a**^{**} is not necessary and rather causes a decrease in overall yield of *N*-aryl-1,2,3,4-tetrahydroisoquinoline **114a**^{**}. Next, instead of an intermediate work-up and subsequent change of the solvent system, the required reagents for the cyclisation (triethylsilane/TFA) were added in a one-pot procedure directly into the reaction mixture of the SUZUKI cross-coupling (Table 3, entry 4; following conditions in entry 1). The reaction was stopped when even after 72 h formation of the cyclised product (**114a**^{**}) was not clearly visible on TLC. Nevertheless, after FCC **114a**^{**} could be isolated in a very poor yield of 6 %.^[132]

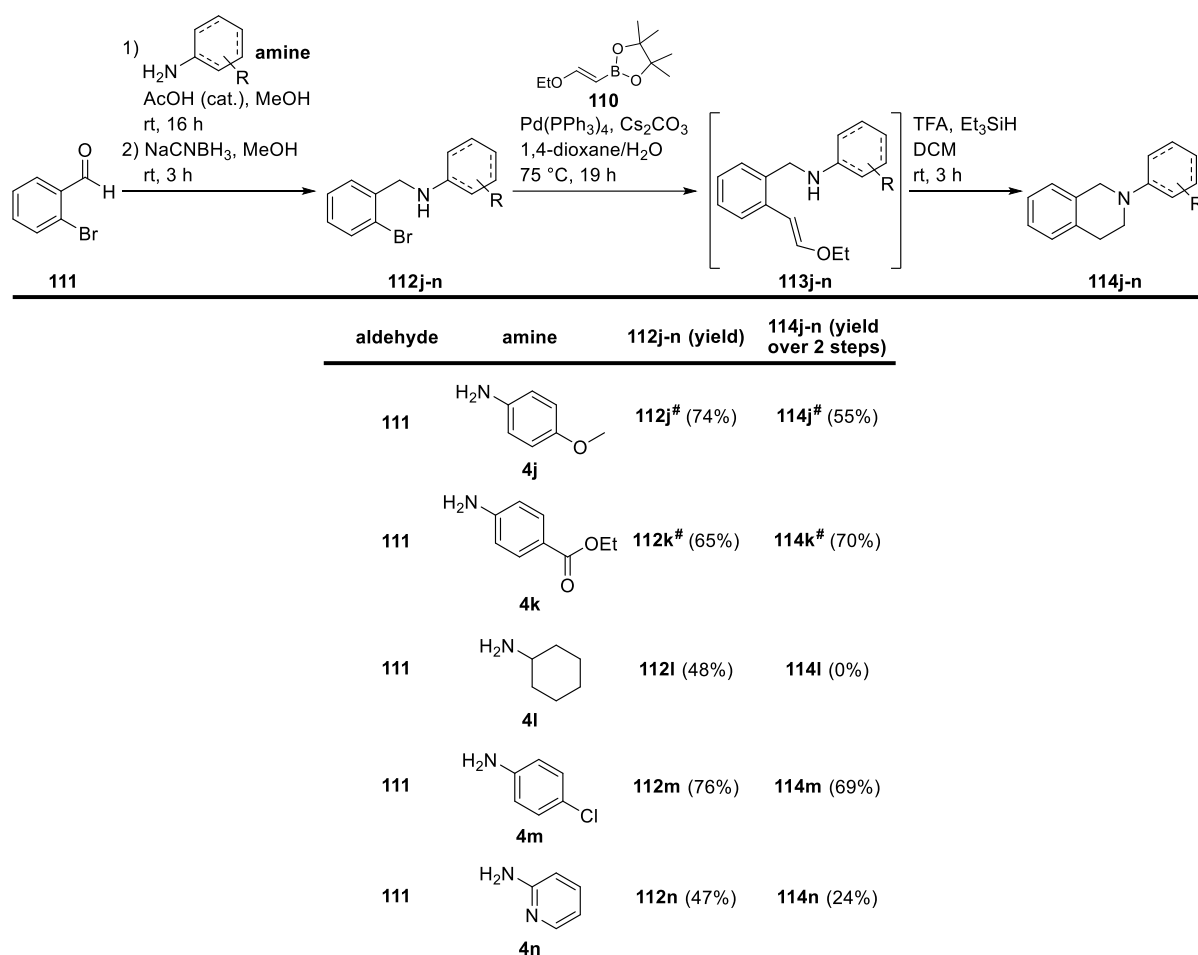
The mechanism of the cyclisation reaction using triethylsilane/TFA proceeds as follows. First, β -protonation of the ethoxyvinyl moiety in **113a**^{**} results in a carbenium-oxonium ion. Next, an intramolecular cyclisation under formation of a C-N bond with the amino group occurs. And finally, O-protonation, subsequent elimination of ethanol, and hydride transfer from

triethylsilane leads to the desired *N*-aryl-1,2,3,4-tetrahydroisoquinolines. Hence, another experiment should investigate whether the 3,4-dehydro analogue of **114a[#]**, an *N*-aryl-1,2-dihydroisoquinoline, would be obtained when only TFA, but no triethylsilane was used (Table 3, entry 5; following conditions published for the synthesis of 1-oxo-1,2-dihydroisoquinolines by SCHÜTZ *et al.*^[133]). Therefore, crude enol ether **113a[#]** was treated with solely TFA at 0 °C. After 3.5 h the starting material was fully consumed and the only product that could be isolated from the product mixture in pure form was, surprisingly, *N*-aryl-1,2,3,4-tetrahydroisoquinoline (**114a[#]**) (Scheme 50) with a poor yield of 6 % over both steps.^[132]

With the optimised cyclisation conditions in hand (Table 3, entry 2), a variety of *N*-aryl-1,2,3,4-tetrahydroisoquinolines and related heterocycles was synthesised starting from variably substituted *ortho*-brominated aromatic aldehydes and primary aromatic amines (Scheme 51 and Scheme 52).



Scheme 51: Application of the novel method for the synthesis of *N*-aryl-1,2,3,4-tetrahydroisoquinolines starting from variably substituted aromatic aldehydes (cf. lit.^[132]).



Scheme 52: Application of the novel method for the synthesis of *N*-aryl-1,2,3,4-tetrahydroisoquinolines starting from variably substituted aromatic amines (except cyclohexylamine (**4l**)) (cf. lit. ^[132]).

For details on these investigations see the publication in *SYNTHESIS*^[132] and the bachelor's thesis of RICKY WIRAWAN.^[131] In summary, Scheme 51 and Scheme 52 show that a variety of *N*-aryl-1,2,3,4-tetrahydroisoquinolines with yields ranging from 24 – 70 % over two steps starting from the corresponding *N*-aryl 2-bromobenzylamines could be synthesised. Almost all used *ortho*-brominated aromatic aldehydes and primary aromatic amines, and even heteroaromatic compounds could successfully be converted into their corresponding *N*-aryl-1,2,3,4-tetrahydroisoquinolines and related compounds. An increase in the yield of the final products was seen when anilines with electron-withdrawing substituents were used. Unsuccessful reactions or low yields for the cyclisation after Suzuki cross-coupling were observed in case of 6-phenyl-5,6,7,8-tetrahydro[1,6]naphthyridine **114i** (0 %) and *N*-pyridyl-1,2,3,4-tetrahydroisoquinoline **114n** (24 %) where it might in part be due to protonation of the pyridine-containing intermediates by TFA in the cyclisation step. Also, cyclisation of aliphatic amine *N*-cyclohexyl-2-bromobenzylamine **112l** did, as expected, not result in the desired product **114l**, most likely because the amine is protonated by TFA and hence prevented from nucleophilic attack at the protonated enol ether.^[132]

It should be noted that some of the desired *N*-aryl-1,2,3,4-tetrahydroisoquinolines experienced oxidative degradation and should be stored under inert conditions after preparation.

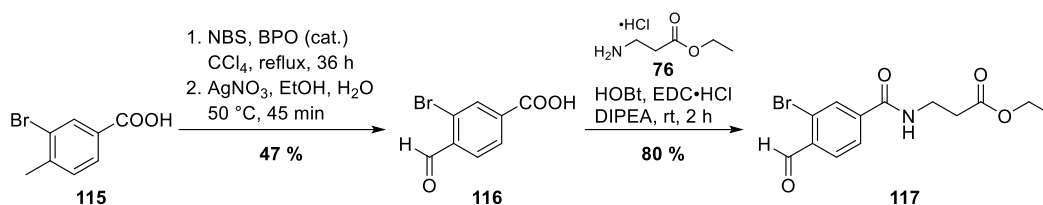
Even though the SUZUKI reaction and final cyclisation gave only moderate to good yields over two steps, this protocol is nevertheless more effective than previous approaches to the target chemotype, due to good availability of the building blocks and the very small number of steps. In conclusion, a convenient and short approach for the synthesis of *N*-aryl-1,2,3,4-tetrahydroisoquinolines and related heterocyclic ring systems with broad variability of substituents in both aromatic rings and high functional group tolerance was developed.^[132]

This method could now be applied on a desired analogue within the set of rigidised analogues of balsalazide.

3.2.1.3. Analogues with a rigidised central unit

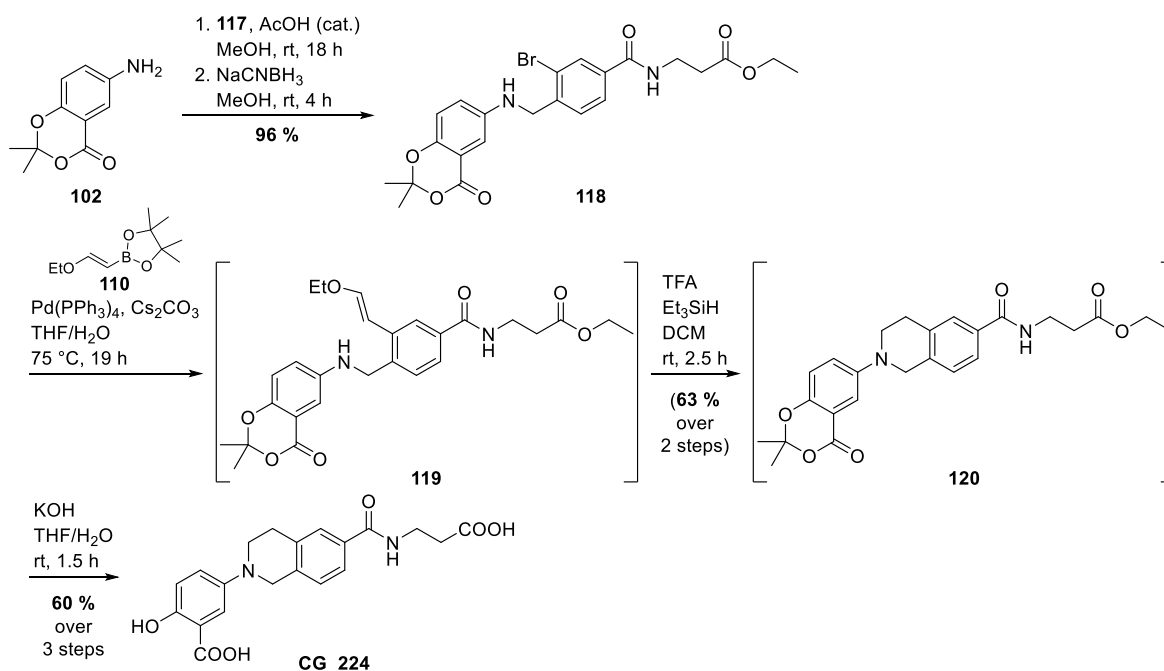
Structures with a rigidised central unit should not only replace the azo motif but at the same time rigidise the structure. This design is based on the concept of the free energy difference that describes the binding of an inhibitor to an enzyme. This thermodynamic quantity is consisting of an entropic and an enthalpic component. The enthalpic one is for any reasonable inhibitor negative due to attractive interactions between enzyme residues and the inhibitor. A bound inhibitor is conformationally confined by the enzyme, and therefore the binding results in an entropy loss for the inhibitor. If the inhibitor is rigidised, the entropy loss which occurs during the binding process is less severe and the binding free energy more favourable. Since starting materials with the optimum substitution patterns were limited, to begin with, only two analogues were designed and synthesised to explore this theory of rigidisation.

For the first analogue (**CG_224**) bearing the *N*-aryl-1,2,3,4-tetrahydroisoquinoline motif, the newly developed synthetic procedure described in Chapter 3.2.1.2 was applied. For this approach, an appropriately substituted 2-bromobenzaldehyde was needed. Therefore, 3-bromo-4-methylbenzoic acid (**115**) was converted into the corresponding 2-bromobenzaldehyde **116** following a patent from NOVARTIS AG from 2004 (Scheme 53).^[139] After benzylic dibromination with NBS, catalytic amounts of BPO in CCl₄ under reflux conditions, the desired aldehyde **116** was constructed with silver nitrate in a mixture of ethanol and water within 45 minutes at 50 °C.^[139] After a general work-up, the crude product was obtained in good purity in 47 % yield.^[139] The product is not stable under FCC conditions, wherefore minor impurities should be left in the product if they do not negatively influence following reactions. Subsequent amide coupling with β -alanine ethyl ester hydrochloride (**76**) under standard coupling conditions gave 2-bromobenzaldehyde **117** in 80 % yield after FCC purification.



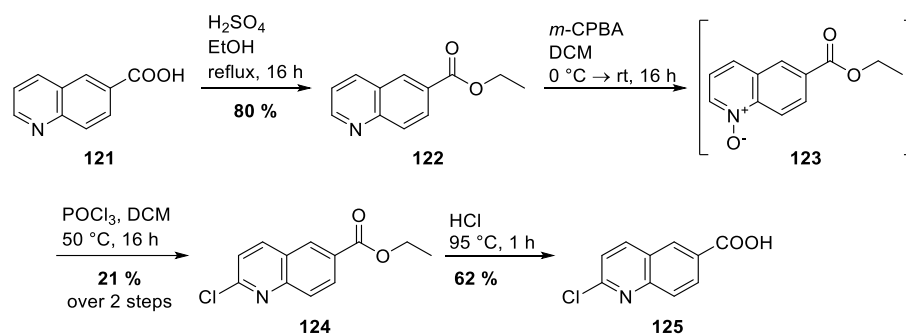
Scheme 53: Preparation of *ortho*-bromobenzaldehyde intermediate **117**.

The following steps were performed following the newly developed protocol for the preparation of *N*-aryl-1,2,3,4-tetrahydroisoquinolines (see Chapter 3.2.1.2 and SYNTHESIS publication^[132]). Reductive amination with previously prepared amine **102** (Scheme 44) and aldehyde intermediate **117** gave benzylamine **118** in 96 % yield. SUZUKI cross-coupling with 2-ethoxyvinyl pinacolboronate (**110**) afforded enol ether **119** as indicated by TLC ($R_f = 0.55$ (EtOAc/hexanes 60:40)). The following cyclisation of the crude enol ether (**119**) with TFA and triethylsilane generated *N*-aryl-1,2,3,4-tetrahydroisoquinoline **120**. Initially, **120** should be isolated and the yield determined. It was possible to purify **120** by FCC and the product was obtained in 63 % yield over two steps, but this product is not stable and decomposes rather quickly. This is probably the reason why several attempts to deprotect supposedly purified **120** under alkaline or acidic standard conditions failed. Hence, it was decided to directly deprotect crude **120** with KOH in THF/water. After FCC, **CG_224** was obtained in 60 % yield over three steps (Scheme 54). Unfortunately, this product is also highly unstable, especially at room temperature. Therefore, after drying under high vacuum, the product immediately had to be stored at $-20\text{ }^\circ\text{C}$ and tested shortly after preparation.



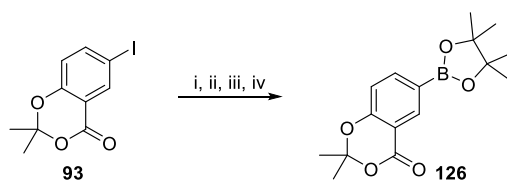
Scheme 54: Preparation of *N*-aryl-1,2,3,4-tetrahydroisoquinoline **CG_224**.^[132]

The second analogue ought to contain a fully aromatic rigidised core, a quinoline. This quinoline motif should be combined with the salicylic acid moiety by a SUZUKI cross-coupling reaction. The required quinoline precursor was synthesised as depicted in Scheme 55 starting from 6-quinolinecarboxylic acid (**121**). The carboxylic acid was protected as an ethyl ester by treatment with sulfuric acid in ethanol under reflux for 16 h according to a procedure for the preparation of this compound (**122**) from BACCON-SOLLIER *et al.*^[140] After FCC, product **122** was obtained in 80 % yield. Following a patent from CANCER RESEARCH TECHNOLOGY LIMITED from 2015,^[141] **122** was then converted into the *N*-oxide using *m*-CPBA and subsequently, without purification, into the 2-chloro substituted quinoline **124**. Purification by FCC afforded **124** in 21 % yield over two steps.^[141] It should be noted that pure **124** could not be obtained by using EtOAc/hexanes as eluent for FCC but only by using EtOAc/toluene. The free carboxylic acid **125** was then generated by adding pure conc. HCl and heating the reaction mixture to 95 °C for 1 h (62 % yield).^[141] In the following, after SUZUKI-cross coupling the benzamide side chain should be constructed.



Scheme 55: Preparation of 2-chloro quinoline-6-carboxylic acid (**125**).

Pinacol boronate **126**, carrying the acetonide protected salicylic acid motif, was then synthesised for the SUZUKI reaction. In a first approach, a borylation procedure for this molecule by PAUDYAL *et al.*^[142] was applied using bis(pinacolato)diboron (B_2pin_2) as borylation reagent and $\text{PdCl}_2(\text{dppf})\cdot\text{DCM}$ as catalyst (Scheme 56, i). Unfortunately, after 2 h at 80 °C only traces of the desired product could be detected on TLC. Instead, product **126** seemed to have undergone a SUZUKI cross-coupling with the starting material **93** right away resulting in dimerisation of **93** in 34 % yield after FCC (false product formation verified by NMR and mass spectrometry). To possibly avoid this homobiaryl formation, triethylamine was exchanged for potassium acetate and 1,4-dioxane with its higher boiling point of 101 °C (MeCN 82 °C) was used as the solvent (Scheme 56, ii). Indeed, with these modified reaction conditions the desired borylated product **126** was the main product.



- i) B_2Pin_2 , $PdCl_2(dppf) \cdot DCM$, Et_3N , MeCN, 80 °C, 2 h, **traces**
 ii) B_2Pin_2 , $PdCl_2(dppf) \cdot DCM$, KOAc, 1,4-dioxane, 80 °C, 16 h, **22 %**
 iii) HBPin, $Pd(PPh_3)_4$, Et_3N , 1,4-dioxane, 80 °C, 16 h, **traces**
 iv) HBPin, $Pd(PPh_3)_4$, Et_3N , 1,4-dioxane, 80 °C, 3 h → used as crude intermediate

Scheme 56: Attempts for the preparation of pinacol boronate **126**.

Difficulties arose when the product should be isolated by FCC. All impurities could be removed except remaining unreacted B_2pin_2 (Figure 16, signal at 1.26 ppm in the 1H -NMR spectrum).

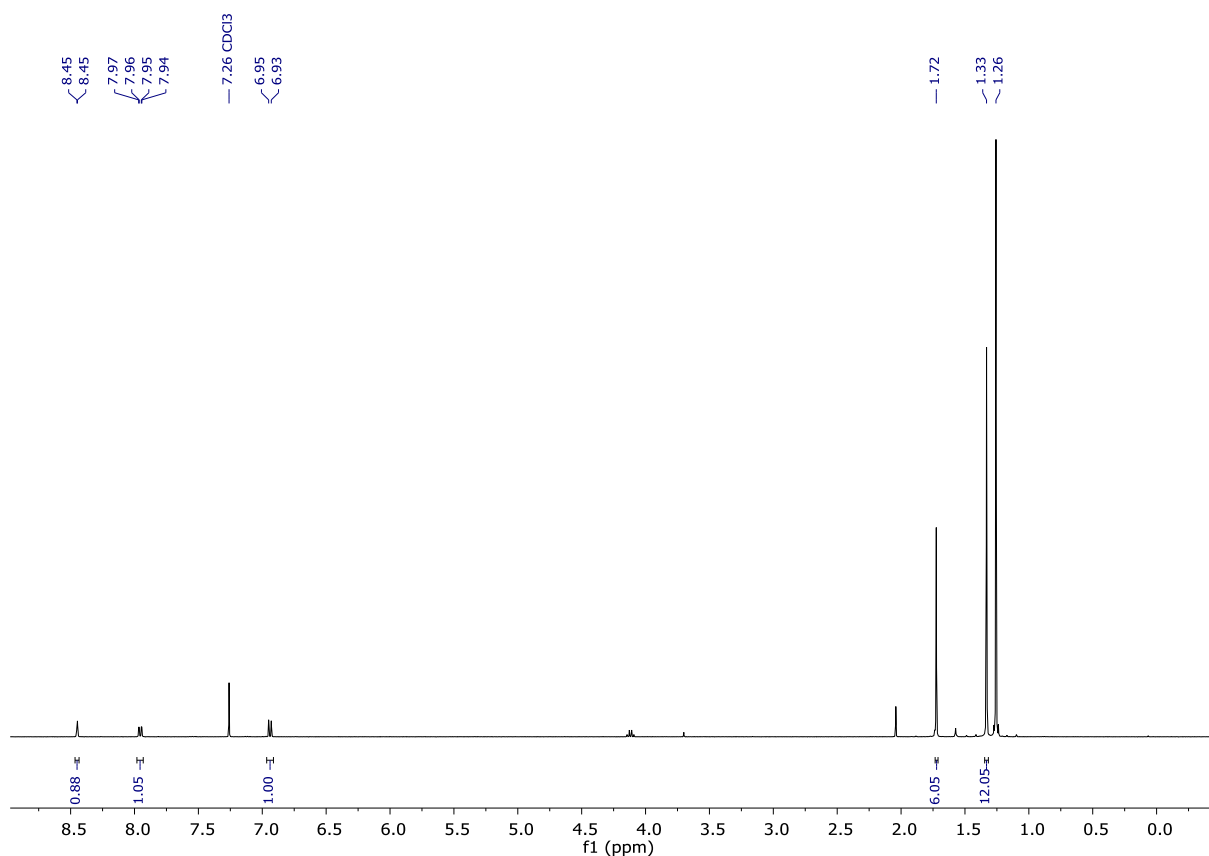


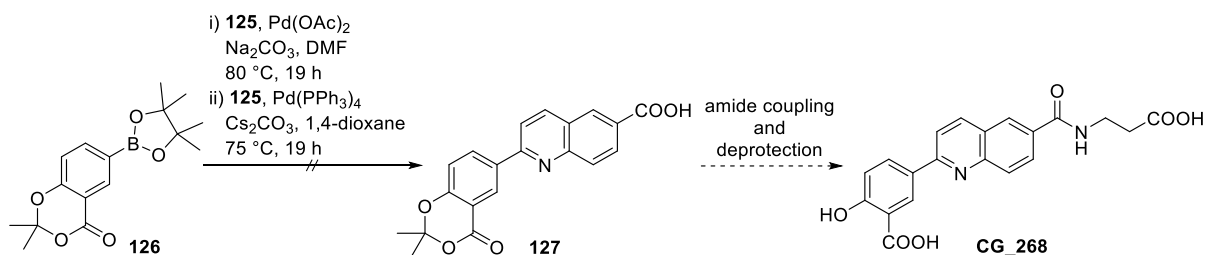
Figure 16: 1H -NMR of the borylated product **126** after one FCC showing starting material B_2pin_2 as impurity (1.26 ppm).

A literature search revealed that this is an often occurring problem and one publication by GALLEGO and SARPONG^[143] offers a solution as long as the desired product does not contain oxidisable functionalities. After a short column chromatography of the crude borylated product, it should be diluted again with ethyl acetate and 2-iodoxybenzoic acid (IBX) should be added and the reaction mixture be heated to 80 °C for 2 h. This approach was used and this time, after FCC, the desired pure product **126** was isolated in 22 % yield. To increase this moderate yield and to avoid difficult purifications, another attempt involved the exchange of B_2pin_2 for

HBpin as the borylation reagent as well as $\text{Pd}(\text{PPh}_3)_4$ for $\text{PdCl}_2(\text{dppf})\cdot\text{DCM}$ as the catalyst according to standard borylation protocols developed in the group of PROF. DR. FRANZ BRACHER by DR. ALEXANDRA KAMLAH^[144] (Scheme 56, iii). First, it was intended to purify the desired compound **126** again, but only traces were obtained after work-up. Therefore, the approach used by DR. CHRISTIAN AIGNER^[145] and DR. KERSTIN RIEDEL,^[146] respectively, was applied. After 3 h at 80 °C, the product was formed, but not isolated. To the reaction mixture all necessary reagents for the following SUZUKI reaction were directly added (Scheme 56, iv and Scheme 59).

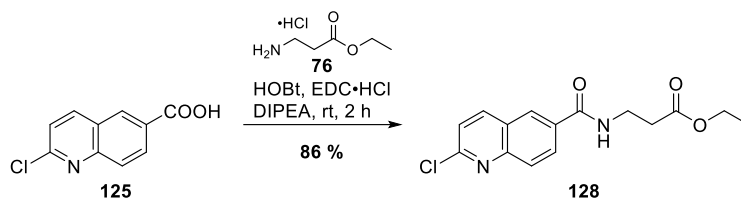
Chloroquinoline **125** should now be coupled with pinacol ester **126** in a SUZUKI cross-coupling, followed by amide coupling and alkaline deprotection to generate **CG_268** (Scheme 57). To see, whether the SUZUKI cross-coupling would be possible at all with a chloro substituted quinoline, this reaction was performed with pure **126**.

When using $\text{Pd}(\text{OAc})_2$ as catalyst and sodium carbonate as base following a procedure by PAUDYAL *et al.*^[142] only traces of product **127** were found by mass spectrometry but the desired product (**127**) could not be isolated in a sufficient amount for characterisation (Scheme 57, i). Therefore, it was decided to use standard conditions applied in the group of PROF. DR. FRANZ BRACHER for SUZUKI cross-coupling reactions with $\text{Pd}(\text{PPh}_3)_4$ and cesium carbonate in 1,4-dioxane (Scheme 57, ii). Unluckily, this led to similar results: minor amounts of one product that was faintly visible on TLC were isolated, but NMR analysis showed only unidentifiable peaks and **127** could not be synthesised this way.



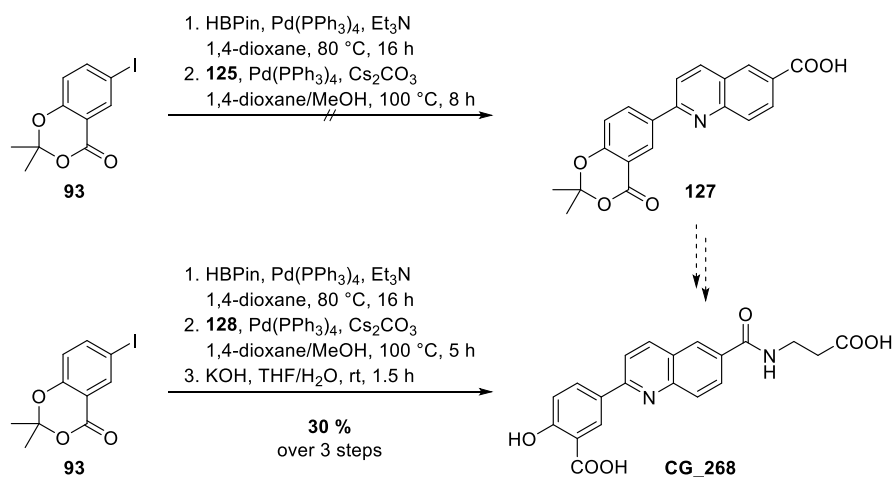
Scheme 57: Failed attempt for the synthesis of arylquinoline **CG_268**.

So far, it was still not clear if the reason for these unsuccessful attempts was the coupling partner or the conditions used. Therefore, the last attempts should now be made in a one-pot reaction manner as mentioned for Scheme 56, iv. This should be applied to the chloroquinoline with the free carboxylic acid (**125**) that was used so far and in addition, **125** should be amide coupled before SUZUKI cross-coupling to avoid the presence of the free carboxylic acid. For the amide coupling by now established conditions with HOBt and EDC·HCl were used and **128** was obtained after FCC in 86 % yield (Scheme 58).



Scheme 58: Preparation of intermediate amide **128**.

For the preparation of quinolines **127** and **CG_268**, respectively, were now employed the same conditions for the borylation reaction of **93** and the following SUZUKI cross-coupling with **125** and **128**, respectively, (Scheme 59). In case of **125** (Scheme 59, top), TLC indicated that the borylated product **126** was formed, but again in presumably small amounts. The subsequent SUZUKI cross-coupling then as well showed formation of the desired product **127** on TLC. Unfortunately, mass spectrometry revealed that in addition to **127** a variety of deprotected and/or newly methyl ester protected compounds were generated. But because of the necessary following amide coupling of the quinolinecarboxylic acid, this approach could not be further pursued, since the benzoic acid of the salicylic acid moiety had to stay protected. An additional full deprotection and re-protection of the compound seemed uneconomic regarding time and reagents. Therefore, the same procedure was applied to quinolinecarboxamide **128** instead (Scheme 59, bottom). This time, after borylation, the following SUZUKI cross-coupling again showed the formation of several products on TLC. Mass spectrometry analysis clearly indicated the formation of a variety of deprotected and/or newly methyl ester protected compounds. Beneficially, because the amide coupling had been performed already, another alkaline deprotection step was added after an aqueous work-up to the crude product mixture of the SUZUKI cross-coupling. The desired quinoline **CG_268** was obtained in 30 % yield over three steps.



Scheme 59: Preparation of quinoline **CG_268**.

In summary, 13 analogues with a modified central azo unit were designed and synthesised as shown in Figure 17.

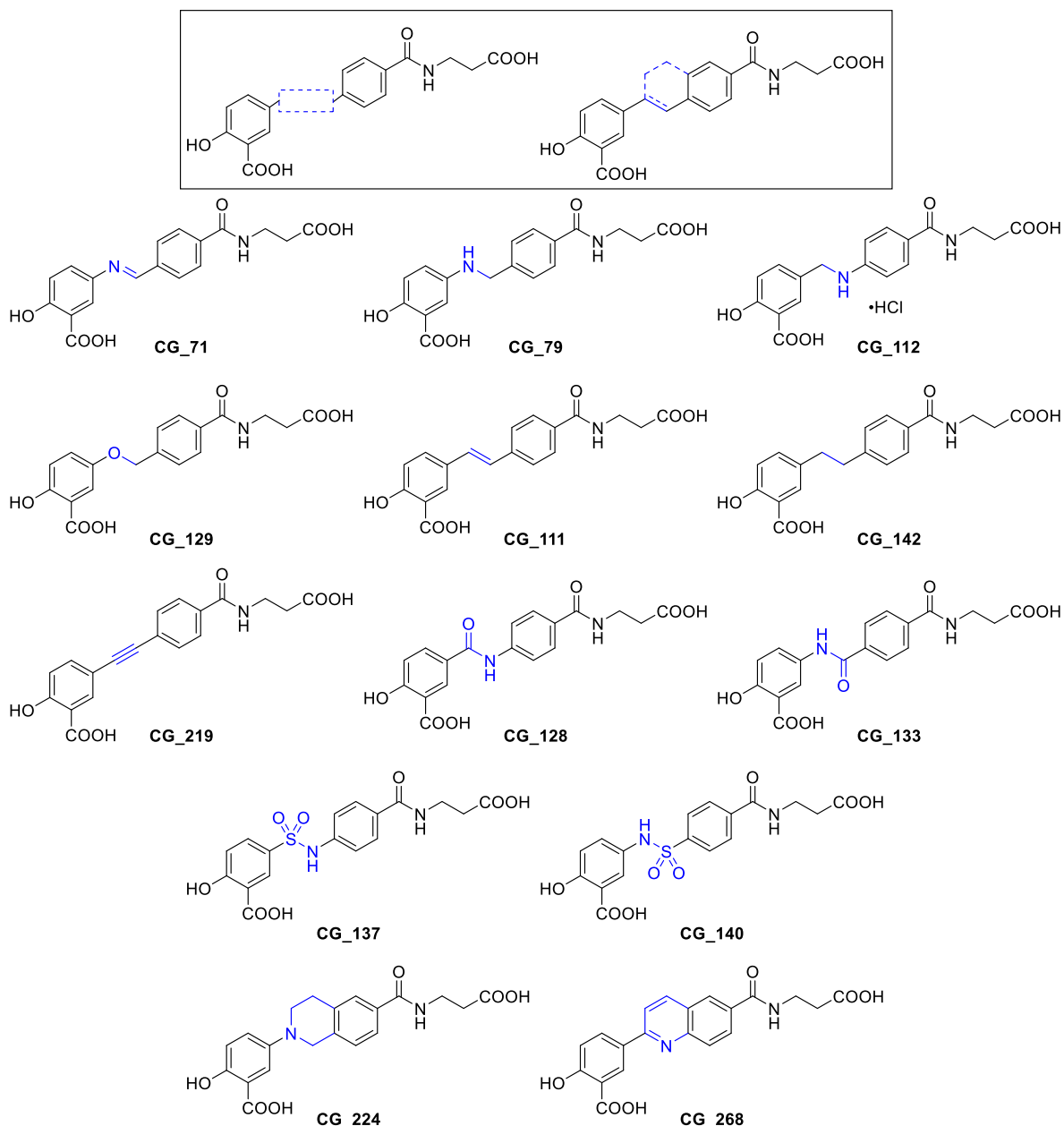


Figure 17: Synthesised analogues bearing a modification of the central azo unit of balsalazide.

These analogues with an open chained or a rigidised azo group modification were then tested for Sirt5 inhibitory activity in comparison with balsalazide. In addition, the two analogues imine **CG_103** and ether **CG_94** still carrying protecting groups, which could not be cleaved off, were included (Figure 18).

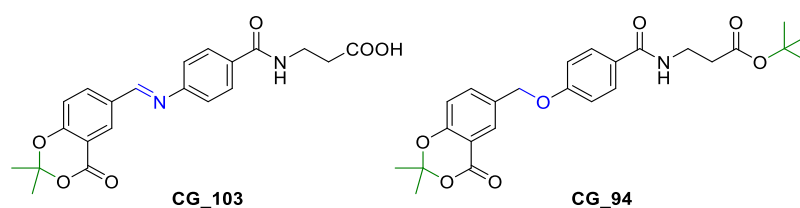


Figure 18: Imine **CG_103** and ether **CG_94**, both still carrying their protecting groups.

3.2.2. Inhibitory activity against Sirt5

These synthesised analogues bearing a modification of the central azo unit were then compared in their inhibitory activity with the lead structure balsalazide in an enzyme-based fluorometric assay (Figure 11 and see Chapter 6.1.3 for detailed procedures). The replacement of the core azo unit of balsalazide comprises an essential step towards a more drug-like inhibitor of Sirt5.

The compounds were again tested at a final assay concentration of 50 μM and the results of this single-concentration measurement are depicted in Figure 19 and

. Nicotinamide and suramin were included as positive controls.

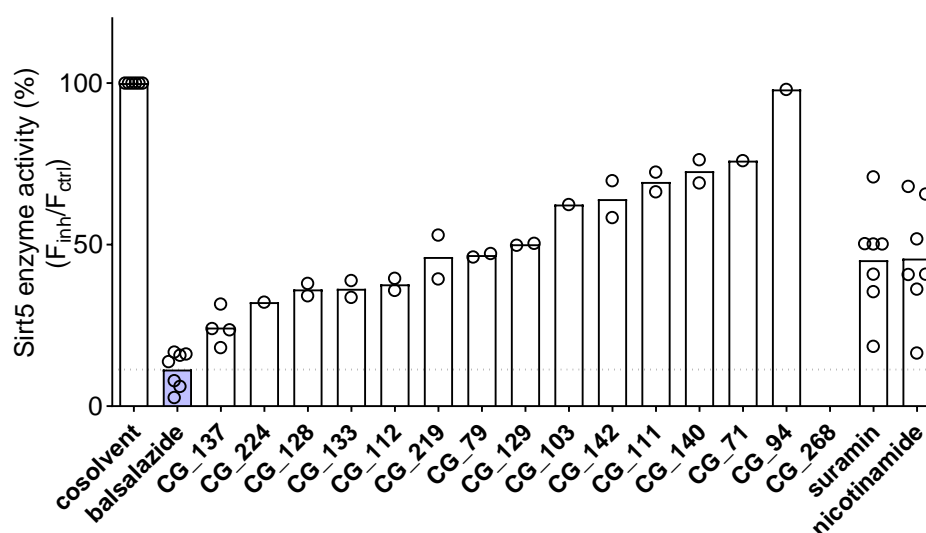


Figure 19: Residual Sirt5 enzyme activity after 1 h incubation with the inhibitors in % ($F_{\text{inh}}/F_{\text{ctrl}}$). The known non-selective inhibitors nicotinamide and suramin were included as positive controls. Fluorescence measured upon incubation with the cosolvent DMSO alone (no inhibitor) was set to 100 % enzyme activity. All inhibitors were tested at 50 μM final concentration. Quinoline **CG_268** showed auto-fluorescence and thereby could not be measured with this assay setup. F = fluorescence units; $\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$; number of biological replicates stated with circles (○) for each bar.

Table 4: Sirt5 inhibition in %, including balsalazide and positive controls nicotinamide and suramin for comparison. Values represent means of technical and biological replicates. *n.d. = not determinable.

Compound name	Inhibition in %	Compound name	Inhibition in %
balsalazide	89	CG_133	64
CG_71	27	CG_137	75
CG_79	54	CG_140	29
CG_111	32	CG_142	37
CG_112	63	CG_219	54
CG_128	65	CG_224	68
CG_129	51	CG_268	n.d.*
CG_94	6	CG_103	40
nicotinamide	54	suramin	55

These results clearly show that similar to the modifications performed before, also the central azo group cannot lightheadedly be replaced, since all analogues had a weaker inhibitory activity than the lead structure balsalazide (89 %). In more detail, analogues **CG_94** and **CG_103**, which still carry their protecting groups, exhibited a significant loss in activity (6 % and 40 % Sirt5 inhibition at 50 μ M, respectively). Especially **CG_94**, which bears a *tert*-butyl ester instead of the free terminal carboxylic acid essentially lost activity completely. This again verifies the high importance of this carboxylic acid and its interaction in balsalazide with Arg105 and Tyr102 in the binding pocket of Sirt5.

Imine **CG_71**, missing only one nitrogen in the spacer, was the weakest inhibitor in this set of analogues with 27 % Sirt5 inhibition. On the first look, one could assume that amines **CG_79** and **CG_112** (54 % and 63 %), which only differ in the position of the secondary amine in the spacer, showed that an additional hydrogen bond donating ability improves potency compared to the imine analogue **CG_71** (27 %). Nevertheless, they still show a significant decrease in activity compared to balsalazide. On a second look, taking ether **CG_129** into account acting solely as a hydrogen bond acceptor again, this theory was disproved. Because ether **CG_129** showed with 51 % Sirt5 inhibition comparable potency with amine **CG_79** (54 %). Analogues lacking HBDs and HBAs simultaneously, **CG_111**, **CG_142** and **CG_219**, also showed interesting results. Stilbene **CG_111** is, like balsalazide, *E*-configured, but lost inhibitory activity almost completely (only 32 % Sirt5 inhibition). The same was observed for fully flexible alkane **CG_142** with an only minor better inhibition of 37 % at 50 μ M.

Interestingly, alkyne **CG_219**, which lost flexibility even more than balsalazide or stilbene **CG_111**, resulted in the best potency within these three analogues with 54 % Sirt5 inhibition.

This could be because the alkyne, a bioisoster of a phenyl ring, now changed the overall geometry of the molecule. Two of the most potent analogues of this set were amides **CG_128** and **CG_133** with 65 % and 64 % inhibition, respectively. These were only overtopped by sulfonamide **CG_137**, which inhibited Sirt5 by 75 % at 50 μM . This analogue showed very promising potency against Sirt5, and especially compared to its laterally reversed analogue **CG_140**, which was among the weakest inhibitors (29 %). Why these two differ so drastically in their potency remained unclear but could eventually be enlightened through computational chemistry experiments (see Chapter 4.3.1). Nevertheless, **CG_137** showed only marginally reduced activity compared to balsalazide in this single-point measurement, which could possibly result in a better IC_{50} value. The last two analogues of this set were the rigidised analogues **CG_224** and **CG_268**. Unfortunately, quinoline **CG_268** showed significant auto-fluorescence in this experimental setup and the inhibitory strength could thereby not be measured but should be determined in an assay system that is not based on fluorescence. The results obtained for tetrahydroisoquinoline **CG_224** (68 %), although being the best inhibitor after **CG_137**, should be interpreted with caution. This compound decomposes relatively fast at room temperature when dissolved in DMSO and it is not known how the aqueous environment of the assay could additionally influence its stability. Therefore, it is not clear, in which state the compound existed during measurement. If it was still intact, the compound is still not suited for further development towards potent inhibitors of Sirt5. In contrast, with 74 % Sirt5 inhibition sulfonamide **CG_137** will be used for further modifications and in the course of in-depth biological investigations.

In summary, these results of the azo analogues cannot easily be explained by interactions within the binding pocket of Sirt5 on the basis of the docking calculations presented in Figure 8 (Chapter 2). In the docking pose of balsalazide (Figure 8b) no interactions of the azo spacer with side chains of Sirt5 are visible. Therefore, further experiments had to give more insights into these observations (Chapter 4).

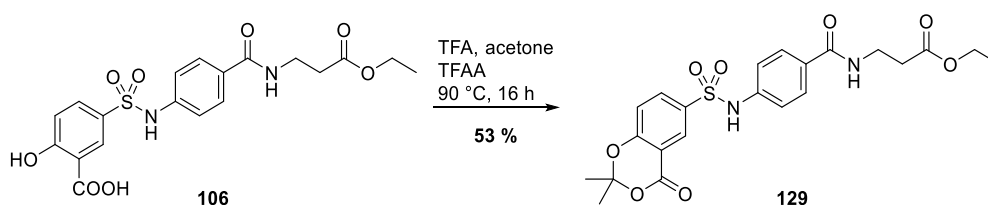
3.3. Bioisosteric variations and modifications based on previous results

Because of the good results obtained with analogues of the previous chapters, a few variants were designed based on these structures to explore structure-activity relationships further. In addition, selected functional groups of balsalazide should be replaced by bioisosteric groups to possibly improve potency.

3.3.1. Synthesis

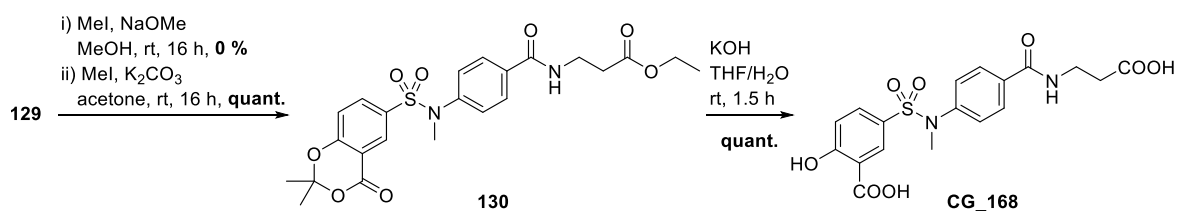
3.3.1.1. Analogues based on previous results

Sulfonamide **CG_137** showed good potency in a comparable range with balsalazide (75 % and 89 %, respectively) and was the best variant of the set of open-chained variations of the azo spacer (Chapter 3.2). As mentioned before, on the other hand, the laterally reversed sulfonamide **CG_140** was significantly less potent with only 25 % Sirt5 inhibition. To explore possible interactions of **CG_137** with the enzyme, it was decided to remove the hydrogen bond donating abilities of the secondary sulfonamide spacer by *N*-methylation. For this purpose, the salicylic acid moiety of precursor **106** (Scheme 47) had to be protected as an acetonide to avoid undesired *O*-methylation at this site. This was accomplished using now established standard conditions with TFA, acetone and TFAA at 90 °C (Scheme 60). Acetonide **129** was obtained in 53 % yield and could then be *N*-methylated.



Scheme 60: Preparation of acetonide protected sulfonamide **129**.

Based on a published procedure by GIANOTTI *et al.*^[147] *N*-methylation of the sulfonamide group was first attempted by using methyl iodide as methylating agent and *in situ* prepared sodium methoxide as base in methanol at room temperature. Unfortunately, this procedure resulted in a variety of deprotected, newly methyl protected and/or sulfonamide methylated products, but none of them being the desired product **130** (Scheme 61). The reason could be the choice of base. Sodium methoxide cannot only act as a base but also as a nucleophile and thereby cause numerous side products as it was the case here. Consequently, the base was exchanged for potassium carbonate and acetone was chosen as the solvent while methyl iodide was still used as the methylating reagent. These conditions have been successfully employed in the group of PROF. DR. FRANZ BRACHER before. This time, the desired product **130** was obtained in quantitative yield. Final alkaline ester and acetonide deprotection gave the desired *N*-methylated sulfonamide **CG_168** also in quantitative yield.



Scheme 61: Preparation of methylated sulfonamide **CG_168**.

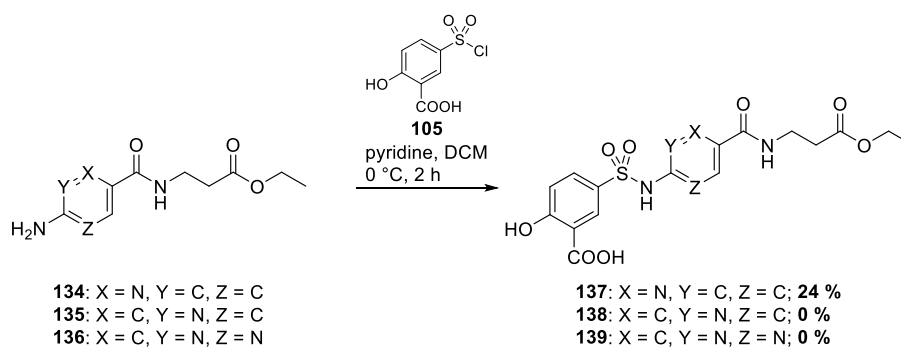
With sulfonamide being a spacer that now opens synthetic possibilities for different variations regarding the rest of the molecule, the next variations should concern the non-salicylate phenyl ring. Here, the goal was to include heteroatoms and vary between 5- and 6-membered rings. This intention was based on the fact, that phenyl rings can be replaced by bioisosteres with similar electronic and sterical properties such as thiophenes or pyridines, whereby the biological effects can be preserved or even potency can be improved.^[148-150] Therefore, this should be accomplished with pyridine, pyrimidine and thiophene rings. If these variations showed promising results in the enzyme assay more analogues could be designed. The phenyl ring of the salicylate unit was not replaced by heterocycles due to the lack of appropriate starting materials still bearing the hydroxy and carboxy groups.

The first three analogues were to bear pyridine rings and a pyrimidine ring, respectively, with the nitrogen atom(s) at different ring positions. These were chosen due to availability of starting materials. In a first step, amide couplings using standard conditions with β -alanine ethyl ester hydrochloride (**76**) afforded amides **134** (89 % yield), **135** (17 % yield), and **136** (44 % yield) starting from the corresponding 5-aminopyridine-2-carboxylic acid (**131**), 6-amino-3-pyridinecarboxylic acid (**132**), and 2-aminopyrimidine-5-carboxylic acid (**133**) (Scheme 62).



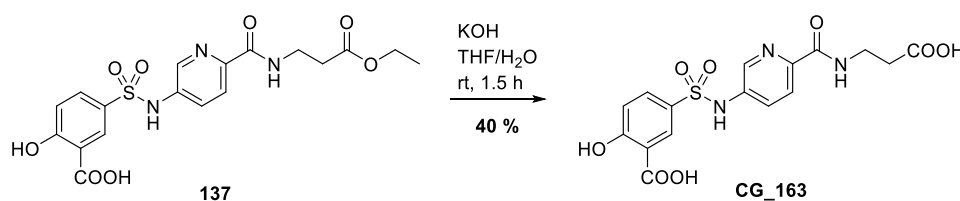
Scheme 62: Amide coupling reactions for the preparation of pyridine **134**, pyridine **135** and pyrimidine **136**.

These heterocycles should then each undergo sulfonamide coupling with commercially available 5-chlorosulfonyl-2-hydroxybenzoic acid (**105**) with the conditions used for the preparation of sulfonamide **106** (Scheme 63 and cf. Scheme 47). Unfortunately, sulfonamide coupling was only successful in case of pyridine **134**, giving **137** in a low yield of 24 %. For pyridine **135** and pyrimidine **136**, respectively, the desired product could neither be detected by mass spectrometry nor be isolated by FCC. A possible reason is the position of the nitrogen atom(s) in *ortho* position to the ring nitrogen, since this reduces the nucleophilicity of the amino group, compared to the *meta* position in **134**. With one example for a 6-membered heterocycle (**137**) in hand, the synthesis of the other two analogues was not further pursued.



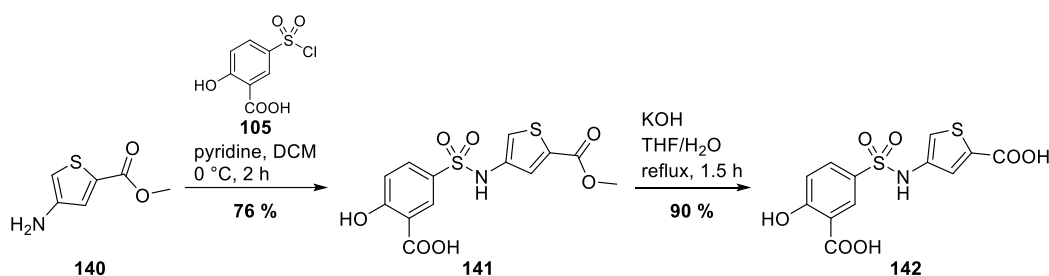
Scheme 63: Failed attempts for the synthesis of pyridine **138** and pyrimidine **139** and successful attempt for the preparation of pyridine **137** via amide coupling.

The ethyl ester of pyridine **137** was cleaved using KOH in THF/water at room temperature (Scheme 64). After 1.5 h, carboxylic acid **CG_163** was obtained in 40 % yield.



Scheme 64: Preparation of carboxylic acid **CG_163** via alkaline deprotection.

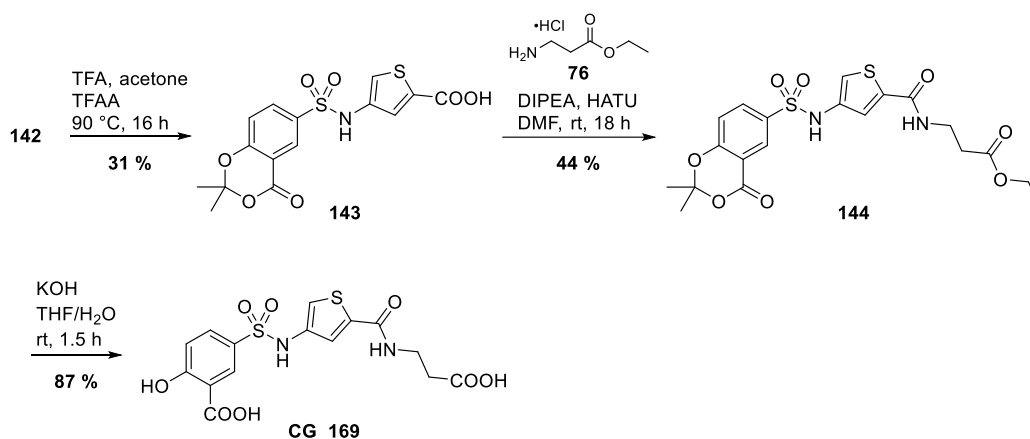
The following 5-membered heterocycles should consist of a thiophene ring with variation of the sulfur position. Depending on the availability of starting materials, the synthetic strategy differs slightly between the analogues. As for the previous syntheses of **106** and **137**, the sulfonamide spacer for the first analogue was constructed with 5-chlorosulfonyl-2-hydroxybenzoic acid (**105**) in DCM in the presence of pyridine starting from methyl 4-aminothiophene-2-carboxylate (**140**) (Scheme 65). Sulfonamide **141** was successfully generated in 76 % yield. In a next step, the methyl ester was hydrolysed to the free carboxylic acid **142** under alkaline deprotection conditions in 90 % yield.



Scheme 65: Preparation of compound **142**.

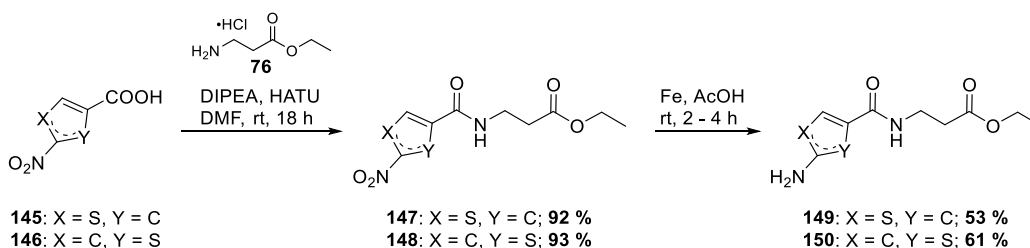
For the construction of the β -alanine derived side chain, the salicylic acid moiety had to be protected. Hence, acetonide **143** was prepared from **142** using TFA, acetone and TFAA in a low yield of 31 %. The following amide coupling with β -alanine ethyl ester hydrochloride (**76**) was performed according to the synthetic procedure of amide **46** (Scheme 19) with HATU and

DIPEA. Product **144** was obtained in 44 % yield. The final desired thiophene **CG_169** was afforded in 87 % yield by removing the protecting groups under alkaline conditions (Scheme 66).



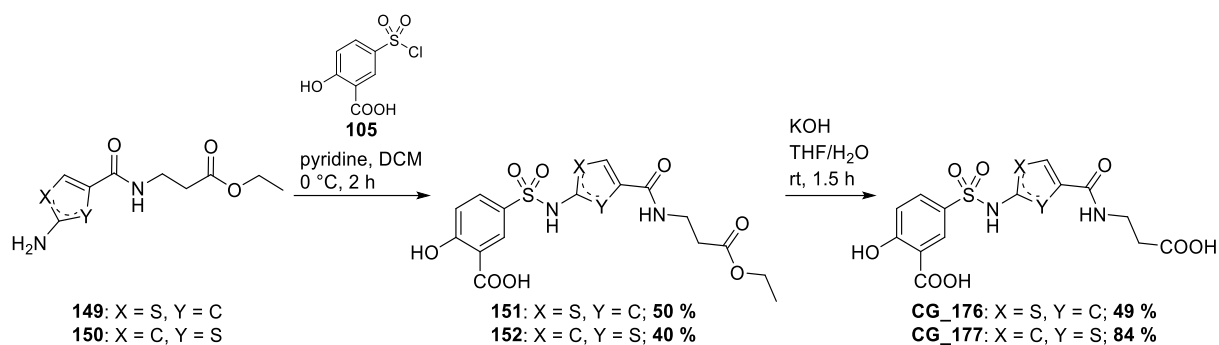
Scheme 66: Preparation of thiophene **CG_169**.

Compared to **CG_169**, the side chain in thiophenes **CG_176** and **CG_177** was generated in the first step (Scheme 67). Here again, the same protocol with DIPEA and HATU was employed starting from 5-nitrothiophene-3-carboxylic acid (**145**) and 5-nitrothiophene-2-carboxylic acid (**146**), giving **147** and **148** in 92 % and 93 % yield, respectively. Subsequent reduction of the nitro group to the aminothiophenes, sulfonamide coupling, and lastly, alkaline deprotection could be performed for both analogues in the same manner (Scheme 67 and Scheme 68). For reduction of the nitro group, iron in acetic acid was used instead of hydrogenation conditions due to an otherwise possible reductive desulfurisation. Amines **149** and **150** were obtained in 53 % and 61 % yield, respectively.



Scheme 67: Preparation of precursors **149** and **150**.

The sulfonamide spacers in **151** and **152** were then prepared according to sulfonamide coupling reactions used before with 5-chlorosulfonyl-2-hydroxybenzoic acid (**105**) in DCM in the presence of pyridine. Compounds **151** and **152** were obtained in 50 % and 40 % yield, respectively. Finally, alkaline deprotection generated the desired final thiophenes **CG_176** and **CG_177** in 49 % and 84 % yield, respectively (Scheme 68).



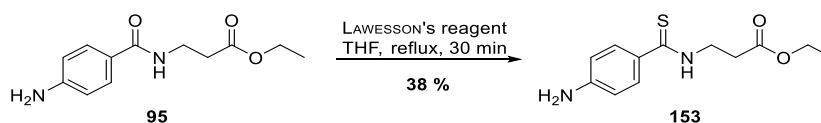
Scheme 68: Preparation of target thiophene compounds **CG_176** and **CG_177**.

The very promising sulfonamide spacer opened the synthetic possibility to explore the non-salicylate phenyl ring and thereby these heterocyclic sulfonamide analogues could be prepared. At the same time more analogues were synthesised carrying bioisosteric functional groups in the β -alanine derived side chain and the salicylic acid part of the molecule where the original azo group spacer should be kept in place.

3.3.1.2. Bioisosteric analogues

The first analogue of this set was inspired by suggestions in various publications in literature that thioureas as well as thioamides are promising variants, which presumably increase affinity to the Sirt5 protein target. For the thiourea-containing analogue **CG_150**, it was already possible to prove this statement^[93] wrong, at least in case of the balsalazide motif and Sirt5. To see, whether this is also the case for a balsalazide analogue containing a thioamide instead of the amide in the side chain, **155** was designed (Scheme 70). In contrast to **CG_150**, this analogue carries a thioamide in the presumably ideal position for a hydrogen bond to the hydroxyl group of the ribose unit of NAD⁺.

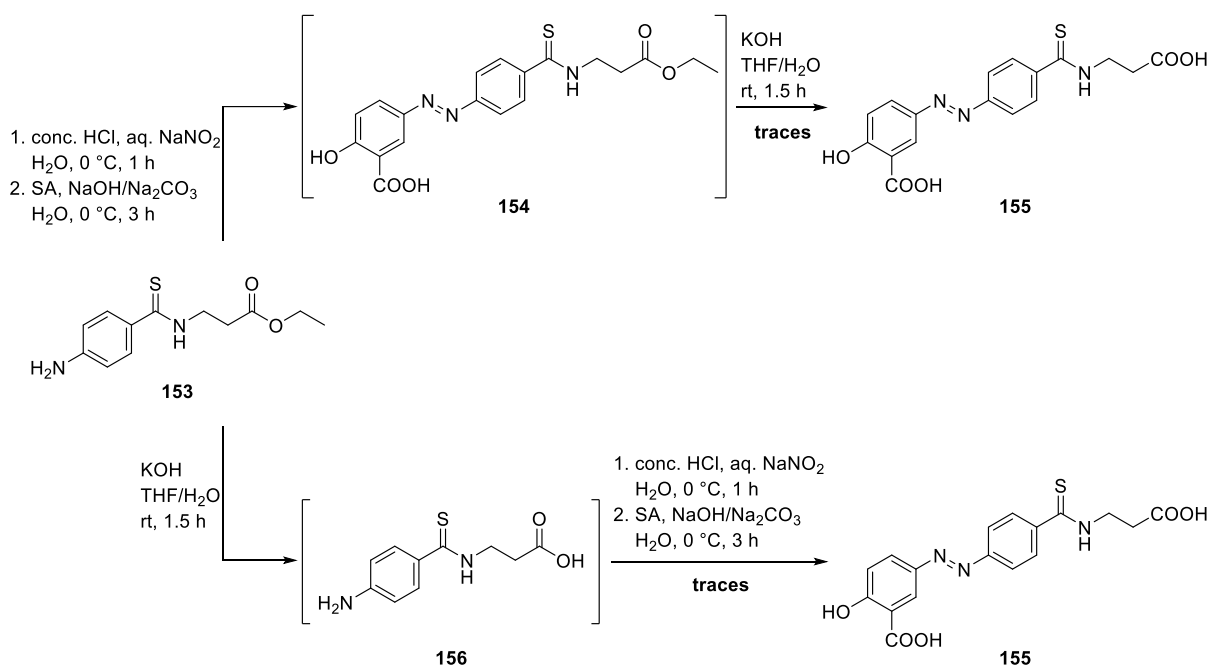
First, previously prepared aminobenzamide **95** (Scheme 41) was converted into the corresponding thioamide **153** in 38 % yield using LAWESSON's reagent according to standard procedures used in the group of PROF. DR. FRANZ BRACHER. The O/S exchange had to be performed at the very beginning of the synthetic sequence due to the incompatible azo bond and functional groups (phenolic OH, carboxylic acid) prone to be otherwise converted themselves.



Scheme 69: Preparation of thioamide **153**.

Starting from thioamide **153** two different approaches were explored for the synthesis of compound **155** (Scheme 70). These only differ in the order of azo coupling and alkaline deprotection steps. If alkaline deprotection is performed first (Scheme 70, bottom), the desired

free carboxylic acid **156** is generated in good amounts as indicated by LC-MS analysis. Due to its zwitter ionic character, this product was not purified after deprotection but used in the azo coupling as crude product. Unfortunately, after azo coupling a mixture of the desired product **155** ($m/z = 374$ $[M+H]^+$) and a product with $m/z = 358$ $[M+H]^+$ was identified *via* mass spectrometry analysis. The same result was obtained when the azo coupling was performed first and the alkaline deprotection second (Scheme 70, top). Here, already the azo coupling to **154** showed several products with similar molecular weights. In both cases the desired thioamide **155** could only be isolated in traces. The major side product with $m/z = 358$ $[M+H]^+$ was identified by NMR spectroscopy and mass spectrometry as balsalazide itself. Apparently, the azo coupling converts the thioamide partially back to the carboxamide.

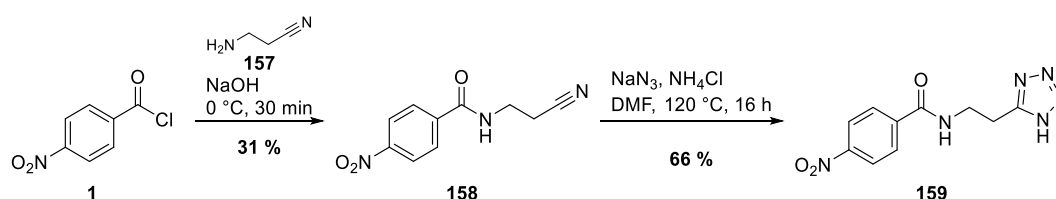


Scheme 70: Failed attempts to prepare thioamide **155**.

Sulfur in the context of specific functional groups is often discussed controversially in literature. Molecules containing thioamides and different related heterocycles such as thiohydantoin (see compound **40b**, Scheme 17) are sometimes called “controversial scaffolds” and are often put in the context with pan-assay interference compounds (PAINS). Furthermore inclusion of functional groups like thioamides or thioureas (see compound **CG_150**, Scheme 18) is often advised against due to e.g. toxicity.^[105, 151-152] Therefore, the synthesis of a thioamide-containing analogue was no longer pursued.

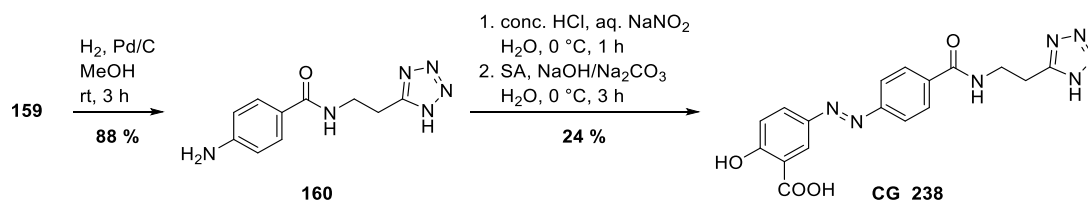
Nevertheless, another bioisostere concerning the side chain was designed and synthesised – a tetrazole analogue (**CG_238**). This bioisostere replaces the as indispensable identified carboxylic acid of the side chain in balsalazide. Tetrazoles have a moderately acidic pK_a of about 5 (like carboxylic acids)^[81] and show hydrogen bond accepting as well as donating

abilities, thus making them good candidates for bioisosterism as exemplified by antihypertensive agents like losartan or the diuretic azosemid.^[153] The tetrazole was synthesised starting from 4-nitrobenzoyl chloride (**1**) in two steps as illustrated in Scheme 71. Nitrile **158** was prepared as described by UEDA *et al.*^[154] from 4-nitrobenzoyl chloride (**1**) and 3-aminopropionitrile (**157**) in 10 % aq. sodium hydroxide. Purification by FCC and additional recrystallisation from hot acetone gave **158** in a moderate yield of 31 %. The tetrazole unit was then synthesised based on a procedure in a patent from GLAXO GROUP LIMITED from 2003,^[155] starting from nitrile **158** with sodium azide and ammonium chloride in DMF at 120 °C. Tetrazole intermediate **159** was obtained in 66 % yield.



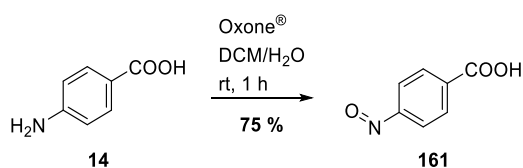
Scheme 71: Preparation of precursor tetrazole **159**.

The nitro group in **159** was then reduced into the corresponding aryl amine **160** using standard hydrogenation conditions with H₂ and Pd/C, resulting in 88 % yield. Subsequent azo coupling with salicylic acid afforded the desired tetrazole analogue **CG_238** in a low yield of 24 % (Scheme 72).



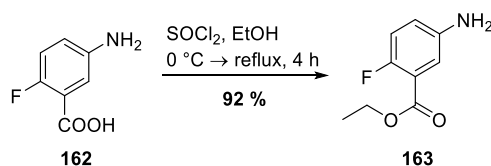
Scheme 72: Preparation of tetrazole **CG_238**. SA = salicylic acid.

Two more compounds should focus on bioisosterism regarding the salicylic acid part of balsalazide. One of them carrying a fluoro substituent in the position of the phenolic hydroxy group (**CG_267**). For the preparation of this analogue, a MILLS coupling should be employed in the manner of the synthesis of **CG_24** and **CG_25** (Scheme 7). For this, literature known nitroso derivative **161**^[156] was prepared from 4-aminobenzoic acid (**14**) using Oxone[®] in DCM/water in 75 % yield (Scheme 73).



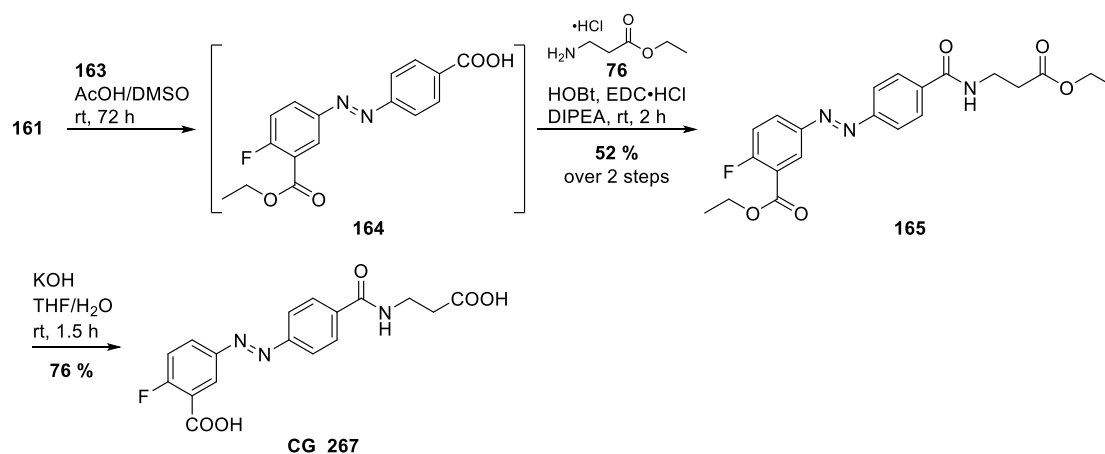
Scheme 73: Preparation of nitroso derivative **161**.

Furthermore, aminobenzoic acid ester **163** was prepared from the corresponding carboxylic acid **162** as described in a patent from BAYER PHARMA AKTIENGESELLSCHAFT from 2017^[157] with thionyl dichloride in ethanol in 92 % yield (Scheme 74).



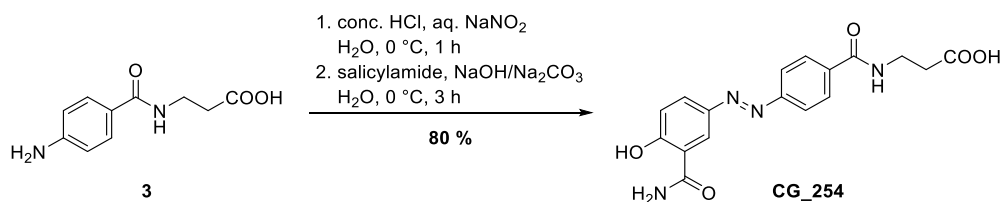
Scheme 74: Preparation of ethyl ester **163**.

These two precursors could now undergo MILLS coupling to form the azo bond (Scheme 75). As mentioned before, the conditions that were used for the preparation of **CG_24** and **CG_25** were applied here as well. The formation of the desired azobenzene **164** was confirmed by mass spectrometry analysis and the crude product was directly amide coupled (under standard conditions with HOBt and EDC·HCl) for ease of purification. Product **165** was obtained in 52 % yield over two steps after FCC. Lastly, alkaline deprotection of both ester units afforded the desired azobenzene **CG_267** in 76 % yield.



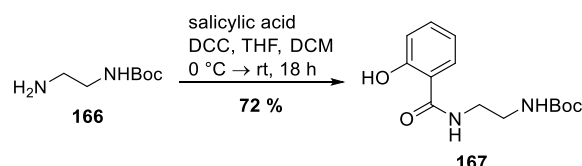
Scheme 75: Preparation of fluoro analogue **CG_267**.

Another interesting bioisostere concerning the salicylic acid moiety of the molecule and its synthesis is depicted in Scheme 76. Analogue **CG_254** is bearing a primary salicylamide motif instead of the salicylic acid moiety. A general azo coupling starting from previously prepared amine **3** (Scheme 2) with salicylamide afforded **CG_254** in a good yield of 80 %.



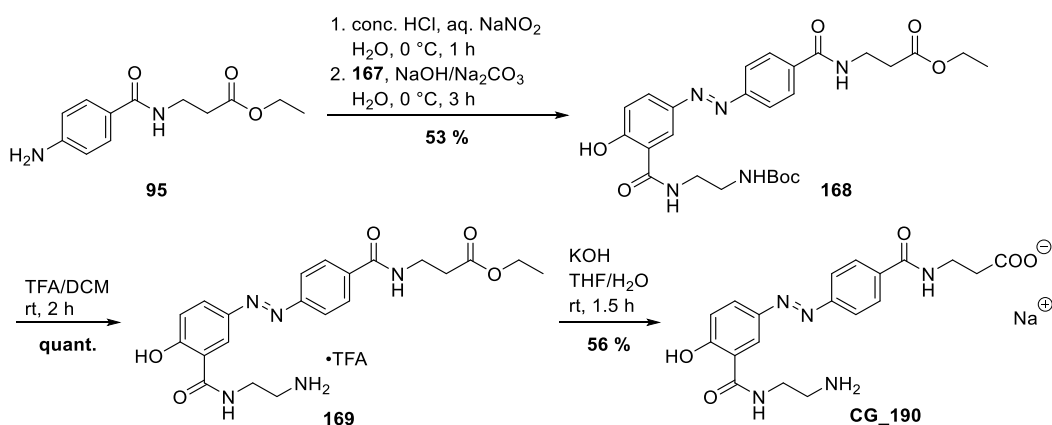
Scheme 76: Preparation of salicylamide bioisostere **CG_254**.

This leads to the last analogue that was synthesised primarily for a chemical proteomics experiment (for details see Chapter 4.4). Here, a linker unit should be incorporated in balsalazide to be able to couple it to beads without affecting the affinity of the molecule to Sirt5. From previous results, it was concluded that minor modifications on the salicylic acid moiety of the molecule are tolerated to some extent. Therefore, an ethylenediamine linker should be attached to the benzoic acid resulting in immobilisable *N*-(2-aminoethyl)salicylamide **CG_190** as illustrated in Scheme 77 and Scheme 78. In a first step, the desired ethylenediamine linker was introduced in an amide coupling of salicylic acid and *N*-Boc-ethylenediamine (**166**) described in a patent from BRISTOL-MYERS SQUIBB COMPANY from 2005.^[158] Here, coupling reagent DCC was used in THF/DCM and salicylamide **167** was obtained in 72 % yield.^[158]



Scheme 77: Preparation of salicylamide **167**.

A subsequent azo coupling with previously prepared intermediate **95** (Scheme 41) and salicylamide **167** afforded compound **168** in a moderate yield of 53 %. Boc protection was removed using TFA/DCM, giving TFA-salt **169** in quantitative yield. The ethyl ester was cleaved in a final alkaline deprotection, resulting in the desired coupleable azobenzene **CG_190** (56 % yield).



Scheme 78: Preparation of coupleable balsalazide amide **CG_190**.

Here it should be noted that purification as well as NMR analysis bore several difficulties. Fortunately, precursor **169** could be purified by FCC before, and ester hydrolysis was performed without the formation of major side products. Therefore, the difficulty that remained was the removal of excess salt in the crude product of **CG_190** because it could not be transferred into the organic phase but instead stayed in the aqueous phase. For that, the crude reaction mixture was adjusted to pH 5 (calculated isoelectric point of the product) with 1 N aq.

NaOH and 1 N aq. HCl and minor organic impurities were removed by washing this aqueous phase with EtOAc. The aqueous phase was then concentrated *in vacuo*. With crude **CG_190** was then successfully performed an SPE (solid phase extraction) purification. Figure 20 depicts the $^1\text{H-NMR}$ spectrum of the purified product in $\text{DMSO-}d_6$. Surprisingly, the two amides' NH groups did not occur in approximately the same shift as for precursor **169** (8.0 – 9.5 ppm), but one of the amide hydrogens, from the amide of the linker moiety, appeared above 11.5 ppm. Initially, it was not clear if this was really the amide or if it was the phenolic OH group or the carboxylic acid. A $^{15}\text{N-NMR}$ analysis should give further insight but the first measurements could not quite answer the question.

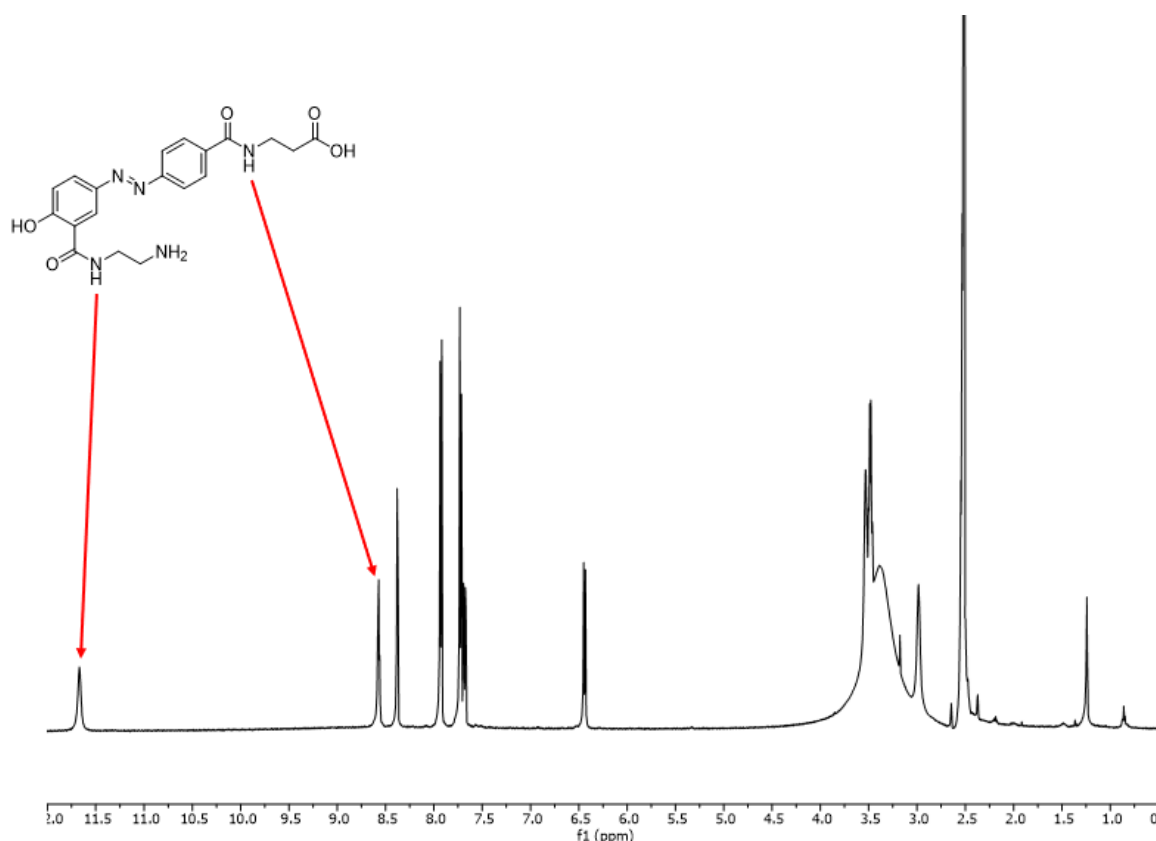


Figure 20: $^1\text{H-NMR}$ spectrum of **CG_190** in $\text{DMSO-}d_6$.

Therefore, another experiment within the NMR tube should solve the problem (Figure 21). This experiment included a titration with 2 N aq. HCl. Hydrochloric acid was added per 0.25 eq/0.5 eq with respect to **CG_190** into the NMR tube. After each step, a $^1\text{H-NMR}$ spectrum was recorded, and the results are shown in Figure 21. It could be observed that the amide of the linker moiety moves more and more upfield in the direction where it would be expected to appear (ca. 9 ppm). At the same time a new signal appeared at 13 ppm from either the carboxylic acid or the phenolic hydroxy group. All other signals were only minimally influenced by the addition of HCl except the amine at ca. 6.5 – 7 ppm, which gets slowly protonated.

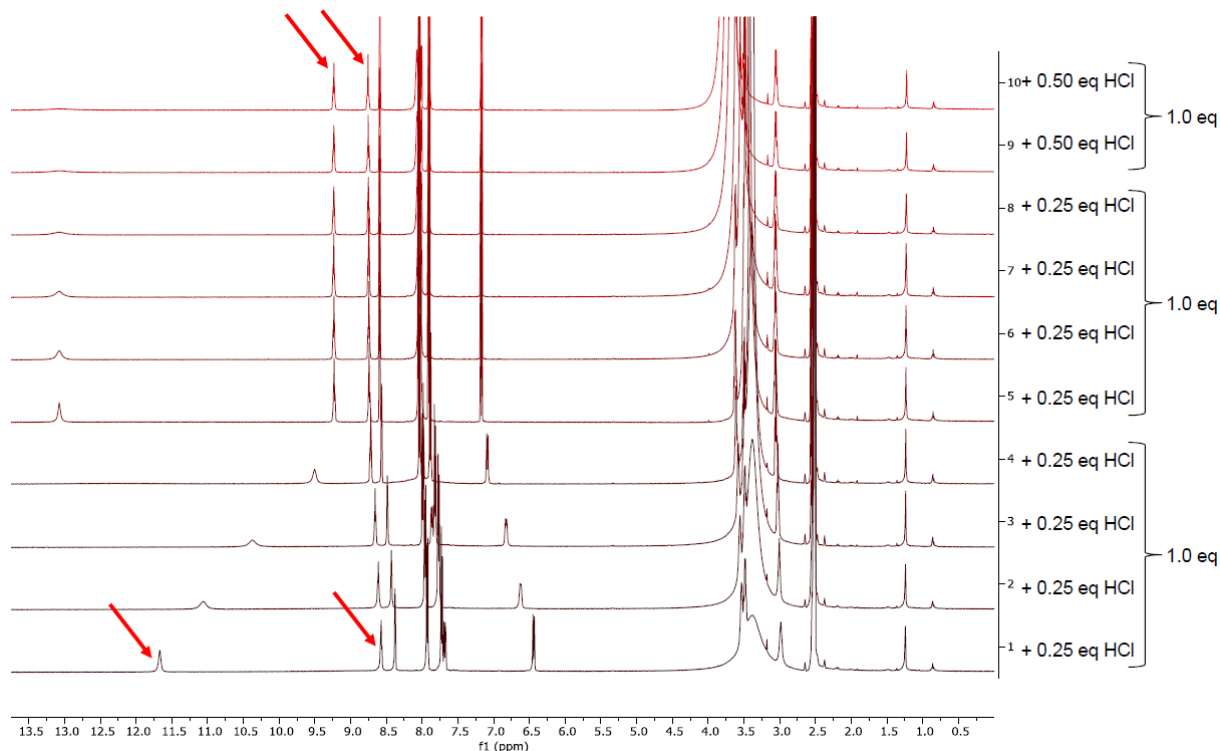


Figure 21: $^1\text{H-NMR}$ titration experiment with 2 N aq. HCl of **CG_190** in $\text{DMSO-}d_6$.

After the addition of three equivalents of HCl in total, no further changes were observed. Another $^{15}\text{N-NMR}$ now clearly identified the signal at ~ 9.2 ppm in the new $^1\text{H-NMR}$ spectrum (Figure 21, top), which originally appeared at ~ 11.7 ppm (Figure 21, bottom), as the amide hydrogen of the ethylenediamine linker. Overall, these experiments revealed that compound **CG_190** in $\text{DMSO-}d_6$ in the NMR tube presumably formed an intramolecular hydrogen bond between the phenolic hydroxy group and the amide of the linker (Figure 22). This results in a downfield-shift of the amide proton of the ethylenediamine linker and the appearance of the presumably phenolic proton more upfield only after addition of acid. Furthermore, this titration experiment showed that the product was formed as the sodium salt after work-up and purification.

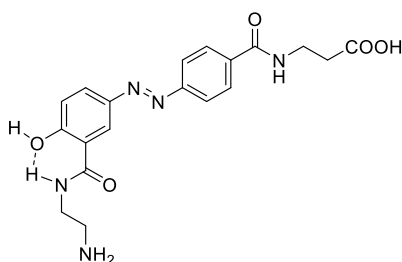


Figure 22: Possible intramolecular hydrogen bond formation of compound **CG_190** in $\text{DMSO-}d_6$ in the NMR tube.

In summary, nine analogues were synthesised with bioisosteric variations or modifications based on results from previous chapters (Figure 23).

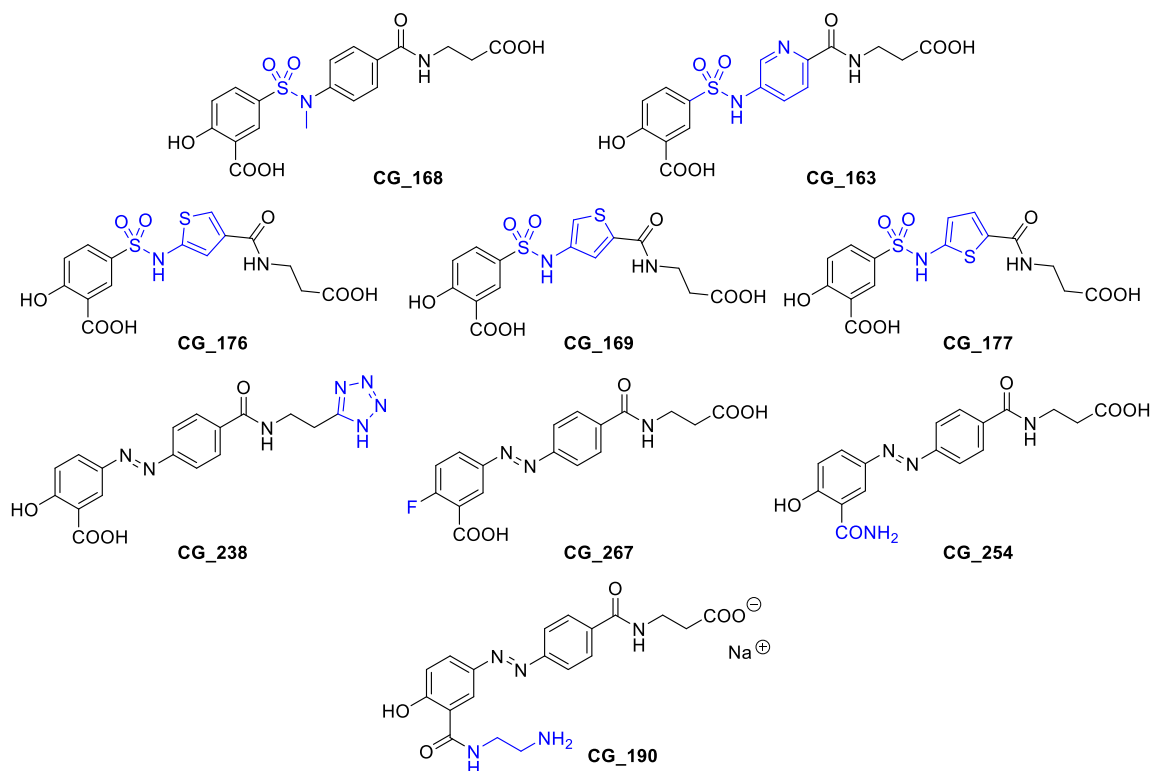


Figure 23: Synthesised analogues with bioisosteric variations or modifications based on results from previous chapters.

3.3.2. Inhibitory activity against Sirt5

The fluorescence-based enzyme assay was here again performed at 50 μM final assay concentration for all nine analogues with bioisosteric variations or modifications based on results from previous chapters and the results are shown in Figure 24 and Table 5.

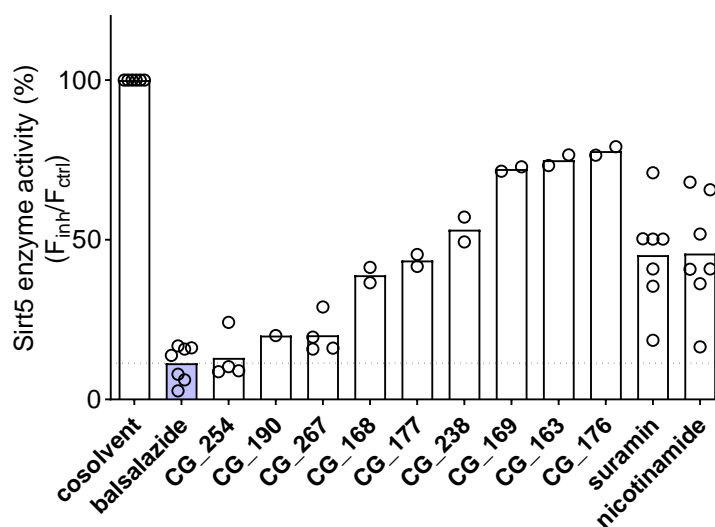


Figure 24: Residual Sirt5 enzyme activity after 1 h incubation with the inhibitors in % ($F_{\text{inh}}/F_{\text{ctrl}}$). The known non-selective inhibitors nicotinamide and suramin were included as positive controls. Fluorescence measured upon incubation with the cosolvent DMSO alone (no inhibitor) was set to 100 % enzyme activity. All inhibitors were tested at 50 μM final concentration. F = fluorescence units; $\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$; number of biological replicates stated with circles (○) for each bar.

Table 5: Sirt5 inhibition in %, including balsalazide and positive controls nicotinamide and suramin for comparison. Values represent means of technical and biological replicates.

Compound name	Inhibition in %	Compound name	Inhibition in %
balsalazide	89	CG_177	57
CG_163	25	CG_190	80
CG_168	61	CG_238	47
CG_169	28	CG_254	87
CG_176	22	CG_267	80
nicotinamide	54	suramin	55

Overall, with these analogues the Sirt5 inhibitory activity could not be increased. First of all, compound **CG_168**, bearing an *N*-methylated sulfonamide, was prepared to investigate, whether the hydrogen bond donating ability was important for high affinity. And indeed, Sirt5 inhibition dropped from 75 % for **CG_137** to 61 % for **CG_168** at 50 μ M. Computational chemistry calculations gave further insight into this observation (see Chapter 4.3.1). Furthermore, replacement of the non-salicylate phenyl ring by a pyridine ring (**CG_163**) decreased potency significantly to only 25 %. This was in the same range as inhibitors **CG_169** and **CG_176** with a thiophene instead of the phenyl ring. These exhibited only 28 % and 22 % Sirt5 inhibition, respectively. These results are in alignment with the results obtained for analogues with modifications in the β -alanine derived side chain (see Chapter 3.1.2). Only thiophene **CG_177** showed moderately good inhibition with 57 % at 50 μ M. In conclusion, these analogues clearly showed that changes on the non-salicylate phenyl ring are also not tolerated.

Bioisosteric compounds **CG_238**, **CG_254**, and **CG_267** verified the hypotheses presented in Chapter 3.1. Compared to balsalazide (89 %), tetrazole **CG_238** showed, with a drop in inhibition by ca. 40 % to only 47 % Sirt5 inhibition that the terminal carboxylic acid has to stay unchanged and cannot be replaced by classical bioisosteric functional groups. The hypothesis that the salicylic acid part of the molecule can to some extent be modified was also verified by fluoro analogue **CG_267** and primary salicylamide **CG_254** with 80 % and 87 % inhibition at 50 μ M. Nevertheless, the results showed that these functional groups could be changed but should not, if not necessary. And for one molecule this was indeed necessary: coupleable aminoethyl amide analogue **CG_190**. This compound was synthesised with the goal to perform chemical proteomics experiments. It was then once tested to see, whether the newly incorporated linker moiety negatively influences the affinity to Sirt5. The measurement showed that it does not or only minimally influence the inhibitory activity of the lead structure

balsalazide. With 80 % Sirt5 inhibition at 50 μM , **CG_190** stayed in the range of balsalazide and could be used for further experiments.

3.4. Variations of the core azo group (2)

With the theories verified that changes on the β -alanine derived side chain as well as on the salicylic acid moiety mostly negatively influence the inhibitory activity against Sirt5, it was decided to explore modifications once again on the azo group. Already the first set (1) of analogues with open-chained and rigidised spacers (see Chapter 3.2) brought with sulfonamide **CG_137** forth one inhibitor with a more drug like spacer unit and with good potential for further improvements. The following chapter focuses on all variants where the central core azo unit was replaced by heteroaromatic spacers as depicted in Figure 25. With these disubstituted 5-membered heteroaromatic rings, the angle between the salicylate unit and the benzamide part is changed and the resulting analogues are more rigidised since they are hetero-triaryls. Heteroaromatic spacers have proved successful drug-like spacers in previous and ongoing projects in the group of PROF. DR. FRANZ BRACHER.

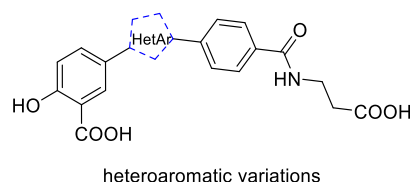


Figure 25: General structure of the analogues with a heteroaromatic replacement of the azo spacer.

3.4.1. Synthesis

3.4.1.1. Analogues with heteroaromatic variations of the azo moiety

Nowadays there is a considerable number of synthetic procedures known for the synthesis of the desired heterocyclic spacers, especially for disubstituted 5-membered heteroarenes. One famous approach is the concept of a HUISGEN 1,3-dipolar cycloaddition as exemplarily depicted with the retrosynthesis of an isoxazole in Figure 26. For this example, a terminal alkyne and a usually *in situ* prepared nitrile oxide are used to form the desired isoxazole in a 1,3-dipolar cycloaddition.

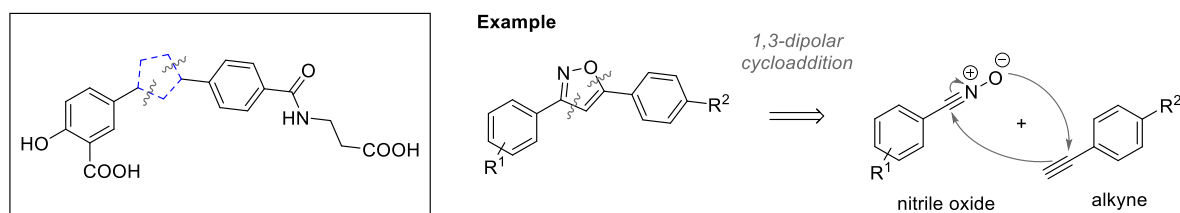
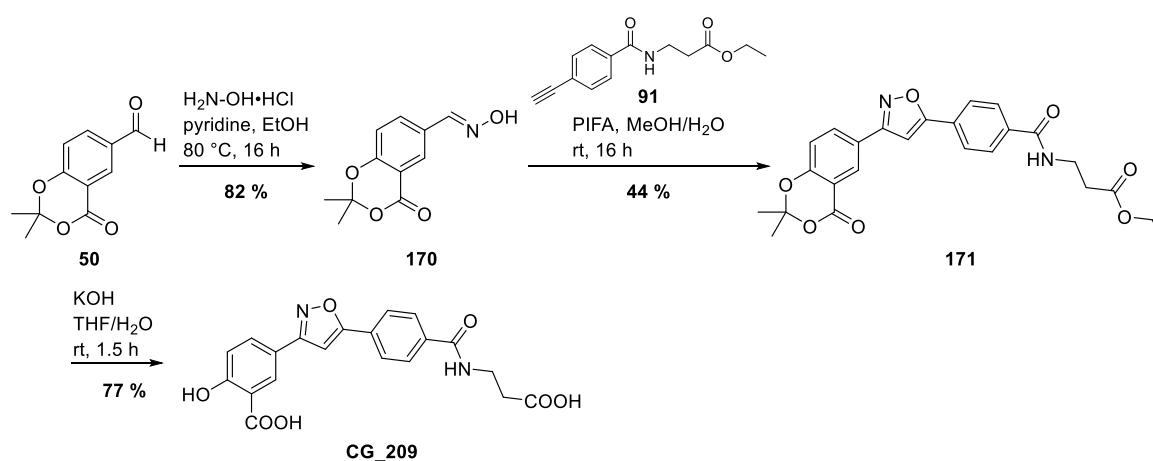


Figure 26: Retrosynthesis of 5-membered heterocycles *via* 1,3-dipolar cycloaddition exemplified with a 3,5-diaryl isoxazole.

The first targeted heteroaromatic analogue with an isoxazole as the spacer replacement was indeed successfully synthesised following the 1,3-dipolar cycloaddition (Scheme 79). For this purpose, previously prepared aldehyde **50** (Scheme 21) was converted into the corresponding aldoxime **170** (82 % yield) following its preparation by PAUDYAL *et al.*^[142] The construction of the isoxazole moiety with previously prepared terminal alkyne **91** (Scheme 39) was performed applying a procedure from JAWALEKAR *et al.*^[159] Compared to the literature procedure with different precursors, in this case stirring of the reaction mixture had to be continued overnight after adding the last portion of phenyliodine bis(trifluoroacetate) (also known as PIFA – a hypervalent iodine compound used as an oxidising agent for converting the aldoxime into a nitrile oxide *in situ*). Intermediate **171** was then obtained after FCC in a moderate yield of 44 %. Deprotection generated the target isoxazole **CG_209** in 77 % yield.



Scheme 79: Preparation of isoxazole **CG_209**.

The next variation contained one more heteroatom than isoxazole, whereby all of them were nitrogens: 1,2,3-triazole **CG_220** (Scheme 80). As one of the most commonly known examples for the aforementioned 1,3-dipolar cycloaddition, triazoles are prepared from an alkyne and an organoazide precursor. Therefore, in the first step, azide **172** was synthesised from previously prepared primary amine **102** (Scheme 44) following a procedure published by YOON *et al.*^[160] using hydrochloric acid, sodium nitrite and sodium azide. In this publication, the authors intended to perform click chemistry as well to prepare analogues of *i.a.* lavendustin C, a structure very similar to core elements of balsalazide as depicted in Figure 27. They evaluated these small molecules against HCT116 colon cancer and CCRF-CEM leukemia cell lines.

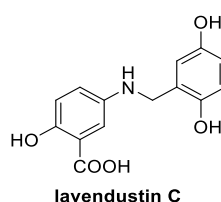
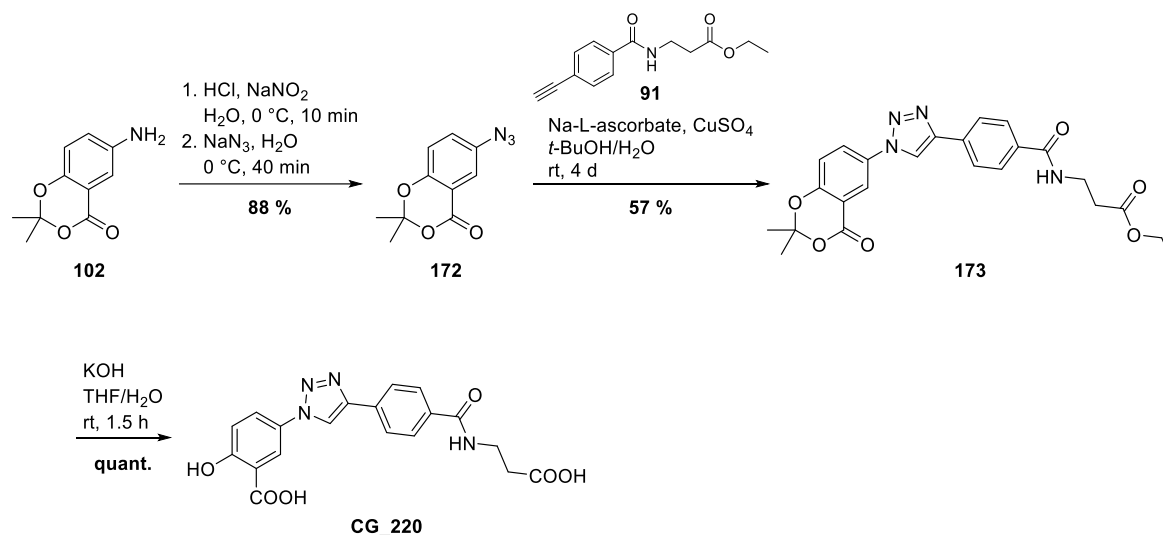


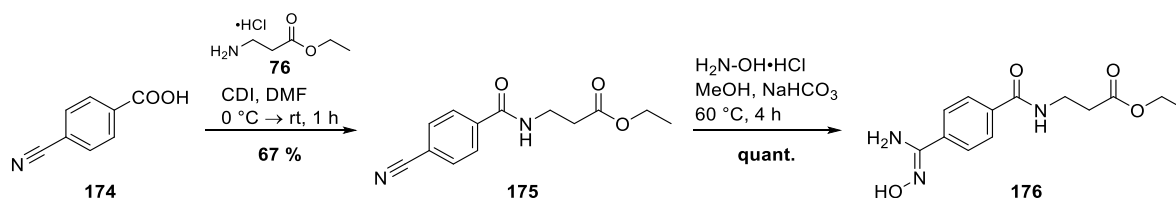
Figure 27: Structural formula of lavendustin C.

During their studies, the authors could show that the carboxylic acid of the salicylic acid moiety had to be protected for a successful click reaction. But the phenolic hydroxy group apparently does not influence the reaction as their azide only consisted of a methyl ester and not the acetonide protected version. Azide **172** was obtained in a good yield of 88 %. Subsequent cycloaddition could then be performed according to a general procedure by YOON *et al.*^[160] again. The reaction between azide **172** and alkyne **91** in the presence of a catalytic amount of copper sulfate and the mild reducing agent sodium ascorbate afforded pure 1,4-disubstituted regioisomer **173** after FCC in 57 % yield. Deprotection with KOH in THF/water gave the desired triazole **CG_220** in quantitative yield.



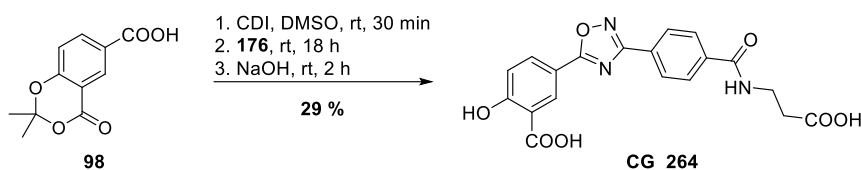
Scheme 80: Preparation of triazole **CG_220**.

One more analogue of balsalazide with three heteroatoms in the spacer, a 1,2,4-oxadiazole, was then synthesised. This time, the amidoxime route was chosen instead of the 1,3-dipolar cycloaddition. The first desired precursor (**176**) towards oxadiazole **CG_264** should carry the *N*-aroyl- β -alanine derived side chain as well as the amidoxime moiety. Starting from 4-cyanobenzoic acid (**174**), the side chain was here constructed in an amide coupling using β -alanine ethyl ester hydrochloride (**76**) and carbonyldiimidazole (CDI) as the coupling reagent, which will also be used in the formation of the 1,2,4-oxadiazole itself. CDI is known to react fast and generate two equivalents of imidazole *in situ*, wherefore no additional base is required. After stirring for 1 h, the desired product **175** was obtained without the need of further purification in 67 % yield. Applying a procedure described by CANESCHI *et al.*,^[161] the reaction of nitrile **175** with hydroxylamine hydrochloride in MeOH in the presence of sodium bicarbonate afforded amidoxime **176** in quantitative yield (Scheme 81).



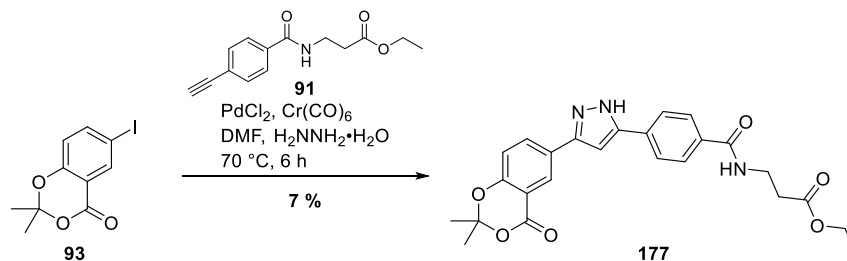
Scheme 81: Preparation of amidoxime **176**.

The preparation of 1,2,4-oxadiazole **CG_264** was then performed following a general procedure published by SHARONOVA *et al.*^[162] (Scheme 82). This one-pot procedure consisted of the activation of the previously prepared carboxylic acid **98** (Scheme 42) with CDI, followed by reaction with amidoxime **176** to form an intermediate *O*-acylamidoxime, and subsequent cyclodehydration with sodium hydroxide. The last step with NaOH had in the present case another advantage of hydrolysing the protective groups as well, whereby the desired 1,2,4-oxadiazole **CG_264** was obtained after FCC without the need of an additional deprotection step in 29 % yield.



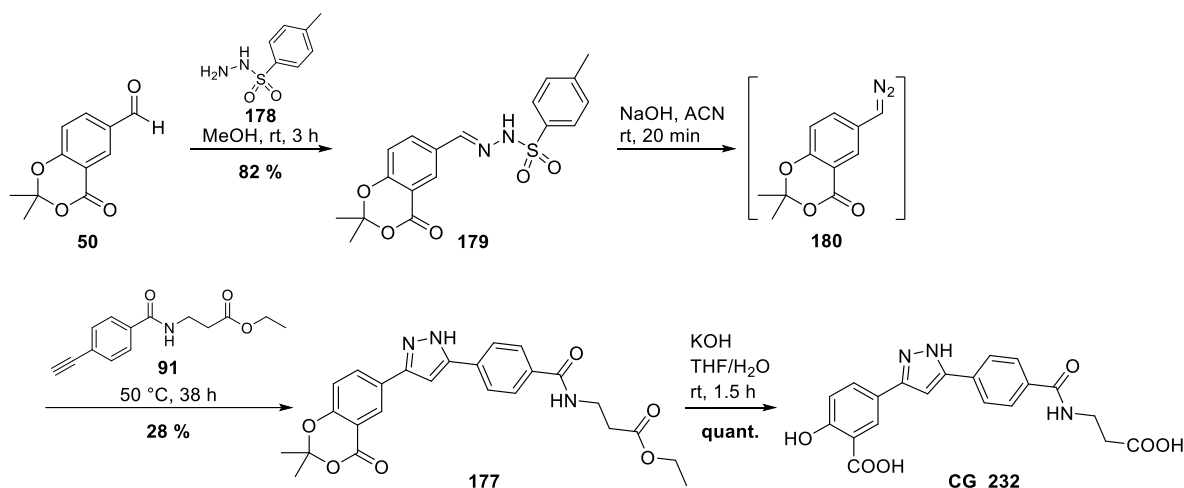
Scheme 82: Preparation of 1,2,4-oxadiazole **CG_264**.

The heteroaromatic spacers of the above mentioned three analogues consisted solely of hydrogen bond acceptors. Therefore, one more heterocyclic analogue was designed consisting of a pyrazole unit, which has the ability of accepting as well as donating hydrogen bonds. There are several synthetic procedures described in literature for the construction of 3,5-diarylpyrazoles, and the first attempt for the synthesis of pyrazole **177** consisted of a “*palladium catalyzed one-pot four component carbonylation reaction*” published by IRANPOOR *et al.*^[163] Here, aryl iodide **93** (Scheme 40) was reacted with terminal alkyne **91** in the presence of chromium hexacarbonyl (as substituent for gaseous carbon monoxide) and aqueous hydrazine solution. Unfortunately, pyrazole **177** was obtained in only 7 % yield (Scheme 83).



Scheme 83: Preparation of pyrazole **177** using PdCl₂ and Cr(CO)₆.

Therefore, another strategy was again pursued: 1,3-dipolar cycloaddition, here utilising a diazo compound as the 1,3-dipole. For this purpose, tosylhydrazone **179** was prepared according to a typical procedure published by WANG *et al.*^[164] from previously prepared aldehyde **50** (Scheme 21) and *p*-toluenesulfonyl hydrazide (**178**) in MeOH. The product (**179**) was obtained in 82 % yield. The following cycloaddition to give the pyrazole was then performed following a procedure derived from an originally one-pot method published by AGGARWAL *et al.*^[165] with alkyne **91** in the presence of NaOH. In the first attempt, following the literature very closely, **177** was isolated in only 7 % yield again. Hence, the procedure was slightly modified. In detail, in the presence of NaOH, first 0.5 equivalents of **179** were converted into the corresponding diazo compound within 20 min at room temperature. After this time, alkyne **91** was added and the reaction mixture stirred at 50 °C for 6 h. Then again 0.5 equivalents of the diazo compound were prepared from **179** with NaOH in MeCN and added to the reaction mixture. After 16 h, this procedure was repeated once more to add in total 1.5 equivalents of the tosylhydrazone **179**. The reaction was then again stirred for 16 h at 50 °C (38 h in total). Pyrazole **177** was obtained after FCC in an increased yield of 28 %. A final alkaline deprotection gave the desired product **CG_232** in quantitative yield (Scheme 84).



Scheme 84: Preparation of pyrazole **CG_232**.

In summary, further four analogues with a heteroaromatic replacement of the core azo unit were designed and synthesised (Figure 28).

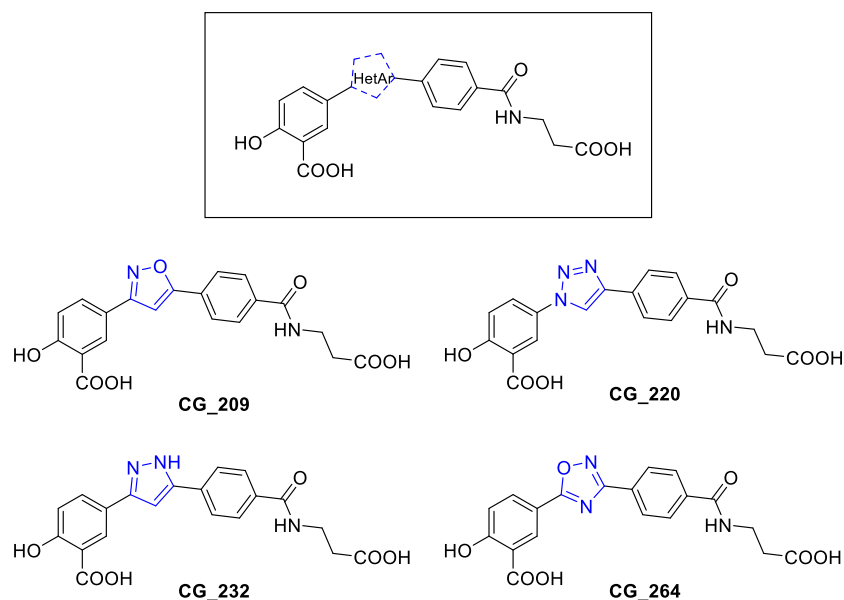


Figure 28: Synthesised analogues bearing a heteroaromatic modification of the central azo unit of balsalazide.

3.4.2. Inhibitory activity against Sirt5

With this last set of analogues of balsalazide, which were synthesised during this thesis, their inhibitory activity against Sirt5 was again tested using the fluorescence-based enzyme assay kit. The determination was performed in a single-point measurement at 50 μM final assay concentration and the results are shown in Figure 29 and Table 6, including balsalazide as well as nicotinamide and suramin as positive controls.

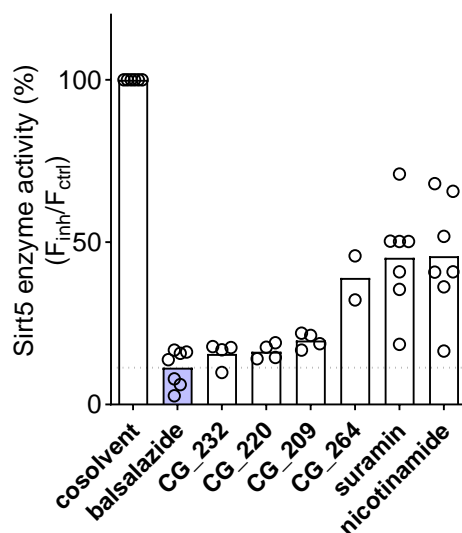


Figure 29: Residual Sirt5 enzyme activity after 1 h incubation with the inhibitors in % ($F_{\text{inh}}/F_{\text{ctrl}}$). The known non-selective inhibitors nicotinamide and suramin were included as positive controls. Fluorescence measured upon incubation with the cosolvent DMSO alone (no inhibitor) was set to 100 % enzyme activity. All inhibitors were tested at 50 μM final concentration. F = fluorescence units; λ_{ex} = 360 nm, λ_{em} = 460 nm; number of biological replicates stated with circles (○) for each bar.

Table 6: Sirt5 inhibition in %, including balsalazide and positive controls nicotinamide and suramin for comparison. Values represent means of technical and biological replicates.

Compound name	Inhibition in %
balsalazide	89
CG_209	80
CG_220	84
CG_232	84
CG_264	61
nicotinamide	54
suramin	55

These four analogues proved most promising with only one of them (1,2,4-oxadiazole **CG_264**) below 80 % Sirt5 inhibition at 50 μM . The other three compounds being isoxazole **CG_209**, triazole **CG_220**, and pyrazole **CG_232** resulted in very good inhibition values of 80 %, 84 %, and 84 %. Hence, these analogues were finally in the range of balsalazide (89 %). Therefore, with heteroaromatic variations it was after all possible to replace the core azo unit without losing affinity for Sirt5. The successful choosing of e.g. triazoles as spacer replacements was already published in studies concerning resveratrol analogues by PAGLIAI *et al.*^[166] and previously mentioned lavendustin analogues by YOON *et al.*^[160] Furthermore, it was investigated in the context of histone deacetylase inhibitors by PIRALI *et al.*^[167] and CHEN *et al.*^[168] For these three best analogues (**CG_209**, **CG_220** and **CG_232**) an IC_{50} could now be determined and further experiments could be performed to gain more insight into the physiological role of Sirt5.

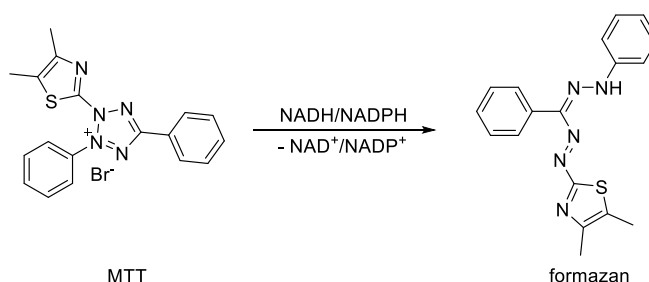
4. Further experiments and in-depth characterisation of selected compounds

This chapter focuses on additional experiments that were carried out for selected synthesised compounds of the previous chapters. Here, not only further studies regarding their inhibitory activity as well as subtype selectivity among sirtuins will be discussed but also newly developed computational methods that can pave the way for an easier design of future analogues and derivatives. Also, routine testing for cytotoxicity and antimicrobial effects will be included and chemical proteomics experiments to identify the inhibitors' target proteins in cell lysates.

4.1. Routine testing for cytotoxicity and antimicrobial effects

4.1.1. MTT assay

Before future experiments in cell culture can be performed (e.g. live cell imaging), the cytotoxic activities of the compounds had to be evaluated. Since this MTT assay belongs to the routine testing in the group of PROF. DR. FRANZ BRACHER, not only selected potent Sirt5 inhibitors were tested but all final compounds. The MTT assay was performed by MARTINA STADLER according to a method of MOSMANN^[169] using HL-60 cells. Triton X-100 was included as a positive control (for a detailed procedure see Chapter 6.1.1). This assay method is based on the reduction of the soluble yellow coloured tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) to the insoluble blue coloured formazan as depicted in Scheme 85.



Scheme 85: Reduction of MTT to a formazan.

This reduction occurs solely in metabolically active cells in the presence of the reducing agents NADH and NADPH, respectively. The amount of generated formazan is measured photometrically and the extent of absorbance then correlates with cell viability. This way, an IC_{50} value can be derived that shows the cytotoxic activity of the compounds. The underlying mode of action of the corresponding compound, which leads to cell death, cannot be determined with this setup.

The MTT assay revealed that none of the final compounds showed cytotoxic effects (all IC_{50} values $>50 \mu\text{M}$). This information is important for future cell culture experiments. It has to be noted that at this point, it is not clear if the lack of cytotoxicity was due to insufficient cell penetration e.g. due to high polarity of the free carboxylates. If this was the case, further optimisations of the inhibitors could increase cell permeability, for example by applying prodrug strategies.

4.1.2. Agar diffusion assay

Another routine assay is the agar diffusion assay, which was also performed by MARTINA STADLER with all final compounds prepared during this thesis. This qualitative assay should lead to the conclusion which compounds inhibit the growth of microorganisms. For potentially

orally available drugs it is desired to have no such effects to avoid the risk of damaging the intestinal flora and cause digestion problems.

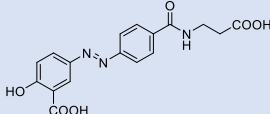
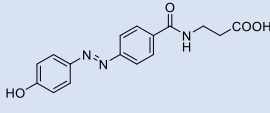
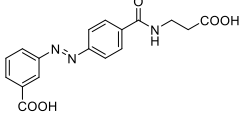
The antimicrobial effect of the test compounds was analysed on various model germs growing on medium containing agar (for details see Chapter 6.1.2). If a compound disturbs the growth of selected germs it would result in inhibition zones that can be measured. A quantitative assessment of the antimicrobial effect cannot be made since the diameters of the inhibition zones are also dependent on the diffusion properties of every single compound on the aqueous medium. As positive controls, clotrimazole was included for the antimycotic effect and tetracycline hydrochloride was used to see an antibacterial effect.

None of the final compounds showed noteworthy antimicrobial effects in this routine testing.

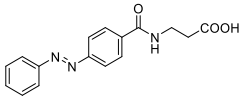
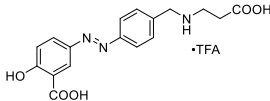
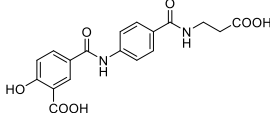
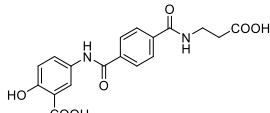
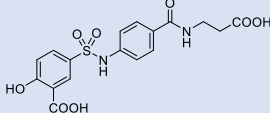
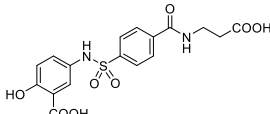
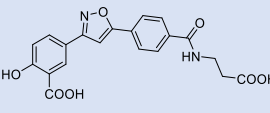
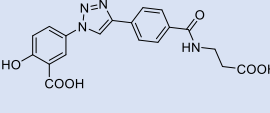
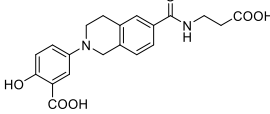
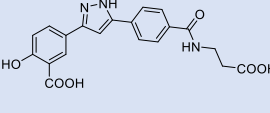
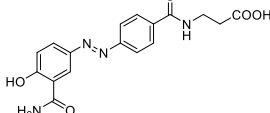
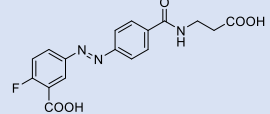
4.2. Investigation of the subtype selectivity over Sirt1, 2, and 3

With the promising results of the inhibition of Sirt5 at 50 μM (see Chapters 3.1.2, 3.2.2, 3.3.2, 3.4.2), the subtype selectivity was then also determined for selected compounds as shown in Table 7. This assay was performed by NATHALIE WÖSSNER in the group of PROF. DR. MANFRED JUNG (Albert-Ludwigs-University Freiburg). All selected inhibitors were tested at 50 μM final assay concentration against class I sirtuins Sirt1, 2, and 3 using an acetylated synthetic substrate,^[63] including nicotinamide and suramin as positive controls. For this assay molecules were chosen that showed high potency against Sirt5 (e.g. **CG_21**, **CG_137** and **CG_220**), but also a negative control with low Sirt5 inhibitory activity (**CG_48**) as well as compounds that were of special interest like inverted, inactive sulfonamide **CG_140** compared to the potent sulfonamide **CG_137**.

Table 7: Inhibition in % of selected small molecules including positive controls nicotinamide and suramin against Sirt1, 2, and 3. *n.i. = inhibition <10 %.

Compound name	Structural formula	Inhibition in % at 50 μM		
		Sirt1	Sirt2	Sirt3
balsalazide		n.i.*	11	n.i.
CG_21		n.i.	26	16
CG_24		n.i.	21	n.i.

Further experiments and in-depth characterisation of selected compounds

CG_25		n.i.	16	n.i.
CG_48		n.i.	19	11
CG_128		n.i.	10	n.i.
CG_133		n.i.	16	n.i.
CG_137		n.i.	14	n.i.
CG_140		n.i.	17	n.i.
CG 209		n.i.	n.i.	n.i.
CG 220		n.i.	15	n.i.
CG 224		14	16	n.i.
CG 232		n.i.	n.i.	n.i.
CG 254		27	41	29
CG 267		n.i.	13	n.i.
nicotinamide	see Chapter 1.4	21	65	48
suramin	see Chapter 1.4	95	72	10

In summary, the obtained percentual inhibition values for Sirt1, 2, and 3 showed all in all very good subtype selectivity for the selected compounds. Highlighted in blue in Table 7 are inhibitors with good stability, high potency against Sirt5 and pronounced subtype selectivity over Sirt1, 2, and 3.

In more detail, in this experimental setup balsalazide showed no inhibition of Sirt1 and Sirt3, and only marginal (11 %) Sirt2 inhibition was observed at 50 μM . Therefore the lead structure balsalazide is indeed a subtype-selective inhibitor of Sirt5.^[40] Amine **CG_48**, which was chosen as a negative control (with only 27 % Sirt5 inhibition at 50 μM), was even less potent on Sirt1, 2, and 3. For the inhibitors **CG_24** and **CG_25**, which lack the phenolic hydroxy group, no inhibition of Sirt1 and Sirt3 was observed. And in addition, with 21 % and 16 % inhibition at 50 μM , **CG_24** and **CG_25** had very low inhibitory activity against Sirt2. Phenol **CG_21** was slightly less subtype-selective and showed 16 % Sirt3 inhibition and even 26 % Sirt2 inhibition.^[40]

Interesting results were obtained regarding the two bioisosteres primary salicylamide **CG_254** and fluoro compound **CG_267**, both with good inhibitory activity against Sirt5 (87 % and 80 %, respectively). While for **CG_267** (in which the phenolic OH is replaced by F) the results were similar to balsalazide with no inhibition of Sirt1 and 3 and only 13 % inhibition of Sirt2, carboxamide **CG_267** lost selectivity to a significant extent. For Sirt1 and 3 were observed 27 % and 29 % inhibition, respectively, and against Sirt2 amide **CG_267** was even more potent with 41 % inhibition at 50 μM . This leads to the conclusion, that with an amide instead of a negatively charged carboxylate, hydrogen bond donors are introduced that not only reduce activity against Sirt5 but also substantially decrease subtype selectivity.

Overall, the analogues with changes on the salicylate unit of the molecule (**CG_21**, **CG_24**, **CG_25**, **CG_254**, and **CG_267**) once again show that these changes result either in reduced Sirt5 inhibitory activity or subtype selectivity or even both. In particular when the hydroxy group is still in place, but the carboxylic acid of the salicylate is missing (phenol **CG_21**) or replaced (primary amide **CG_267**) a decreased subtype selectivity was observed.

Within the set of open chained variations of the core azo group (amides **CG_128** and **CG_133**, and sulfonamides **CG_137** and **CG_140**) the results showed overall very good subtype selectivity. No inhibition of Sirt1 and 2 was observed for all of them. For Sirt2 the best results were obtained with amide **CG_128** with only 10 % inhibition at 50 μM . **CG_133**, **CG_137** and **CG_140** experienced only little potency against Sirt2 (14 – 17 %). This was also the case for rigidised analogue **CG_224**, which showed 16 % Sirt2 inhibition. In contrast, even though this inhibitor did also not inhibit Sirt3, compared to all previous compounds it showed minor inhibitory activity against subtype Sirt1 with 14 % inhibition at 50 μM .

The most promising results from first screenings against Sirt5 were obtained with heteroaromatic spacer variants **CG_209**, **CG_220** and **CG_232** (>80 % Sirt5 inhibition at 50 μM). In this subtype selectivity assay these compounds showed similar if not better selectivity than the lead structure balsalazide itself. Triazole **CG_220** did not inhibit Sirt1 and 3 and showed only 15 % Sirt2 inhibition. Isoxazole **CG_220** and pyrazole **CG_232** did not inhibit any of the tested subtypes at 50 μM .

In summary, the results received here undermined the findings of sulfonamide **CG_137** and heteroaromatic analogues **CG_209**, **CG_220**, **CG_232** being excellent optimised inhibitors. With these modifications of the core azo group the spacer was finally replaced by drug-like functional groups that retained the essential subtype selectivity.

4.1. Determination of IC_{50} values for Sirt5 inhibitory activity

First, the inhibitory activity of balsalazide against Sirt5 was quantitatively compared with the IC_{50} reported by GUETSCHOW *et al.*^[73] Therefore, its IC_{50} value was determined in the fluorometric assay system that was used before for the single-concentration measurements at 50 μM . The resulting inhibition curve is illustrated in Figure 30. In this first measurement, it was concluded that balsalazide indeed is a potent inhibitor of Sirt5 with an IC_{50} of 5.3 μM in this assay system.^[40]

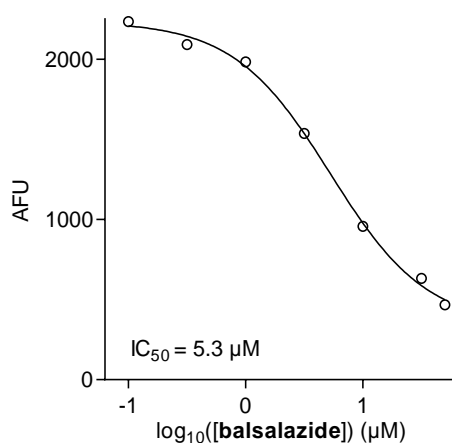
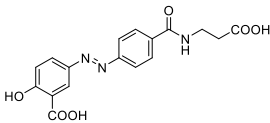
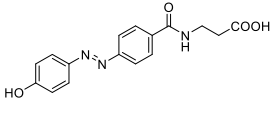
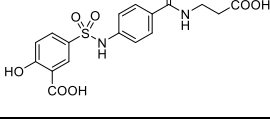
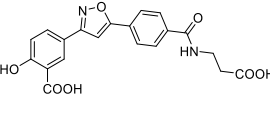
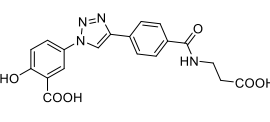
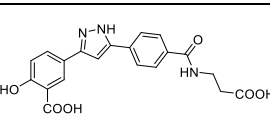
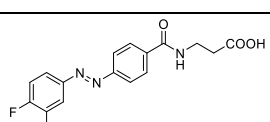


Figure 30: Inhibition of Sirt5 desuccinylase activity by balsalazide determined using the FLUOR DE LYS® SIRT5 fluorometric drug discovery assay kit depicted as dose-response curve with the corresponding IC_{50} value. AFU = absolute fluorescence units.^[40]

In addition to balsalazide, compounds that showed high inhibitory activity against Sirt5 at 50 μM in previous tests were then selected for IC_{50} measurements as well. This should show in more detail whether these inhibitors have similar potency like balsalazide or if they even represent the next generation of drug-like Sirt5 inhibitors. Selected compounds are shown in Table 8.

Table 8: Selected inhibitors for IC₅₀ screening.

Inhibitor name	Structural formula	Inhibition of Sirt5 in % at 50 μ M
balsalazide		89
CG_21		76
CG_137		75
CG_209		80
CG_220		84
CG_232		84
CG_267		80

Even though primary salicylamide **CG_254** also showed high inhibitory activity against Sirt5 with 86 % inhibition at 50 μ M, this compound was not included in these further studies due to its lack in subtype selectivity.

First, the IC₅₀ values of azobenzenes balsalazide, **CG_21**, and **CG_267** were determined and compared to each other. The inhibition at the following final assay concentrations was measured: 100 μ M, 50 μ M, 30 μ M, 10 μ M, 3 μ M, 1 μ M, 0.3 μ M, and 0.1 μ M. The dose-response curves are shown in Figure 31a. The derived IC₅₀ values of phenol **CG_21** and fluoro compound **CG_267** were with 31.5 μ M and 23.6 μ M clearly worse than the one of balsalazide. Unfortunately, this time the IC₅₀ value of balsalazide itself was also only 13.8 μ M (compared to the first measurement with 5.3 μ M shown in Figure 30). This could be due to a different batch of enzyme or any other of the provided substances in the commercial kit. For a first assessment in a purely enzyme-based assay system the values are still in a good range. When taking a

closer look at the obtained curves it can be observed that the curve did not reach a plateau when high concentrations ($>30 \mu\text{M}$) were used. This was further investigated by measuring the IC_{50} value again and including self-absorption tests for balsalazide at every single concentration. The results showed that there was a concentration dependent absorption of the light by balsalazide and presumably this is the case for all azobenzenes leading to slightly falsified values. When the values for self-absorption were added, the curve clearly ended up forming a plateau (Figure 31b). By this adjustment, the obtained IC_{50} value slightly improved to $9.5 \mu\text{M}$.

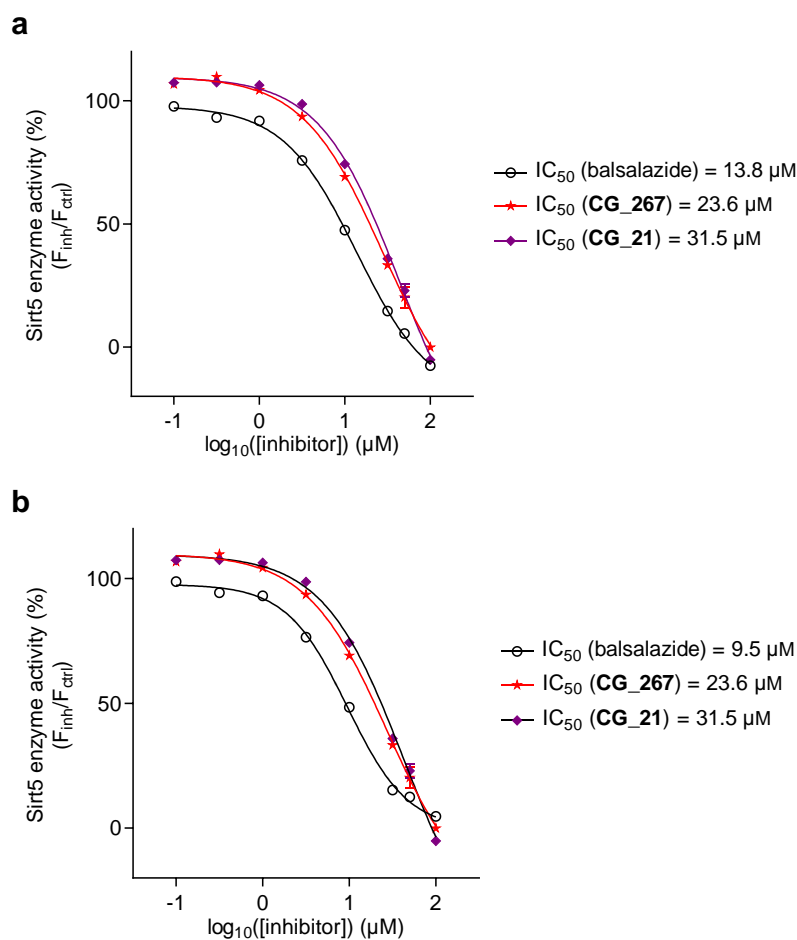


Figure 31: Inhibition of Sirt5 desuccinylase activity by balsalazide, **CG_267** and **CG_21** determined using the FLUOR DE LYS® SIRT5 fluorometric drug discovery assay kit resulting in residual Sirt5 enzyme activity after 1 h incubation with the inhibitors in % ($F_{\text{inh}}/F_{\text{ctrl}}$). **a)** Dose-response curves and corresponding IC_{50} values without including self-absorption and **b)** dose-response curves and corresponding IC_{50} values including self-absorption for balsalazide at all measured concentrations. F = fluorescence units; $\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$.

From these observations it was concluded that one can compare similar azobenzenes among each other at $50 \mu\text{M}$ final assay concentration. When compounds with no self-absorption (compounds where the azo group was replaced) are included in the assay, they can still be compared in a first single-concentration measurement, but it should be noted that the inhibition

of the azobenzenes actually differs by 4 to 10 % at 50 μM . Therefore, this should either be considered or appropriate controls included for adjusting the values.

With these results, balsalazide still being the most potent inhibitor within the azobenzenes, determination of IC_{50} values against Sirt5 of the newly developed and synthesised analogues of balsalazide with a modified spacer was performed. The dose-response curves are depicted in Figure 32. The results showed that the modifications of the central azo motif not only resulted in supposedly optimised metabolic stability but also led to potent Sirt5 small molecule inhibitors with IC_{50} values between 7 and 13 μM . Among these, sulfonamide **CG_137** and isoxazole **CG_209** had the weakest potency against Sirt5 with IC_{50} values of 12.5 μM and 11.5 μM , respectively. In contrast, with 7.2 μM and 8.5 μM triazole **CG_220** and pyrazole **CG_232** showed even better inhibitory activity against Sirt5 than the lead structure balsalazide in this direct comparison.

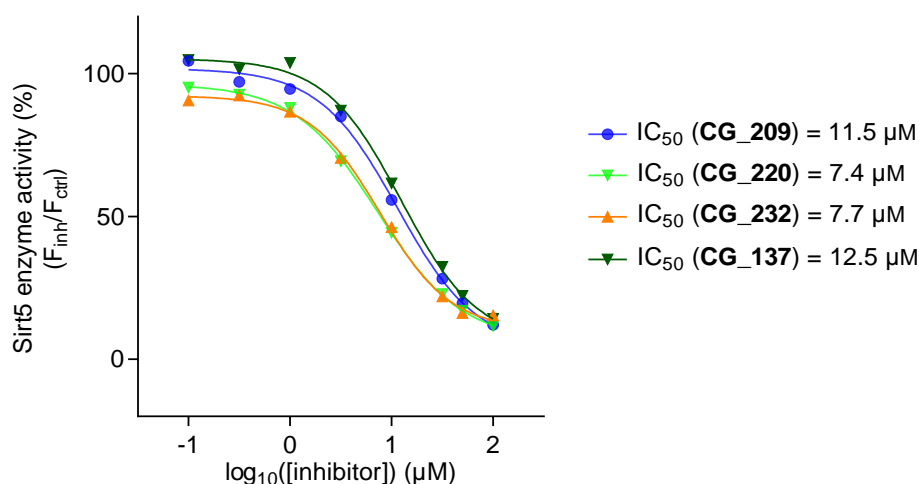


Figure 32: Inhibition of Sirt5 desuccinylase activity by **CG_137**, **CG_209**, **CG_220**, and **CG_232** determined using the FLUOR DE LYS® SIRT5 fluorometric drug discovery assay kit resulting in residual Sirt5 enzyme activity after 1 h incubation with the inhibitors in % ($F_{\text{inh}}/F_{\text{ctrl}}$). Depicted are dose-response curves and corresponding IC_{50} values for each inhibitor. F = fluorescence units; $\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$.

Overall, the synthetic and biological effort led to the finding of at least two new potential second generation Sirt5 inhibitors (**CG_220** and **CG_232**) with high potency and high subtype selectivity over Sirt1, 2, and 3.

4.2. Competition experiments

In an add-on experiment performed by NATHALIE WÖSSNER concerning the inhibition of Sirt5 by balsalazide for the publication in the *EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY*,^[40] the competition towards the cofactor NAD^+ and the synthetic substrate Z-Lys(succinyl)-7-amino-4-methylcoumarin (ZKsA) was tested in a trypsin-coupled assay as shown in Figure 33. In addition to balsalazide, also the azobenzenes phenol **CG_21** as a potent analogue and amine **CG_48** as negative control were included.

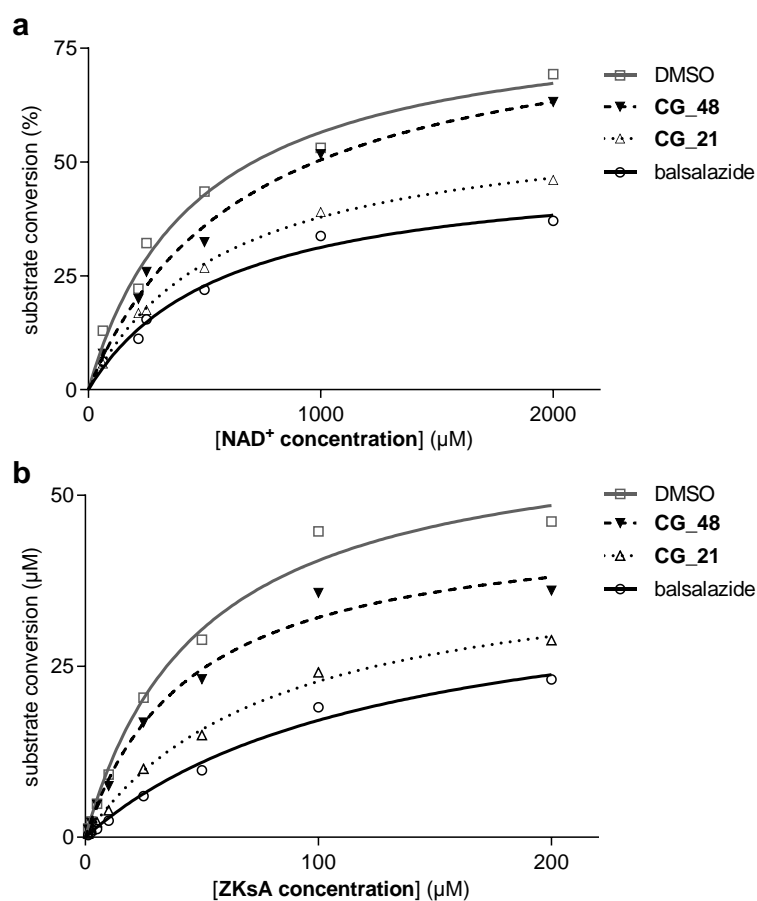


Figure 33: *In vitro* characterisation of balsalazide, phenol **CG_21**, and negative control amine **CG_48** by evaluation of the competition of the inhibitors at 50 μM for the enzyme Sirt5 with (a) increasing concentrations of NAD⁺ or (b) increasing concentrations of the synthetic substrate ZKsA.^[40]

In previous experiments **CG_48** inhibited Sirt5 only by 27 % at 50 μM and therefore the results obtained here were as expected: substrate conversion remained unchanged as compared to only DMSO instead of an inhibitor. For the potent inhibitors balsalazide and phenol **CG_21** an overall decrease in ZKsA conversion was observed – for varying NAD⁺ concentrations as well as for varying ZKsA concentrations. For competitive inhibitors the curve would converge towards the curve of the DMSO control with increasing concentrations of NAD⁺ and ZKsA, respectively. In summary, no competition between these inhibitors and NAD⁺ or ZKsA was seen. This is in accordance with the docking studies (Chapter 2) where in particular a hydrogen bond between NAD⁺ and balsalazide was visible. In general, even though it could not be displaced by the small molecule substrate ZKsA, it is possible that balsalazide does compete with larger peptide substrates, which remains to be elucidated.^[40]

4.3. Computational chemistry

This chapter focuses on calculations regarding the interaction of the herein developed inhibitors with Sirt5 that were performed by JOHANNES DIETSCHREIT and ELI NAYDENOVA in the group of PROF. DR. CHRISTIAN OCHSENFELD (Ludwig-Maximilians-University Munich).

4.3.1. Classification of the mechanism of inhibition

The first set of experiments regarding the classification of the mechanism of inhibition by selected inhibitors was carried out by JOHANNES DIETSCHREIT who investigated the reaction mechanism of Sirt5 in a previous study.^[170] The results obtained in the course of this thesis with compounds developed therein were published in the *EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY* IN 2020^[40] and will only be summarised in the following in order to give a complete overview on the collaboratively gathered evidence on Sirt5 inhibitors.

The desuccinylation reaction of Sirt5 proceeds *via* several steps as depicted in Figure 6 (Chapter 1.4) and for this simulation the first two reactions steps illustrated in Figure 34 were looked at. In this case the energy of the reaction was in the same manner computed for a succinylated substrate (Figure 34a), balsalazide itself (and the inverted amide **CG_186**; Figure 34b), and thiourea **CG_150** (Figure 34c). For computation details see Chapter 6.2.

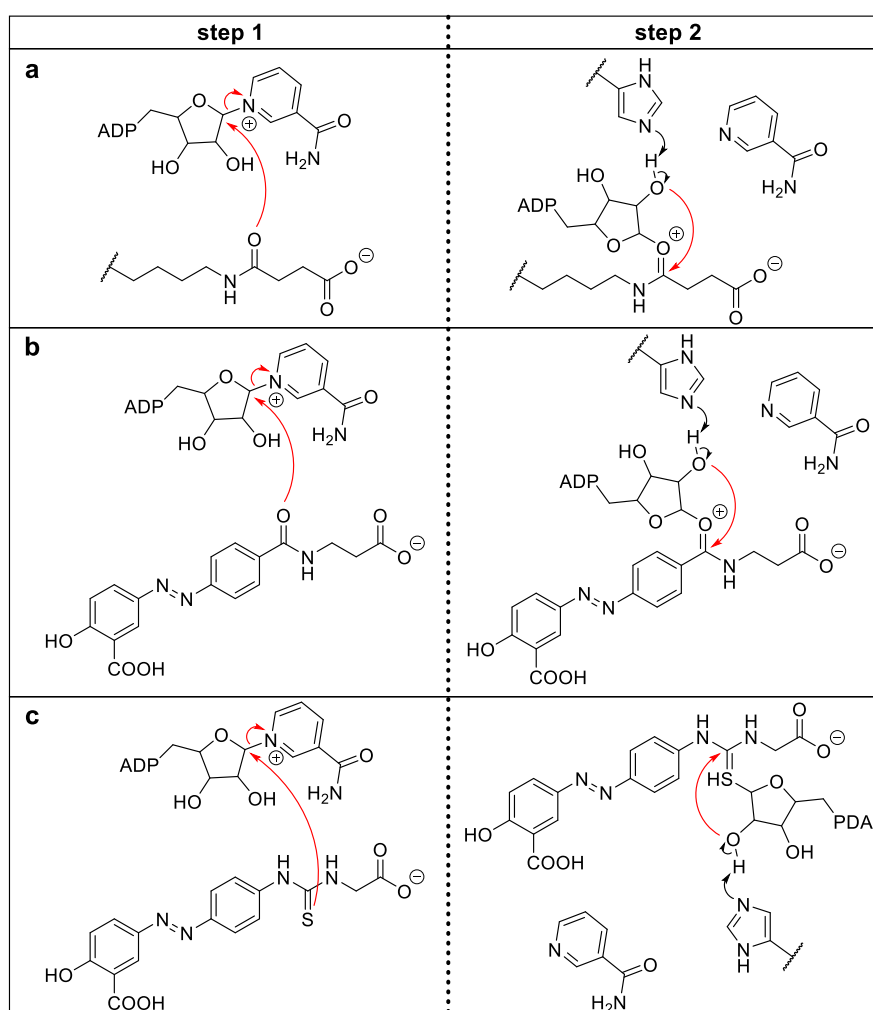


Figure 34: Shown are reaction step 1 (left column) and step 2 (right column) for the reaction catalysed by Sirt5 with the substrates **a**) *N*-succinyl lysine, **b**) balsalazide (inverted amide **CG_186** is very similar), and **c**) **CG_150** as well as His158 (the only protein residue that actively takes part in the reaction). The used reaction coordinate is highlighted in red. Other reaction arrows are not explicitly included in the reaction coordinate (cf. lit. ^[40]).

The results derived from this simulation are shown as reaction curves in Figure 35. In detail, the ϵ -*N*-succinylated peptide showed a stable intermediate after each reaction step (Figure 35a). In contrast, balsalazide did not form a stable intermediate in this simulation (c). Compared to the natural *N*-succinyl substrate, balsalazide contains an inverted amide in its *N*-aroyl- β -alanine side chain. This causes an increased distance in balsalazide between the benzamide carbonyl group and NAD⁺, which adds to the instability of the first intermediate, rendering it a non-covalent inhibitor. Compound **CG_186**, which is with the *N*-succinyl motif closer related to the natural substrates, turned out to be almost inactive in the enzyme-based *in vitro* assay (22 % Sirt5 inhibition at 50 μ M) before. In theory, **CG_186** was expected to show a lower barrier and be deacylated like the natural substrate but here the reaction curves in Figure 35c suggest that it is also an inhibitor due to its extremely high first barrier. This observation is possibly caused by the higher strain due to the bulkiness of the benzene ring as compared to the small lysine side chain of the succinylated peptide substrate. The last analogue **CG_150** carrying the postulated thiourea warhead,^[66, 93, 171] which also showed very low inhibitory activity against Sirt5 with 24 %, formed in these simulations an extremely stable product after the first reaction step (Figure 35d). This would indicate that **CG_150** acts as an irreversible inhibitor.^[40]

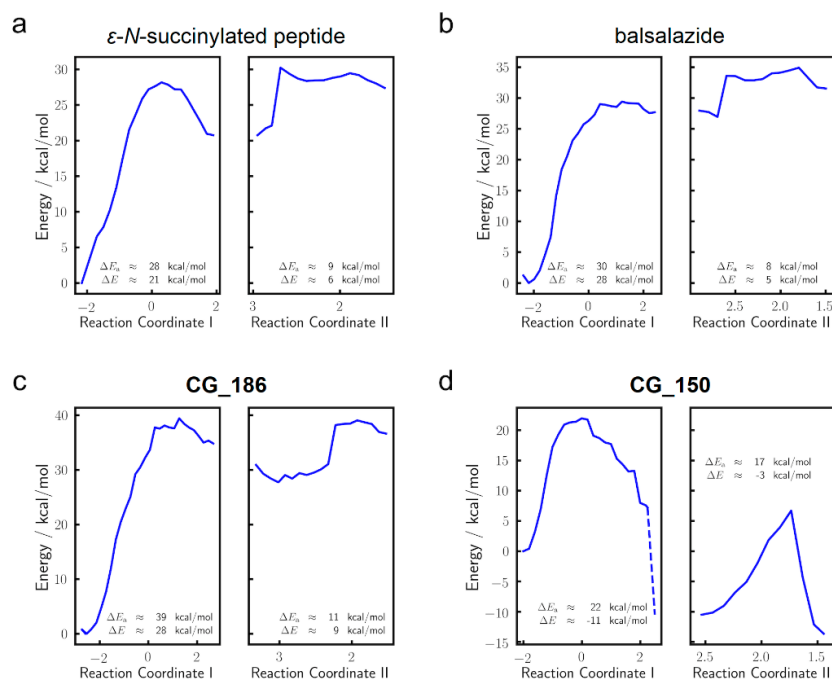


Figure 35: Reaction curves of the first two reaction steps (reaction coordinate I and reaction coordinate II correspond to the red arrows in Figure 34) of the deacylation reaction catalysed by Sirt5. The paths were using adiabatic mapping in a QM/MM setup using B3LYP-D3/def2-svp. Substrates were **a)** the model substrate, **b)** balsalazide, **c)** inverted amide **CG_186**, and **d)** thiourea **CG_150**.^[40]

In summary, comparison of the *in vitro* experimental observations to the simulation results leads to the conclusion that the mode of inhibition or the amount by which the activation barrier

is raised is not decisive for the compound's inhibitory activity. The strength of inhibition is very likely rather dominated by the interaction of functional groups increasing the stability of the inhibitor-enzyme complex and not the aspect whether the inhibitor binds covalently or not.^[40]

4.3.1. Calculations of relative binding affinities for the Sirt5 inhibitors from molecular dynamics (MD) simulations

As aforementioned, the QM/MM reaction path calculations (Chapter 4.3.1) could not provide an explanation for the difference in the stability of the inhibitor-enzyme complex upon a modification of the ligand structure. A different approach was applied by ELI NAYDENOVA to calculate the relative binding free energy difference ($\Delta\Delta G$) between two structurally related inhibitors to assess the binding affinity of a ligand. Using the alchemical non-equilibrium method^[172-173] in conjunction with molecular dynamics simulations, it can be predicted, whether a modification of the lead structure would lead to an increase or a decrease in binding affinity. It is important to note here, that not all desired or synthesised analogues were suitable for the following calculations, as macrocyclisation, ring breaking or a change in the charge state are known to produce significant errors in the estimation of free energies.^[174-175]

The relative binding free energy difference ($\Delta\Delta G$) represents the difference in the binding affinity between two ligands and can be evaluated using the thermodynamic cycle depicted in Figure 36.

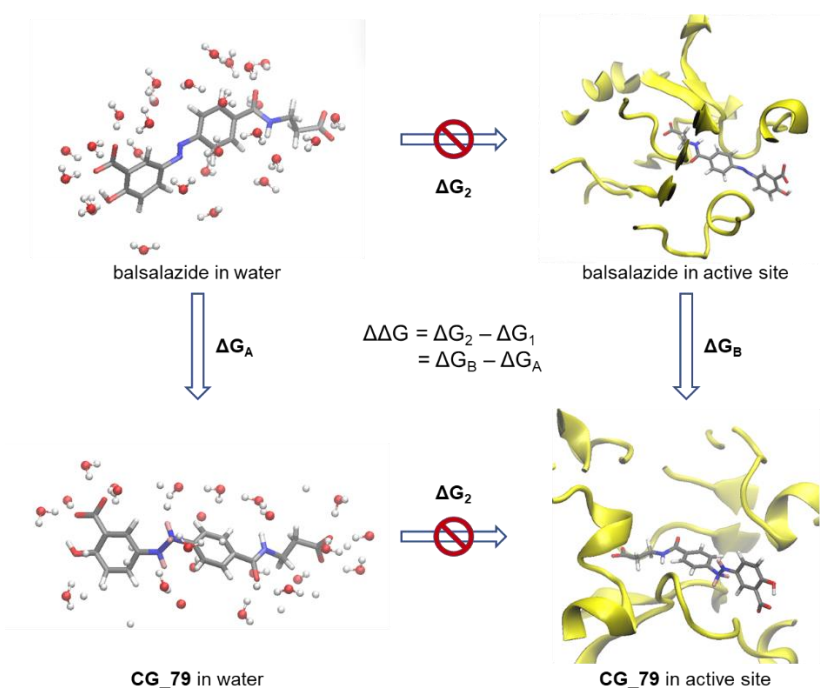


Figure 36: Thermodynamic cycle exemplified with amine **CG_79** with respect to balsalazide. A simulation along the horizontal lines would lead to convergence problems due to the large difference between the end states (water vs. protein). The paths along the vertical lines require smaller changes to the system and are thereby more accessible.

An estimation of the absolute binding affinity of e.g. balsalazide to Sirt5 (ΔG_1 in Figure 36) or **CG_79** to Sirt5 (ΔG_2 in Figure 36) is quite difficult due to the significant change in the environment and the associated convergence problems. Instead, the relative binding affinity ($\Delta\Delta G$) can be calculated by assessing the free energy difference from the transformation of balsalazide to **CG_79** in water (ΔG_A) and in the protein active site (ΔG_B). Using this approach, the relative binding affinities of the Sirt5 inhibitors to a reference compound can be computed. The lead structure balsalazide was selected as the reference ligand and $\Delta\Delta G$ was calculated with respect to this molecule for 20 of the newly synthesised compounds that were suitable for this calculation (Figure 37). A negative value for $\Delta\Delta G$ indicated a higher binding affinity to Sirt5 for the appropriate inhibitor compared to balsalazide.

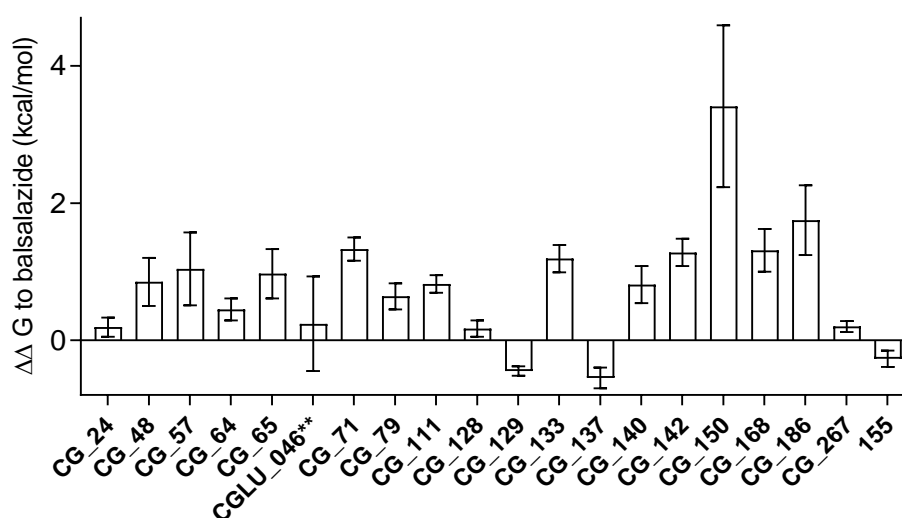


Figure 37: $\Delta\Delta G$ values for selected inhibitors respective to balsalazide in kcal/mol. Negative values signify a higher binding affinity as compared to balsalazide.

For most inhibitors that were less potent *in vitro* than balsalazide, the calculated binding affinity was also slightly lower than for balsalazide. This holds for inhibitor **CG_24**, lacking the phenolic hydroxy group, compound **CG_267**, where this group is replaced by a fluoride, and analogues with modifications in the β -alanine derived side chain: amine **CG_48**, ether **CG_57**, ketone **CG_64**, alcohol **CG_65**, and ethylene analogue **CGLU_046****. Thioamide **155** has a slightly higher binding affinity to Sirt5 than balsalazide according to the simulations, however this result could not be confirmed experimentally as this compound could not be isolated in sufficient amount. In contrast to the calculations presented in Chapter 4.3.1, the predicted binding affinity for thiourea **CG_150** corresponded to the experimental results. This indicates that this computational approach provides more insight into the inhibitory strength than the QM/MM calculations.

Balsalazide analogues with a modified spacer, imine **CG_71**, amine **CG_79**, stilbene **CG_111**, ethylene compound **CG_142**, amides **CG_133** and **CG_128**, and sulfonamide **CG_140**, were

inferior to balsalazide both *in vitro* and *in silico*. For ether **CG_129** there is a significant discrepancy between the predicted and experimental values. Even though the calculations suggested a higher binding affinity for **CG_129** compared to balsalazide, the residual enzymatic activity for this inhibitor is quite high (only 52 % inhibition at 50 μM). This inconsistency is probably due to force field errors.

Looking only at the structures, one could assume that amide **CG_128** and sulfonamide **CG_137** would show similar results. According to the predictions, the binding affinity of **CG_137** is higher and the one for **CG_128** is slightly lower than the binding affinity of balsalazide. This result was partially validated by the enzyme-based experiments since **CG_137** has a higher potency than **CG_128**. However, the simulations seem to overestimate the binding affinity for **CG_137**, as this inhibitor performed worse than balsalazide in the enzyme assay (25 % residual activity for **CG_137** vs. 11 % for balsalazide). A closer look into the structures from the MD simulations reveals that the only hydrogen bond interaction involving the azo-linker of balsalazide with the Sirt5 residues is a water-mediated contact between the backbone of Glu225 (E225) and one of the nitrogen atoms (Figure 38b). In comparison, **CG_137** forms two hydrogen bonds with its sulfonamide group to protein residues: one with the backbone hydrogen of Gly224 (G224) and one with the backbone oxygen of Glu225. The presence of these interactions could explain the strong binding affinity calculated for this compound.

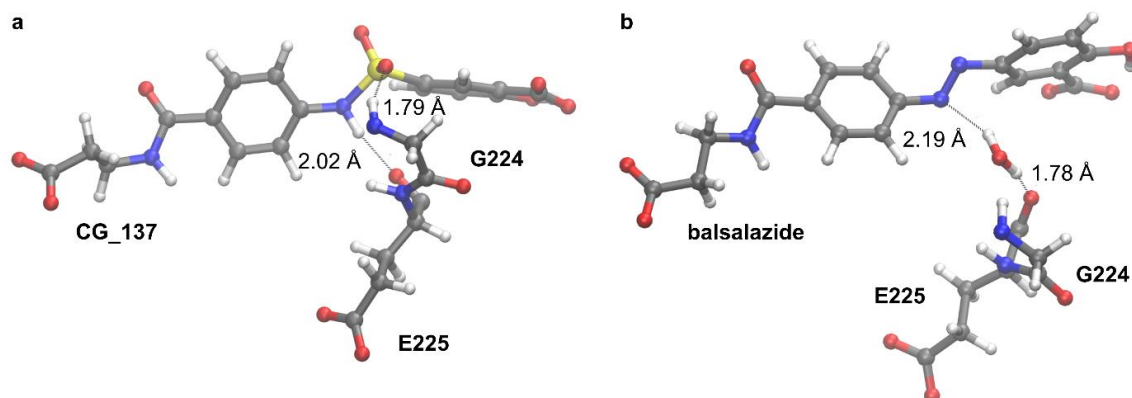


Figure 38: Representative structures from the MD simulations with (a) **CG_137** and (b) balsalazide in the active site of Sirt5.

A methylated sulfonamide (**CG_168**) was designed to investigate, whether the difference between **CG_128** and **CG_137** was caused by the sulfonamide hydrogen. And indeed, the calculated binding affinity and the experimental results were again worse for **CG_168** than for **CG_137** and balsalazide.

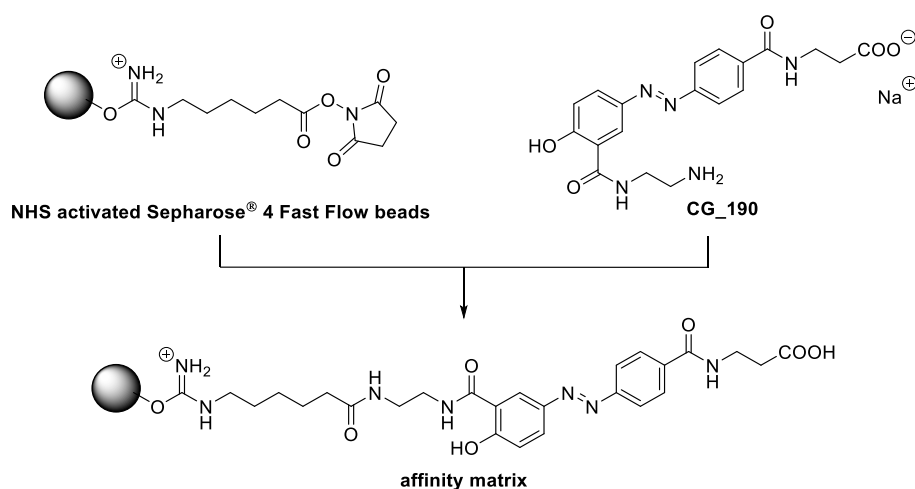
In summary, the herein described method for the calculation of the relative binding affinities of the balsalazide analogues seemed to qualitatively agree with the experimental results and can

be used to predict the affinity of potential Sirt5 inhibitors. The results of the calculations should be handled with care, as the binding affinity could be over- or underestimated and if a structure seems promising, it should be nonetheless synthesised and tested. Furthermore, this method has its limitations regarding structural changes of inhibitors such as ring formation and ring breaking, so that the relative binding affinities of the compounds with heteroaromatic spacers (that were the best inhibitors designed in this thesis) could not be calculated. A co-crystallised structure of one of these inhibitors with Sirt5, however, could be used as a starting point for calculations of the binding affinities of other analogues.

4.4. Target identification *via* pull-down assay and MS analysis

In the following, chemical proteomics experiments were used to verify Sirt5 as a target of balsalazide and to identify possible off-targets in a full cell lysate using an immobilised derivative of balsalazide in a pull-down assay. In addition, competition experiments with free balsalazide and the selected potent inhibitors **CG_220** and **CG_232** should give further insight into the affinity of the different compounds to Sirt5. These experiments were performed by SEVERIN LECHNER in the group of PROF. DR. BERNHARD KÜSTER (Technical University of Munich).

First, an immobilisable derivative of the inhibitor of interest was designed and synthesised as described in Chapter 3.3.1.1. An ethylenediamine linker was attached at a position, which supposedly plays no or only a minor role in the binding of balsalazide to the protein: the carboxylic acid of the salicylic acid unit. For the following pull-down assay, previously synthesised coupleable **CG_190** was amide coupled to *N*-Hydroxysuccinimidyl-Sepharose[®] (NHS) 4 Fast Flow beads for immobilisation as shown in Scheme 86.



Scheme 86: Coupling of **CG_190** to *N*-Hydroxysuccinimidyl-Sepharose[®] (NHS) 4 Fast Flow beads.

The resulting affinity matrix was then incubated with native MCF7 cell lysate with either DMSO as control or different concentrations of competing free balsalazide or compounds **CG_220**

and **CG_232**. MCF7 cells were chosen, since sirtuins, and in particular Sirt5, are overexpressed here.^[176] After incubation, proteins with low affinity to the beads were washed off and remaining bound proteins were denatured, reduced, alkylated and digested with trypsin. These samples were then analysed by LC-MS/MS (for experimental details see Chapter 6.1.1). Of the over 2300 identified proteins in each competition assay, only three proteins showed dose dependent reduction in intensity when incubated with the competing free compounds and can thus be considered to be bound by the molecules (Figure 39 and Figure 41). The proteins were identified as the target Sirt5 and two off-targets: glutaryl-CoA-dehydrogenase (GCD; gene: GCDH) and nucleoside diphosphate kinase (NDPK; gene: NME4).

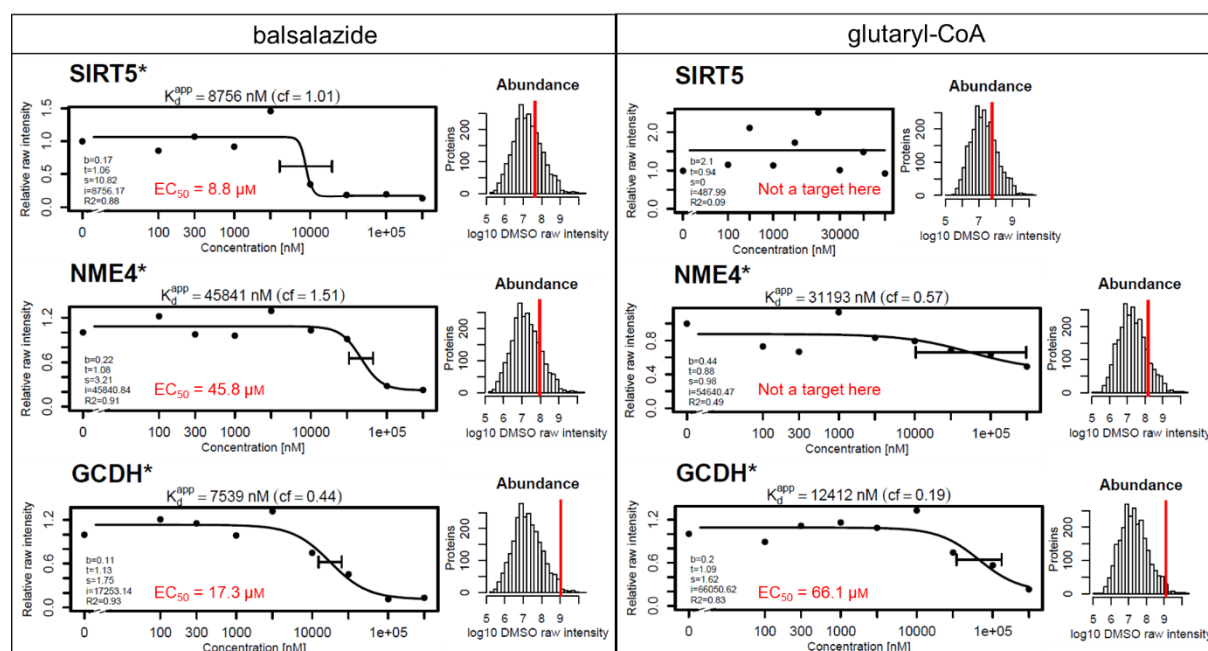


Figure 39: MS analysis results of the competition assay with immobilised **CG_190** vs. balsalazide or glutaryl-CoA depicted as binding curves. Abundance = MS-based raw intensity of the target protein (red line) within the intensity distribution of all proteins in the DMSO control. * indicates that the protein is classified as a high confidence target.

With free balsalazide the competition assay resulted in an EC_{50} value of $8.8 \mu\text{M}$ regarding Sirt5 (Figure 39). This is surprisingly in accordance with the IC_{50} value derived from the enzyme activity assay (Chapter 4.1), since the binding assays performed herein often result in 10 – 100-fold lower affinity compared to enzyme-based assays as described in Chapter 3.1.2. In addition to Sirt5, the proteins NDPK and GCD were also competed and resulted in EC_{50} values of $45.8 \mu\text{M}$ and $17.3 \mu\text{M}$, respectively. This leads to the conclusion that balsalazide also shows binding to these proteins to some extent, but it does not tell if these enzymes are inhibited by balsalazide. A possible explanation for the observation regarding GCD is that balsalazide also fits into the active site of GCD. Therefore, a competition assay with glutaryl-CoA was included, which is the natural substrate of GCD (Figure 39 and Figure 40). Identical

to the binding mode for Sirt5, the terminal carboxylate of balsalazide could mimic the free carboxylic acid group of the glutaryl residue.

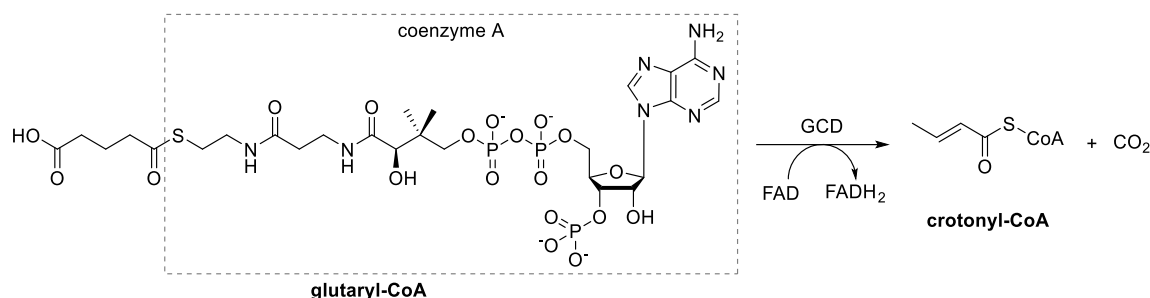


Figure 40: Structure of the natural substrate glutaryl-CoA of the enzyme GCD and the oxidative decarboxylation reaction catalysed by GCD with cofactor FAD.^[177]

The resulting binding curves for glutaryl-CoA (Figure 39) strongly undermine the hypothesis that balsalazide binds into the same binding pocket of GCD because immobilised **CG_190** was displaced with increasing concentrations of glutaryl-CoA. Glutaryl-CoA does not target Sirt5 and NDPK compared to balsalazide.

Additional competition assays with triazole **CG_220** and pyrazole **CG_232** showed that the same proteins were displaced from the affinity matrix as it was the case with balsalazide: Sirt5, NDPK and GCD (Figure 41), undermining molecular modes of action identical to balsalazide for the two advanced analogues.

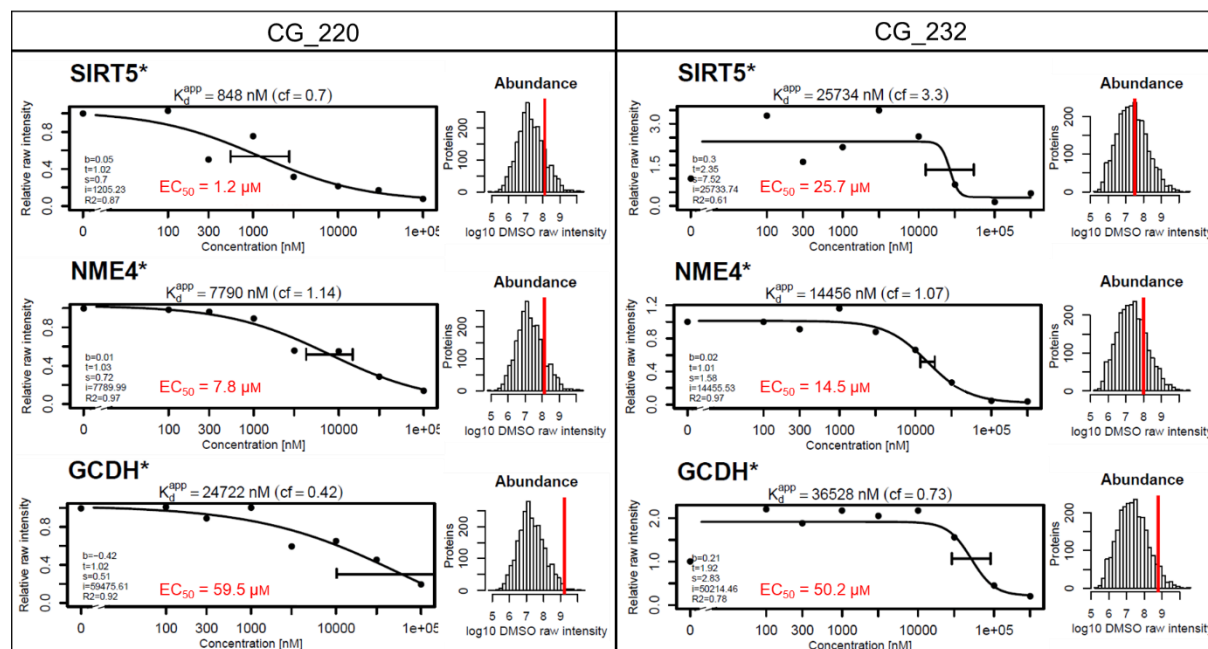


Figure 41: MS analysis results of the competition assay with immobilised **CG_190** vs. **CG_220** or **CG_232** depicted as binding curves. Abundance: MS-based intensity of the target protein (red line) within the intensity distribution of all proteins in the DMSO control. * indicates that the protein is classified as a high confidence target.

The inhibitors triazole **CG_220** and pyrazole **CG_232** were slightly more potent in the previously performed enzyme-based assay than balsalazide. Here within these chemical proteomics experiments, the competition with compound **CG_232** resulted in EC₅₀ values of only 25.7 μM for Sirt5 and 50.2 μM for GCD. For NDPK an EC₅₀ value of 14.5 μM was obtained (balsalazide: EC₅₀ = 45.8 μM). For **CG_220** this experiment resulted in an even better EC₅₀ value of 1.2 μM for Sirt5 and thereby suggesting that this molecule exhibits the strongest binding affinity for Sirt5 within this set of compounds. Regarding NDPK, triazole **CG_220** was also more affine with an EC₅₀ value of 7.8 μM. On the contrary, for the off-target GCD an EC₅₀ value of only 59.5 μM was obtained.

A literature search revealed that for both off-targets (GCD and NDPK) so far no small molecule inhibitors are known and the compounds from this thesis could potentially serve as lead structures for the development thereof. Interestingly, if glutaryl-CoA turnover by GCD was indeed inhibited by balsalazide and its analogues, the concentration of the GCD substrate glutaryl-CoA would be increased, which results in increased (non-enzymatic) glutarylation. In addition, an inhibition of Sirt5 results in decreased deglutarylation and thereby possibly a synergistic effect. But before final assumptions can be made this observation has to be investigated in future experiments.

Overall, these chemical proteomics experiments verified Sirt5 as a target protein for balsalazide as well as for the most potent inhibitors **CG_220** and **CG_232**. In addition, the two off-targets NDPK and GCD were identified.

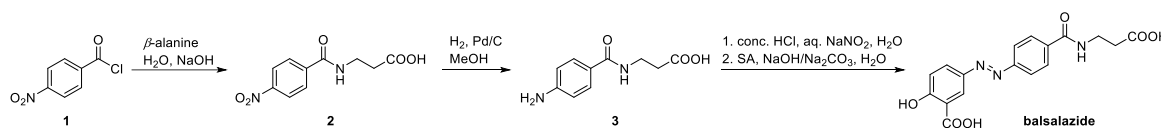
5. Summary

For the research towards novel inhibitors of Sirt5, this thesis was based on the results of a high-throughput screening where balsalazide was identified as a potent inhibitor of Sirt5. To get a closer understanding why this compound is able to inhibit Sirt5, initial docking experiments were performed where a very similar binding mode of a succinylated peptide as the natural substrate compared to balsalazide with Sirt5 in the presence of NAD⁺ was observed. The aim of this thesis was then to synthesise balsalazide to verify the previously reported inhibitory activity and further to design and synthesise various analogues of balsalazide to study structure-activity relationships with Sirt5. Thereby balsalazide should be turned into a potential therapeutic drug for systemic use with a different indication. Several biological experiments and computational methods were exploited to reach this goal.

Parts of these studies were published in the *EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY*^[40] and a general method for the preparation of *N*-aryl-1,2,3,4-tetrahydroisoquinolines developed during this thesis was published in *SYNTHESIS*.^[132]

5.1. Synthesis

Scheme 87 illustrates the synthetic steps for the preparation of balsalazide itself. These steps comprising of an amide coupling, hydrogenative reduction of the nitro group and an azo coupling were used in full or partially as template for the synthetic strategy of the designed analogues of balsalazide.

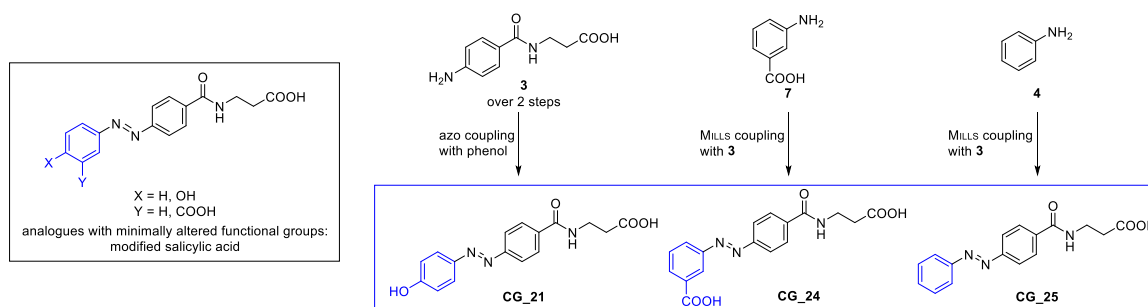


Scheme 87: Synthesis of the lead structure balsalazide in three steps. SA = salicylic acid.

For the following overviews of synthetic sequences to the target analogues of balsalazide only essential steps are illustrated, whereby synthetic details will not be given. The preparation of precursors and intermediates will also not be shown in this summary.

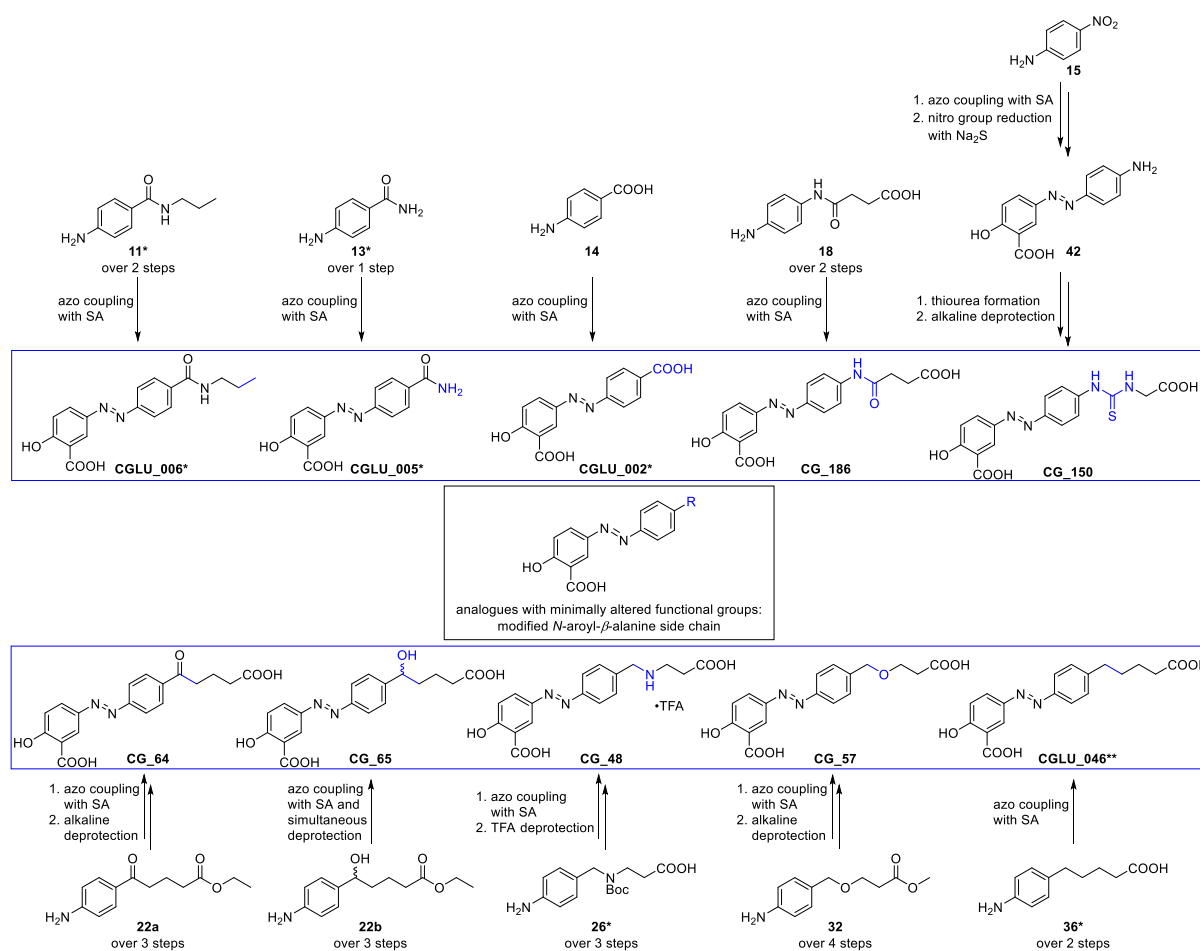
As illustrated in Scheme 88, the first set of analogues with minimal alterations regarding the salicylic acid motif was prepared by either employing the azo coupling reaction or MILLS coupling, resulting in phenol **CG_21**, benzoic acid **CG_24** and phenyl analogue **CG_25**.

Summary



Scheme 88: Overview of the synthesis of analogues with minimally altered functional groups regarding the salicylic acid motif: phenol **CG_21**, benzoic acid **CG_24**, and phenyl **CG_25**. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue.

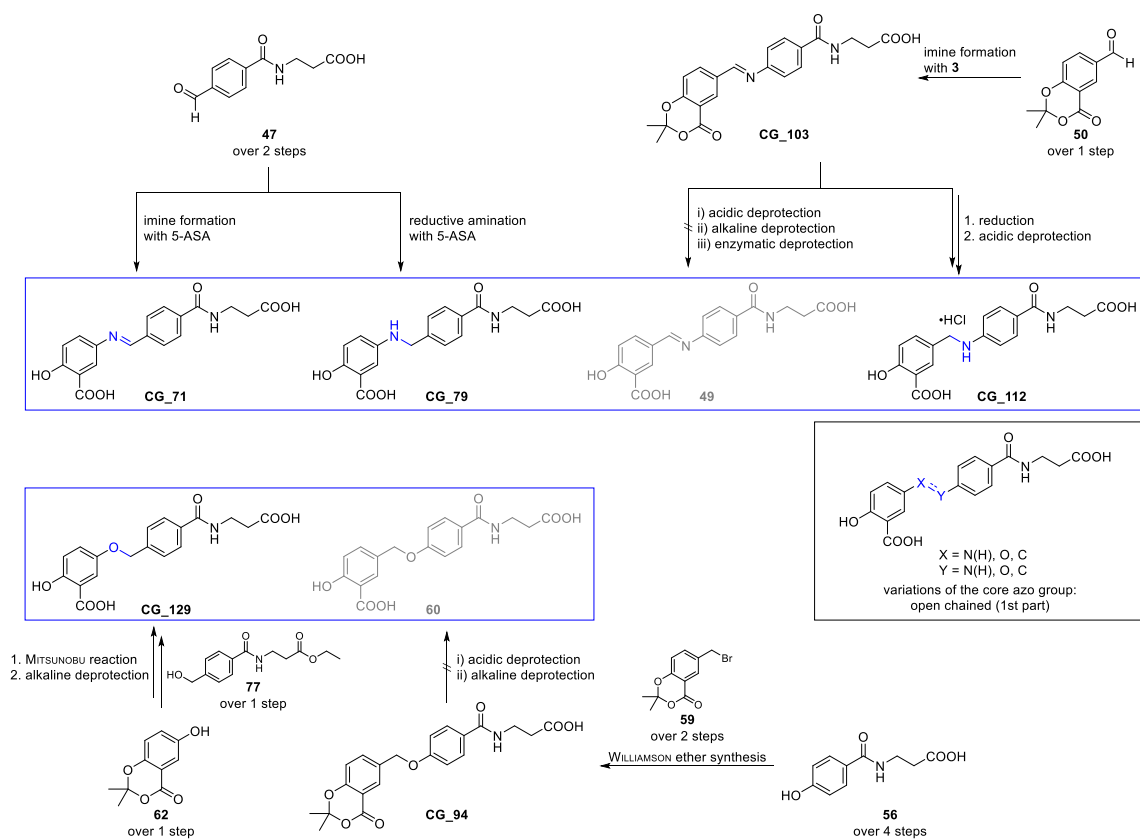
Modification of the *N*-aroyl- β -alanine side chain led to ten additional analogues (Scheme 89). The synthesis of each of them consisted of an azo coupling with salicylic acid (SA) as central step. Most of the starting materials, except *p*-aminobenzoic acid (**14**), had to be prepared in several steps to construct the desired side chain and to form the amine from a nitro group for the azo coupling. Thereby the following variants were obtained: the truncated derivatives carboxylic acid **CGLU_002***, primary amide **CGLU_005*** missing the β -alanine unit (with the crucial distal carboxylate group), and compound **CGLU_006*** where only the distal carboxylic acid is deleted. Further analogues affected the carboxamide by replacing it with an ethylene unit (**CGLU_046****), by creating a secondary benzylamine (**CG_48**), and a benzyl ether (**CG_57**). Deletion of the amide nitrogen led to aromatic ketone **CG_64** and secondary alcohol **CG_65**. Furthermore, thiourea **CG_150** and compound **CG_186** carrying an inverted amide in the form of an *N*-succinylated aromatic amine were synthesised.



Scheme 89: Overview of the synthesis of analogues with minimally altered functional groups regarding the *N*-aryl- β -alanine side chain: **CGLU_006***, **CGLU_005***, **CGLU_002***, **CG_186**, **CG_150**, **CG_64**, **CG_65**, **CG_48**, **CG_57**, and **CGLU_046****. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue. SA = salicylic acid.

Scheme 90 depicts an overview of the synthesis of analogues that contain only one heteroatom in the spacer (C-N or C-O units). The azo group was thereby replaced by an imine (**CG_71**), a more flexible amine (**CG_79** and **CG_112**) and an ether (**CG_129**). These analogues were designed to investigate the effect of an enhanced flexibility of the molecule as well as the importance of hydrogen bond accepting and donating abilities of the spacer. As for most analogues, the synthetic steps required an amide coupling to synthesise the upper part of the molecule with the β -alanine derived side chain and the preparation of the appropriate salicylic acid derivative. Both parts were then combined to construct the spacer unit. Here, this was accomplished either *via* condensation or reductive amination for the imines and amines, respectively, or WILLIAMSON ether synthesis or a MITSUNOBU reaction to form the ether bond. Imine **49** and ether **60** could not be synthesised.

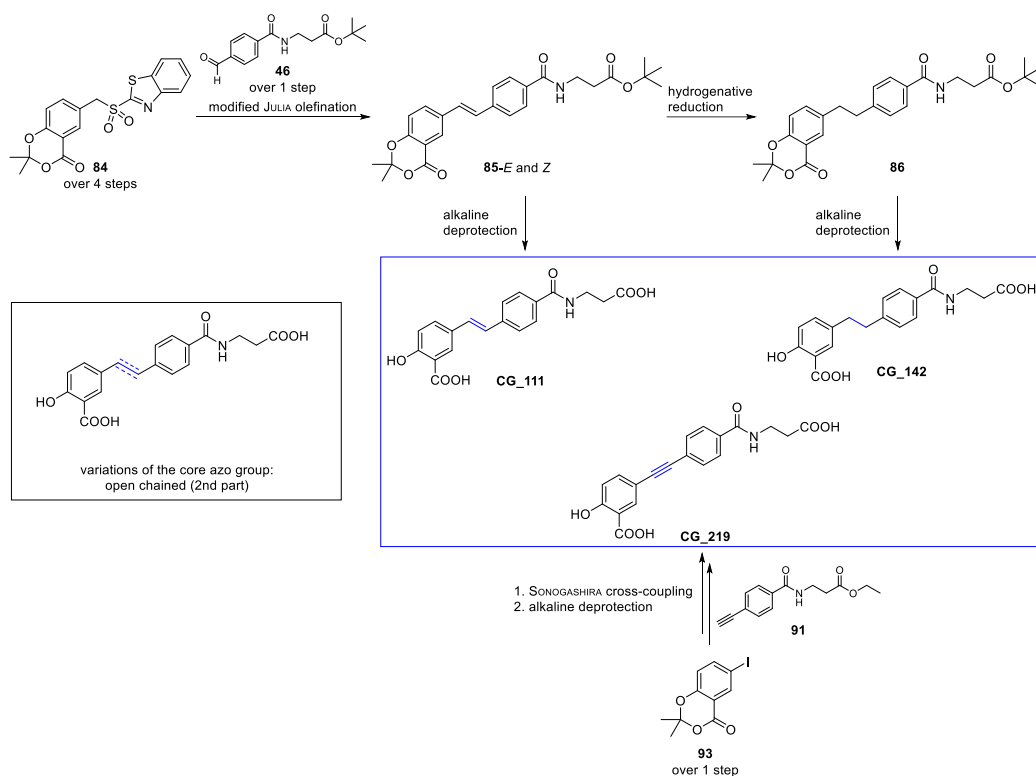
Summary



Scheme 90: Overview of the synthesis of analogues with a modified central azo group containing only one heteroatom: imine **CG_71**, amines **CG_79** and **CG_112**, and ether **CG_129**. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue. Compounds that could not be obtained are marked in grey: imine **49** and ether **60**.

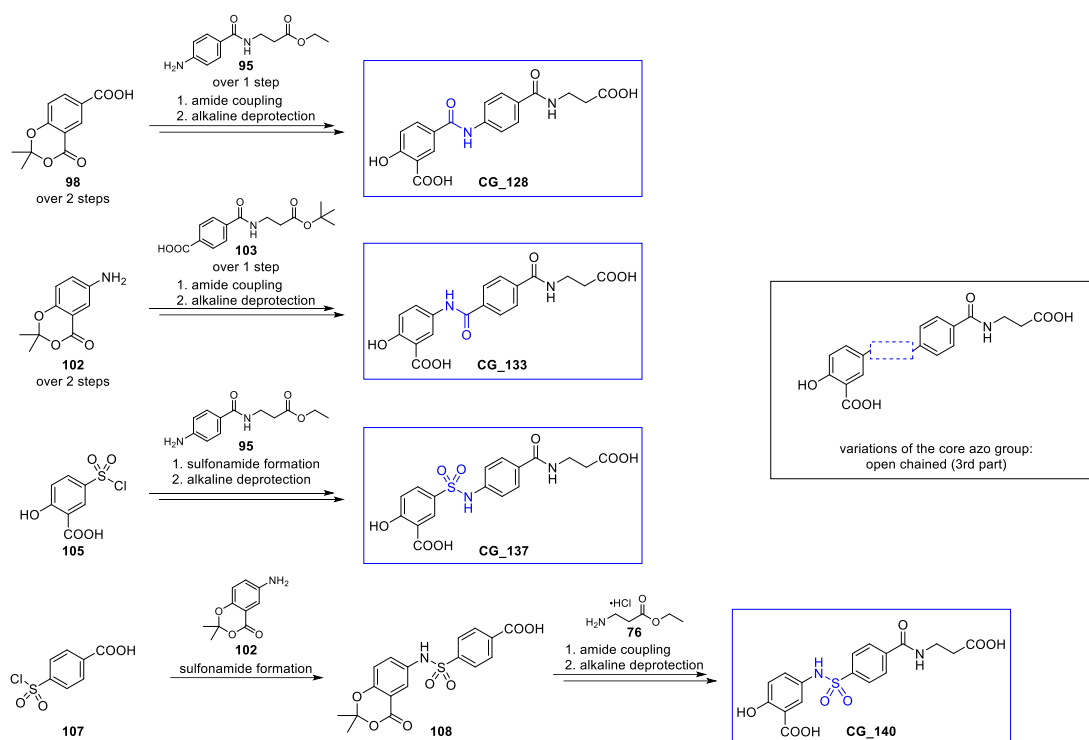
Lacking all hydrogen bond donating and/or accepting abilities, stilbene **CG_111**, alkane **CG_142** and alkyne **CG_219** were synthesised as shown in Scheme 91. Stilbene **CG_111** and alkane **CG_142** were prepared using a modified JULIA olefination as the central step, whereas alkyne **CG_219** was obtained employing a SONOGASHIRA cross-coupling.

Summary



Scheme 91: Overview of the synthesis of analogues with a modified central azo group lacking all heteroatoms in the spacer compared to the azo compound balsalazide: stilbene **CG_111**, alkane **CG_142**, and alkyne **CG_219**. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue.

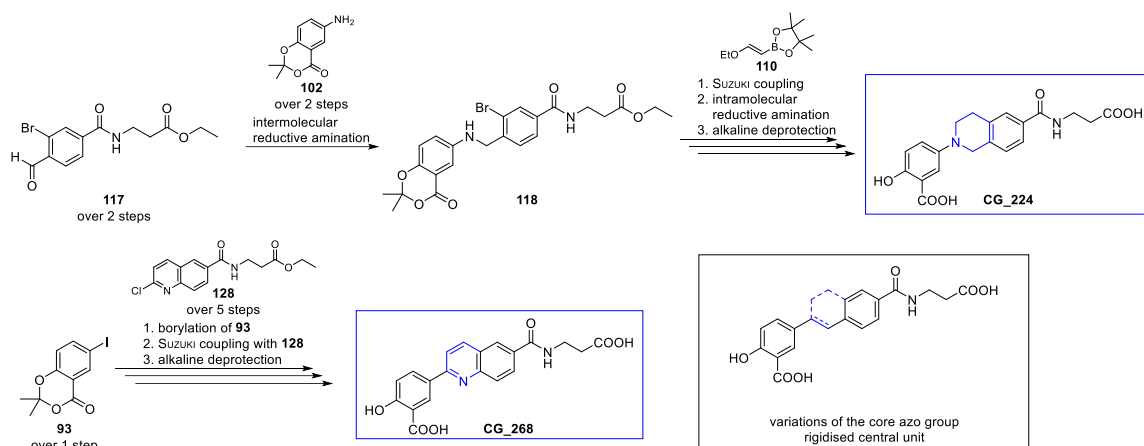
The next analogues were synthesised carrying a spacer unit with more heteroatoms and full flexibility. These four target compounds were amides **CG_128** and **CG_133**, as well as sulfonamides **CG_137** and **CG_140**. Each new spacer was synthesised laterally reversed as well for full investigation of possible interactions with the enzyme's active site. An overview of the synthesis is given in Scheme 92. Standard amide or sulfonamide couplings led to the desired target compounds.



Scheme 92: Overview of the synthesis of analogues where the core azo group was replaced by an amide (**CG_128** and **CG_133**) and a sulfonamide (**CG_137** and **CG_140**), respectively. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue.

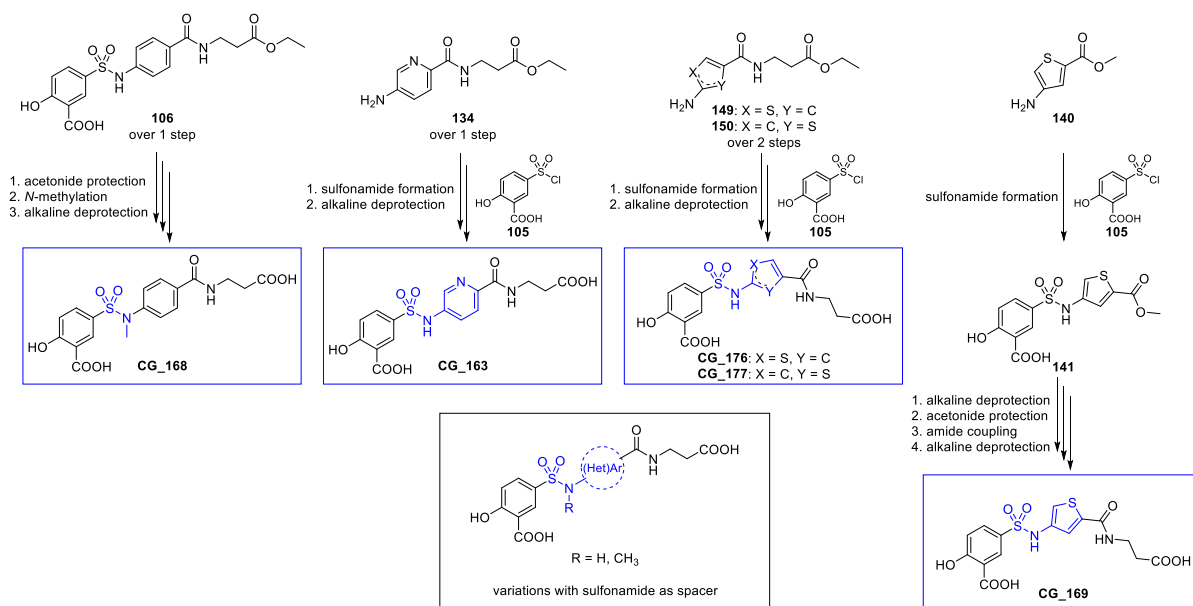
In order to take away the flexibility of the molecule and to reduce the loss of entropy that occurs during the binding to Sirt5, two rigidised compounds were synthesised as illustrated in Scheme 93. While quinoline **CG_268** was obtained using a SUZUKI cross-coupling reaction, analogue **CG_224** was prepared applying a novel general method^[132] for the preparation of *N*-aryl-1,2,3,4-tetrahydroisoquinolines that was developed during this thesis. This new route starts from readily available *ortho*-brominated aromatic aldehydes and primary aromatic amines. Condensation of these building blocks under reductive conditions gives *N*-aryl 2-bromobenzylamines. The C-3/C-4-unit of the tetrahydroisoquinoline is introduced using commercially available 2-ethoxyvinyl pinacolboronate (**110**) under SUZUKI cross-coupling conditions. Finally, the obtained *ortho*-ethoxyvinyl benzylamines are cyclised *via* an intramolecular reductive amination using the combination of triethylsilane/TFA. Tetrahydroisoquinoline **CG_224** was synthesised using this method but is unfortunately unstable.

Summary



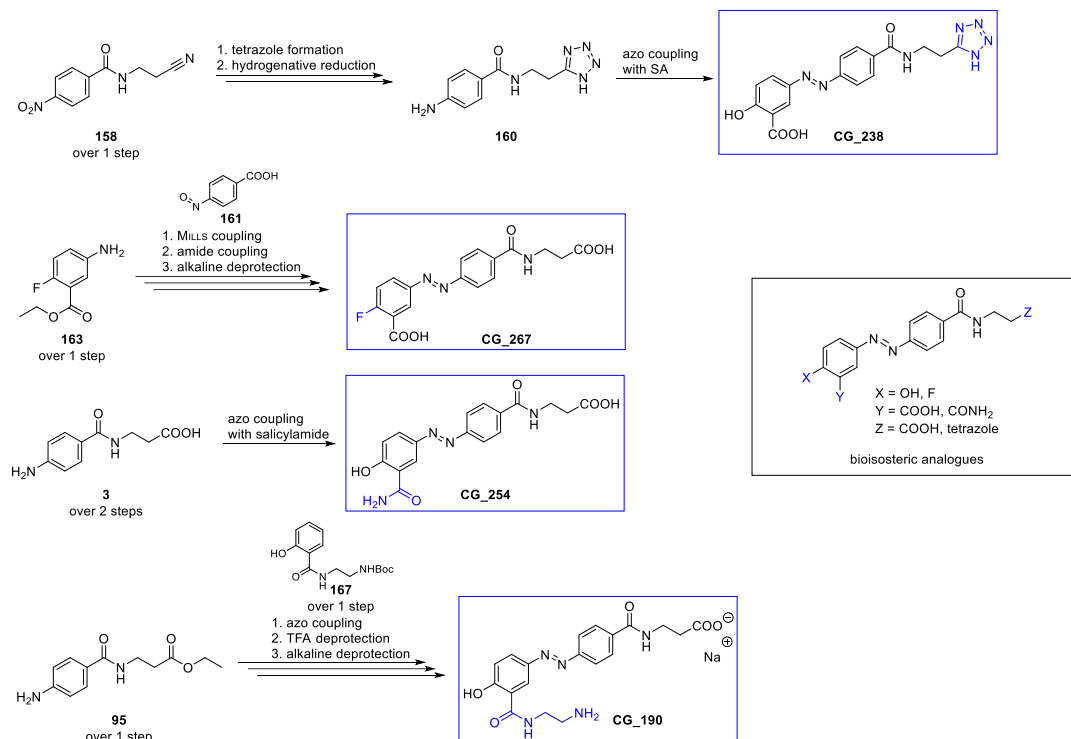
Scheme 93: Overview of the synthesis of rigidised analogues: *N*-aryl-1,2,3,4-tetrahydroisoquinoline **CG_224** and quinoline **CG_268**. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue.

Further five analogues (Scheme 94) were designed because of good results for the inhibitory activity against Sirt5 found for sulfonamide **CG_137**. In **CG_168** the sulfonamide spacer was *N*-methylated, thereby removing the hydrogen bond donating ability. Replacement of the non-salicylate phenyl ring resulted in pyridine **CG_163** as an example for a 6-membered heterocycle and thiophenes **CG_176**, **CG_177**, and **CG_169** as examples for 5-membered heterocycles. The synthetic strategy for these consisted of first, the construction of the side chain in an amide coupling and second, a sulfonamide coupling with a salicylic acid derivative, whereby the order of the required synthetic steps varied based on availability of starting materials.



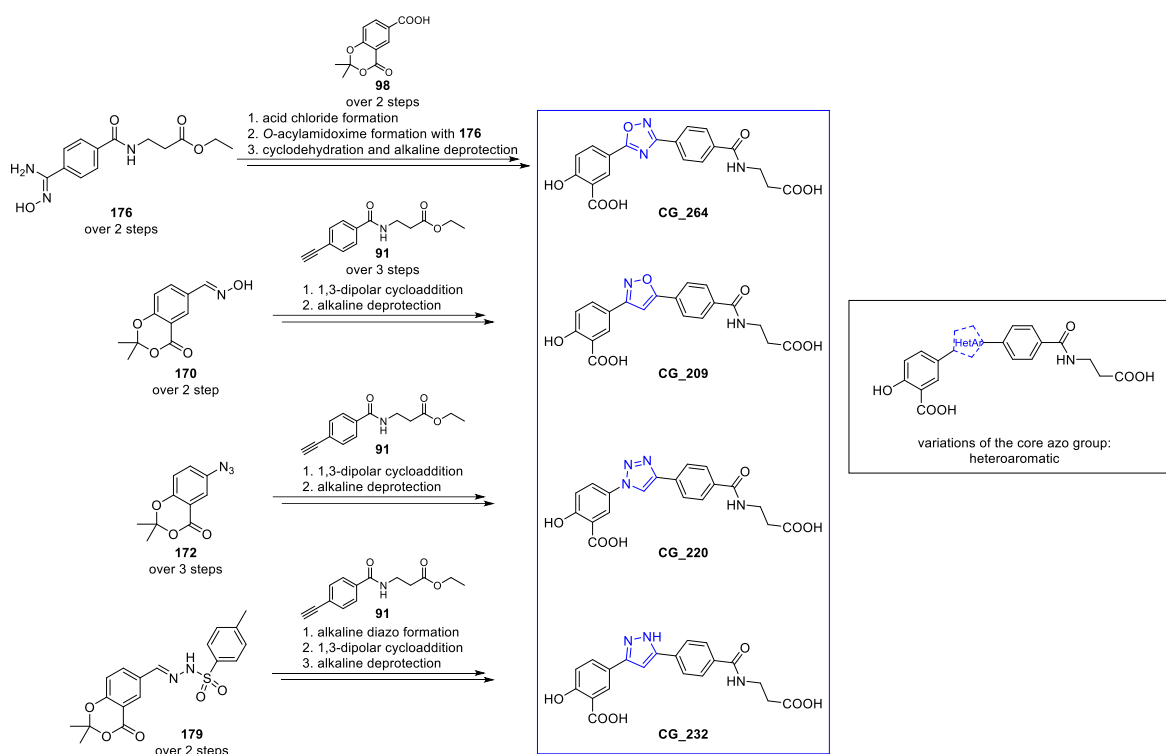
Scheme 94: Overview of the synthesis of derivatives and analogues with the sulfonamide spacer introduced before, focusing on varying the non-salicylate phenyl ring: *N*-methylated sulfonamide **CG_168**, pyridine **CG_163**, and thiophenes **CG_176**, **CG_177**, and **CG_169**. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue.

An overview of the synthesis of bioisosteric variations of balsalazide is illustrated in Scheme 95. The equally acidic tetrazole in analogue **CG_238** was to replace the important distal carboxylate in balsalazide. Fluoro compound **CG_267** and primary salicylamide **CG_264** were designed to explore whether these bioisosteric changes on the salicylic acid part of the molecule could increase affinity. Compound **CG_190** with an ethylenediamine linker was solely prepared for chemical proteomics experiments, where this analogue should be coupled to beads to study the possible enrichment of target proteins of balsalazide. Again, an azo coupling or MILLS coupling was the central step to these analogues.



Scheme 95: Overview of the synthesis of bioisosteric analogues carrying the azo group as spacer again: tetrazole **CG_238**, fluoride **CG_267**, amide **CG_254** and ethylenediamine **CG_190**. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue. SA = salicylic acid.

The synthesis of the last set of analogues of balsalazide is shown in Scheme 96. These four analogues were designed bearing aromatic heterocycles as replacements of the core azo group to investigate a different geometry of the molecule and again possible hydrogen bond interactions with the enzyme. The synthesis was performed using HUISGEN 1,3-dipolar cycloadditions (isoxazole **CG_209**, triazole **CG_220**, and pyrazole **CG_232**) or *via* the amidoxime route (1,2,4-oxadiazole **CG_264**).



Scheme 96: Overview of the synthesis of heteroaromatic analogues regarding the core azo group of balsalazide: 1,2,4-oxadiazole **CG_264**, isoxazole **CG_209**, triazole **CG_220** and pyrazole **CG_232**. Desired target compounds are framed with a blue box and the modifications compared to the lead structure balsalazide are also marked in blue.

In summary, balsalazide and 39 analogues thereof were designed and synthesised as depicted in Figure 42. Among them, 13 analogues with minimally altered functional groups except the azo spacer, 12 analogues with an open-chained spacer replacing the azo group and 4 analogues with a sulfonamide spacer and a heterocyclic replacement of the non-salicylate phenyl ring. Furthermore, 4 analogues containing a heteroaromatic spacer, 2 analogues with a rigidised core and lastly, 3 bioisosteric analogues and 1 coupleable analogue of balsalazide were synthesised.

Summary

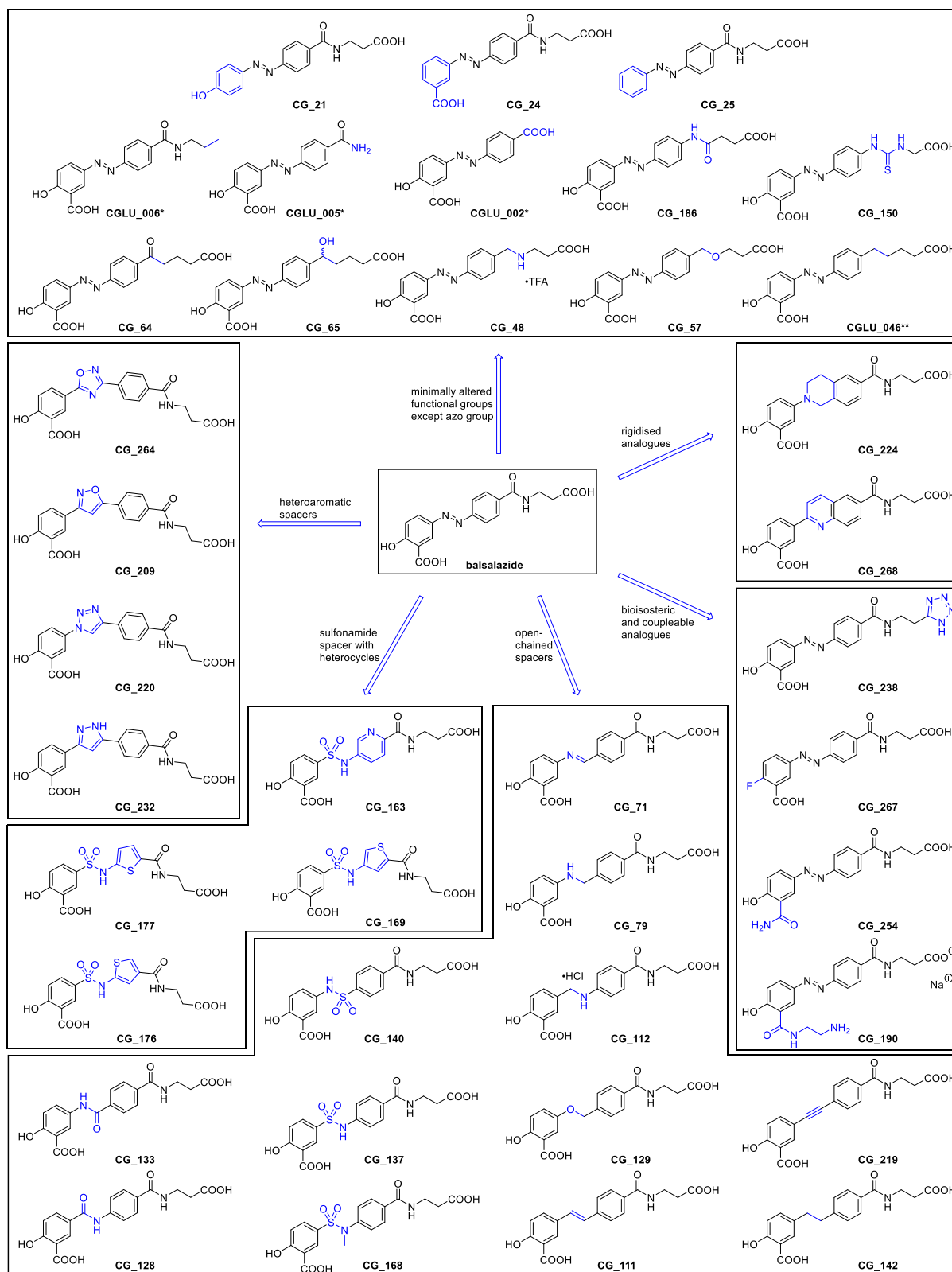


Figure 42: Overview of balsalazide and its 39 analogues that were designed and synthesised during this thesis.

5.2. Biological and theoretical evaluation

Overall, balsalazide and the 39 synthesised analogues were tested for inhibitory activity against Sirt5 in an enzyme-based fluorometric assay in several test campaigns. Each round of

testing included a set of synthesised inhibitors that were designed based on previous test results. This first determination was performed as a single-concentration measurement at 50 μM final assay concentration and the results are illustrated in Figure 43.

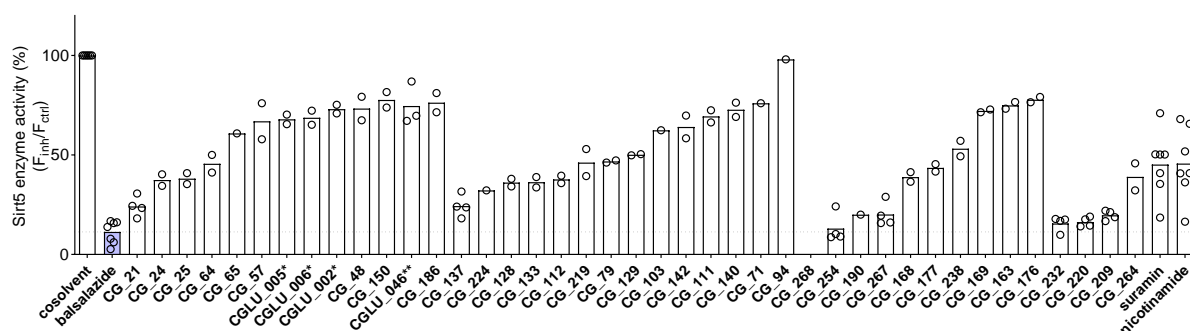


Figure 43: Residual Sirt5 enzyme activity after 1 h incubation with the inhibitors in % (F_{inh}/F_{ctrl}). The known non-selective Sirt5 inhibitors nicotinamide and suramin were included as positive controls. Fluorescence measured upon incubation with the cosolvent DMSO alone (no inhibitor) was set to 100 % enzyme activity. All inhibitors were tested at 50 μM final concentration. Quinoline **CG_268** showed auto-fluorescence and thereby could not be measured with this assay setup. F = fluorescence units; λ_{ex} = 360 nm, λ_{em} = 460 nm; number of biological replicates stated with circles (○) for each bar.

These experiments clearly showed that a) changes on the salicylic acid part of the molecule are tolerated to some extent, b) modifications on the *N*-aroyl- β -alanine side chain result in a significant decrease in activity, and c) the core azo group can successfully be replaced by spacers, which increase the drug-likeness of the inhibitor. Balsalazide, the most promising analogues, and amine **CG_48** (as a negative control) were then chosen for investigation of subtype selectivity over the class I sirtuins Sirt1, 2, and 3 (Table 9). These experiments were performed by NATHALIE WÖSSNER.

Table 9: Inhibition of Sirt1, 2, 3, and 5 by balsalazide and selected analogues. *n.i. = inhibition <10 %.

Compound name	IC ₅₀ [μM] or % inhibition at 50 μM				Compound name	IC ₅₀ [μM] or % inhibition at 50 μM			
	Sirt1	Sirt2	Sirt3	Sirt5		Sirt1	Sirt2	Sirt3	Sirt5
balsalazide	n.i.*	11 %	n.i.	9.5 μM	CG_140	n.i.	17 %	n.i.	29 %
CG_21	n.i.	26 %	16 %	31.5 μM	CG_209	n.i.	n.i.	n.i.	11.5 μM
CG_24	n.i.	21 %	n.i.	63 %	CG_220	n.i.	15 %	n.i.	7.2 μM
CG_25	n.i.	16 %	n.i.	62 %	CG_224	14 %	16 %	n.i.	68 %
CG_48	n.i.	19 %	11	27 %	CG_232	n.i.	n.i.	n.i.	8.5 μM
CG_128	n.i.	10 %	n.i.	65 %	CG_254	27 %	41 %	29 %	87 %
CG_133	n.i.	16 %	n.i.	64 %	CG_267	n.i.	13 %	n.i.	23.6 μM
CG_137	n.i.	14 %	n.i.	12.5 μM					

Here it was revealed that almost all analogues were to some extent subtype-selective. Only one of the selected inhibitors (amide **CG_254**) was considered as not selective due to inhibition values of 27 %, 41 % and 29 % for Sirt1, 2, and 3. Balsalazide itself showed no inhibition of Sirt1 and 3 and only weak inhibition of Sirt2 (11 %). The most promising analogues of balsalazide (phenol **CG_21**, sulfonamide **CG_137**, isoxazole **CG_209**, triazole **CG_220**, pyrazole **CG_232**, and fluoride **CG_267**) were all subtype-selective and in addition showed already very good inhibitory activity against Sirt5. For these, the IC₅₀ values for Sirt5 inhibition were then measured (Table 9).

The derived IC₅₀ values for the selected inhibitors showed that all these compounds represent potent inhibitors of Sirt5. For balsalazide with an IC₅₀ of 9.5 µM a value was calculated that was slightly different from a first measurement with 5.3 µM. But it is still in the range of the most potent inhibitors of Sirt5 known so far. It has to be noted that for precise determination of IC₅₀ values of azobenzenes, the respective concentration-dependent self-absorption has to be taken into account. Azobenzenes **CG_21** and **CG_267** were significantly less potent than balsalazide with IC₅₀ values of 31.5 µM and 23.6 µM, respectively. All analogues with a modified spacer exhibited very good IC₅₀ values in the low micromolar range (≤12.5 µM). With triazole **CG_220** (IC₅₀ = 7.2 µM) and pyrazole **CG_232** (IC₅₀ = 8.5 µM) inhibitors were developed that represent two very promising inhibitors of Sirt5 that can be used for future investigations of the physiological role of Sirt5 and its clinical relevance. In addition, because both carry spacers (triazole and pyrazole) that are easily accessible using 1,3-dipolar cycloadditions, optimisations can simply be performed to possibly increase affinity, and hence potency, further.

In an additional experiment performed by NATHALIE WÖSSNER it was revealed that there is no competition between balsalazide, **CG_21**, and negative control **CG_48** and NAD⁺ or ZKsA. Even though the inhibitors could not be displaced by the small molecule substrate ZKsA, it is possible that balsalazide does compete with larger peptide substrates which remains to be elucidated.

Through calculations of the reaction paths of selected compounds, which were performed by JOHANNES DIETSCHREIT, it was revealed that the inhibition strength for Sirt5 inhibitors is very likely dominated by the interaction of functional groups increasing the stability of the inhibitor-enzyme complex and not the aspect whether the inhibitor binds covalently or not.

Furthermore, a method for calculating the relative free binding affinity of the analogues compared to balsalazide as reference compound performed by ELI NAYDENOVA agreed qualitatively with the potencies obtained in the *in vitro* experiments. Therefore, this method can be used to predict the affinity of potential Sirt5 inhibitors. But it has its limitations regarding structural changes of inhibitors such as ring formation and ring breaking, so that the relative binding affinities of the compounds with heteroaromatic spacers (that were the best inhibitors

designed in this thesis) could not be calculated. A co-crystallised structure of one of these inhibitors with Sirt5, however, could be used as a starting point for calculations of the binding affinities of such analogues. These co-crystallisation experiments are currently still in progress.

A chemical proteomics experiment using a pull-down assay with balsalazide coupled to beads and subsequent MS analysis was performed by SEVERIN LECHNER to identify the target proteins of balsalazide. As targets the desired sirtuin Sirt5, and two more enzymes called glutaryl-CoA dehydrogenase (GCD) and nucleoside diphosphate kinase (NDPK) were found. Competition assays with balsalazide, analogues triazole **CG_220** and pyrazole **CG_232**, and glutaryl-CoA should give further insights into the ligand-protein interaction. These experiments revealed that triazole **CG_220** shows the strongest binding affinity to Sirt5 with an EC_{50} value of 1.2 μM (balsalazide: $EC_{50} = 8.8 \mu\text{M}$ and **CG_232**: $EC_{50} = 25.7 \mu\text{M}$). Furthermore, balsalazide binds GCD in the same manner as its natural substrate glutaryl-CoA. If balsalazide is thereby inhibiting GCD the effects of the inhibition of both enzymes, GCD and Sirt5, could work synergistically. But this hypothesis has to be investigated further.

In summary, the synthetic and biological work carried out during this thesis and additional *in vitro* and computational experiments by cooperating groups resulted in the verification of balsalazide as a potent, subtype-selective Sirt5 inhibitor. It was shown that changes on the existing substituents on both aromatic rings of balsalazide are tolerated only to a very limited extent. In contrast, changes on the core azo group could not only increase the drug-likeness of the desired inhibitors but also potency, since these led with **CG_220** and **CG_232** to the development of at least two even more potent Sirt5 inhibitors with IC_{50} values in the low micromolar range ($<10 \mu\text{M}$) and proven subtype selectivity over Sirt1, 2, and 3. With these analogues subtype selectivity was maintained and inhibitory activity as well as physicochemical properties were improved. Hence, these novel inhibitors can be used as chemical tools for the investigation of the physiological role of Sirt5 but could also serve as lead structures for drug candidates for systemic use.

6. Experimental part

6.1. Biology

6.1.1. Cell-based assays

MTT assay^[169]

HL-60 cells were maintained under standard cell culture conditions in RPMI 1640 medium supplemented with 10 % fetal bovine serum (FBS) without antibiotics. Cells were grown and incubated at 37 °C in a 5 % CO₂ atmosphere.

HL-60 cells were seeded in 96-well plates at 9×10^4 cells/well and incubated for 24 h before treatment with test compounds for 24 h with a final well volume of 100 μ L, 1 % DMSO; three technical replicates; the cosolvent control was treated with 1 % DMSO only. Triton-X 100 was used as a positive control. 3-[4,5-Dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT; 5 mg in 1.0 mL PBS) was added to the cells for 2 h. Then DMSO (190 μ L) was added. After 1 h, absorbance of the MTT metabolite formazan was measured at 570 nm using an MRX Microplate Reader (Dynex Technologies, Chantilly, USA). Absorbance data was averaged over the technical replicates, then normalised to viable cell count from the cosolvent control cells (% control) as 100 %, where 0 % viability was assumed to correspond to absorbance zero.

Chemical Proteomics

Cell culture and lysis:

MCF7 cells were maintained under standard cell culture conditions in Dulbecco's modified eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS). Cells were grown and incubated at 37 °C in a 5 % CO₂ atmosphere. Upon reaching 90 % confluency, cell medium was discarded, cells were washed twice with PBS and then lysed in lysis buffer containing 50 mM Tris-HCl, pH 7.5, 5 % glycerol, 1.5 mM MgCl₂, 150 mM NaCl, 1 mM Na₃VO₄, 0.8 % Igepal, 0.375 mM NaF, 1 mM DTT including protease inhibitors (SigmaFast protease inhibitor tablet S8820) and phosphatase inhibitors (Phosphatase Inhibitor Cocktail 3, Sigma-Aldrich, Munich, Germany). After one freeze-thaw cycle (-80 °C) the lysate was ultracentrifuged for 30 min at 4 °C and 50,000 g and supernatant was stored at -80 °C for further procedures.

Preparation of the affinity matrix:

NHS-Sepharose beads (Amersham Biosciences) were washed with dry DMSO (40 mL/mL beads) and reacted with **CG_190** (1 μ mol/mL beads) for 20 h on an end-over-end shaker at room temperature in the dark in the presence of triethylamine (15 μ L/mL beads) in DMSO (1 vol of DMSO for 1 vol of beads). Aminoethanol (50 μ L/mL beads) was then added to react

remaining NHS-groups, and the mixture was further shaken for an extra 20 h at room temperature in the dark. The beads were then washed with DMSO (30 mL/mL beads) and ethanol (3 x 10 mL/mL beads) and stored in ethanol (1 mL/mL beads) at 4 °C. Aliquots of the supernatants before and after coupling were controlled by LC-MS to conclude of the completion of the reactions.

Competition assay:

The cell lysates were diluted with equal volumes of 1 x compound pull-down (CP) buffer (50 mM Tris-HCl, pH 7.5, 5 % glycerol, 1.5 mM MgCl₂, 150 mM NaCl, 20 mM NaF, 1 mM sodium ortho-vanadate, 1 mM DTT), protease inhibitor (SigmaFast protease inhibitor tablet S8820) and phosphatase inhibitors (Phosphatase Inhibitor Cocktail 3, Sigma-Aldrich, Munich, Germany). If required, lysates were further diluted to a final protein concentration of 5 mg/mL using 1 x CP buffer supplemented with 0.4 % Igepal (CP-0.4).

For selectivity profiling experiments in 96-well plates, the diluted cell mix lysates (2.5 mg of total proteins/well) were incubated for 30 min at 30 °C in an end-over-end shaker with 0 μM (DMSO control), 0.1 μM, 0.3 μM, 1.0 μM, 3.0 μM, 10 μM, 30 μM, 100 μM, or 300 μM of the Sirt5 inhibitor dissolved in DMSO. 18 μL of the affinity matrix were incubated with the lysates at 30 °C for 30 min in a filter plate. The DMSO control lysate was recovered and incubated similarly with 18 μL affinity matrix as a pull-down of pull-down experiment in order to calculate the depletion factor. The beads were then washed (3 times 1 mL of CP-0.4 buffer followed by 2 times 1 mL of CP-0.2) and the bound proteins subsequently denatured in a buffer consisting of 8 M urea, 40 mM Tris-HCl (pH 7.4) and 50 mM DTT at 40 °C and 700 rpm. After adding 4 μL of 550 mM chloracetamide for 30 min at 37 °C, the urea concentration was diluted with 250 μL Tris-HCl (pH 7.4). Denatured proteins were digested by adding 30 μL of 10 ng/μL trypsin and incubating overnight at 700 rpm. The digestion was stopped with 10 μL of 10 % (v/v) formic acid (FA) and peptides were desalted on a Sep-Pak tC18 μElution Plate (Waters). Desalted peptides dried down on a Speedvac and stored at -20 °C for LC-MS/MS measurement.

LC-MS/MS Analysis:

Label-free nano-flow LC-MSMS measurement of peptides was performed on Dionex Ultimate3000 nano HPLC (Thermo Fisher Scientific) coupled to an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific). The peptides were delivered to a trap column (100 μm x 2 cm, packed in-house with Reprosil-Pur C18-AQ 5 μm resin, Dr. Maisch) at a flow rate of 5 μL/min in 100 % solvent A (0.1 % formic acid in HPLC grade water) and separated on an analytical column (75 μm x 40 cm, packed in-house with Reprosil-Gold C18, 3 μm resin, Dr. Maisch) at a flow rate of 300 nL/min using a 51 min gradient ranging from 4 % to 32 % solvent C in B (solvent B: 0.1 % FA and 5 % DMSO in HPLC grade water, solvent C: 0.1 % FA and 5 % DMSO in acetonitrile). The eluent was sprayed *via* stainless steel emitters (Thermo)

at a spray voltage of 2.1 kV and a heated capillary temperature of 275 °C. The instrument was operated in data-dependent mode. Full scan MS spectra (m/z 360 – 1300) were acquired in the Orbitrap at 60,000 resolution using an automatic gain control (AGC) target value of 4e5 charges and a maximum injection time of 50 ms.

For LC-MS2 analysis of the full proteome, up to 12 peptide precursors were selected for fragmentation by higher energy collision-induced dissociation (HCD; 1.7 m/z isolation window, AGC value of 2e5, maximum injection time of 75 ms, Dynamic Exclusion of 30 s) using 30 % normalised collision energy (NCE) and analysed at a resolution of 15,000 in the Orbitrap.

Protein Identification and Quantification:

Protein and peptide identification and quantification was performed using MaxQuant^[178] (Version 1.6.1.0). The tandem MS data was searched against human canonical sequences from the Swissprot database (20193 entries, downloaded 22.03.2016) using the Andromeda search engine.^[179] *N*-terminal acetylation and oxidation of methionine were set as variable modification, while carbamidomethylation of cysteine was set as fixed modification. Trypsin/P was set as proteolytic enzyme. The minimum peptide length was set to seven and all data were adjusted to 1 % protein false discovery rate (FDR). Matching between runs was enabled with a match time window of 0.7 min and alignment window of 20 min.

LC-MSMS data analysis

Subsequent data analysis was performed on identified and quantified protein groups. The relative binding of proteins to the affinity matrix in dependence of free competing compound was calculated as ratio of the raw intensities of the protein at given compound concentration relative to the raw intensity of the DMSO sample. EC_{50} values were derived from a four-parameter log-logistic regression using an internal pipeline that utilises the ‘drc’ package in R.^[180]

To calculate the K_d^{app} value, the EC_{50} value was multiplied with a correction factor derived from the ratio of the amount of protein captured from two consecutive pull-downs of the same DMSO control lysate.^[181] The targets were manually annotated based on the sigmoidal shape of the binding curve with a dose-dependent decrease of binding to the affinity matrix. Moreover, number and dose-dependent decrease of unique peptides and MSMS counts per condition were considered.

6.1.2. Agar diffusion assay

Solutions with 1 % (m/V) compound in DMSO were prepared. Of these solutions 3.0 μ L were given on a test platelet (diameter 6 mm, Macherey-Nagel), equivalent to 30 μ g substance. The same was done for the reference substances clotrimazole (antifungal) and tetracycline (antibacterial). A blind control was conducted with DMSO. The test platelets were then dried

for 24 h at room temperature. Microorganisms were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig and cultivated according to the DSMZ recommendations in liquid culture. For the agar diffusion assay, different agars were required. For *Candida glabrata* (DSM number: 11226), *Hyphopichia burtonii* (DSM number: 70663), *Yarrowia lipolytica* (DSM number: 1345), *Escherichia coli* (DSM number: 426), and *Pseudomonas marginalis* (DSM number: 7527) all-culture agar (AC-agar) of Sigma Aldrich was used. 35.2 g AC-agar and 20 g agar were suspended in 1.0 L water and autoclaved. For *Staphylococcus equorum* (DSM number: 20675) and *Streptococcus entericus* (DSM number: 14446) an agar is likewise prepared from 10.0 g casein peptone, 5.0 g yeast extract, 5.0 g glucose and 5.0 g sodium chloride in 1.0 L water. For *Aspergillus niger* (DSM number: 1988) 32 g potato dextrose agar and 20 g agar in 1.0 L water were used. After treatment in the autoclave 15 mL each of the warm, liquid agar was filled into petri dishes under aseptic conditions and cooled to 8 °C for 1 h. The germs were then brought onto the different agars using cotton swabs. The platelets containing the substances, the reference, and the blind control were put onto the agar. The agar plates were incubated for 36 h at 32 °C (bacteria) or 28 °C (yeasts). Then the diameters of growth inhibition were measured manually.

6.1.3. Enzyme-based assays

FLUOR DE LYS® SIRT5 fluorometric drug discovery assay kit^[40]

For screening the inhibitory activity of synthesised inhibitors against recombinant human Sirt5 a commercially available Sirt5 fluorometric drug discovery kit (BML-AK513) from ENZO LIFE SCIENCES (ELS) AG (Lausen, Switzerland) was used. The assay was performed in Corning ½ volume 96-well black NBS microplates provided in the kit. In brief, for the desuccinylation reaction to take place, potential inhibitors (10 mM DMSO stock solutions) were incubated with Sirt5, FLUOR DE LYS®-Succinyl, and NAD⁺ in assay buffer for 60 min at 37 °C. After this time, the deacylation reaction was stopped by addition of FLUOR DE LYS® Developer and nicotinamide. The mixture was incubated for 15 min. Fluorescence was measured on a FLUOstar Omega microplate reader (BMG Labtech, Ortenberg, Germany) ($\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$) and averaged over the technical replicates. A mixture with only DMSO was used as no inhibition control and set to 100 % enzyme activity. All values were normalised to this cosolvent control after blank subtraction. IC₅₀ values were determined with GraphPad Prism 9.0 software (La Jolla, CA). Dose response curves were fit using a nonlinear log(inhibitor) vs. response model with variable slope.

NAD⁺ competition assay for Sirt5^[40]

NAD⁺ competition was examined using a procedure previously reported for Sirt1^[182] that was adapted for Sirt5. Instead of the deacetylation substrate ZMAL the succinylated variation Z-

Lys(succinyl)-AMC (ZKsA) was used. 2 μL NAD^+ in assay buffer (50 mM Tris-HCl, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl_2 , pH 8.0, and 0.1 % PEG8000) in concentrations varying from 2 mM to 62.5 μM were mixed with the respective inhibitor (50 μM) or DMSO as a control (final DMSO concentration 5 % (v/v)). To start the reaction, ZKsA (100 μM final assay concentration) and 0.1 μL Sirt5 (purchased from ENZO LIFE SCIENCES (ELS) AG, Lausen, Switzerland) were added and the mixture filled up to 20 μL with assay buffer. After incubation for 1 h at 37 °C and 140 rpm, the reaction was stopped by addition of 4 μL of trypsin solution (6 mg/mL in trypsin buffer). After 2 min of incubation at 37 °C and 140 rpm, fluorescence intensity was detected using a BMG POLARstar ($\lambda_{\text{EX}} = 390 \text{ nm}$, $\lambda_{\text{EM}} = 460 \text{ nm}$, BMG POLARstar Optima, BMG Labtech, Germany). An enzyme-free blank control and a control containing AMC (amino-4-methylcoumarin) instead of ZKsA were performed as well. Conversion was calculated in % in relation to the 100 % control (AMC) after subtraction of the blank fluorescence signal. Conversion [%] was then plotted against NAD^+ concentration.

ZKsA competition assay for Sirt5^[40]

Substrate competition towards the synthetic substrate ZKsA was determined using the same protocol as described for NAD^+ competition. NAD^+ concentration was kept constant at 500 μM final assay concentration while varying concentrations of ZKsA (200 – 1.25 μM final assay concentration) were used. All other parameters were identical to the protocol described above. Total amount of formed product [μM] was plotted against applied ZKsA concentration.

SIRT1, 2, and 3 fluorometric enzyme assay^[40]

Inhibition of recombinant Sirt1/2/3 was determined using a homogeneous fluorescence deacetylase assay.^[183] Sirt1/2/3 was mixed with the fluorescent substrate ZMAL (final assay concentration 10.5 μM), NAD^+ (6 mM, final assay concentration 500 μM), the inhibitor in DMSO or DMSO as a control [5 % (v/v)], and filled up with assay buffer (25 mM Tris-HCl, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl_2 , pH 8.0) to a volume of 60 μL , incubated for 4 h (37 °C, 180 rpm), then treated with 60 μL of trypsin solution [50 mM Tris-HCl, 100 mM NaCl, 8 mM nicotinamide, 5.5 U/ μL trypsin, 6.7 % DMSO (v/v)] and incubated again (37 °C, 180 rpm, 20 min). The fluorescence intensity was then measured with a microplate reader (BMG Polarstar, $\lambda_{\text{EX}} = 390 \text{ nm}$, $\lambda_{\text{EM}} = 460 \text{ nm}$). To ensure initial state conditions, the conversion of ZMAL was adjusted to 10 – 30 % substrate conversion without inhibitor. A blank control without enzyme and a 100 % conversion control with the fluorescent metabolite AMC instead of ZMAL were performed. Inhibition rates were calculated in reference to the DMSO control after blank subtraction. All inhibition experiments were run at least twice in duplicates.

6.2. Theoretical Calculations

Docking^[40]

The 3D structures of inhibitors under study were generated from SMILES strings, and a subsequent energy minimisation was carried out using the MMFF94x force field implemented in Molecular Operating Environment System (MOE) 2014.10 (Chemical Computing Group, Montreal, Canada). All compounds were used in their neutral form. A maximum of 100 conformations were generated for each ligand using the Conformational Search module implemented in MOE. Sirt5 protein structure in complex with NAD⁺ and a succinyl-lysine based peptide was downloaded from the Protein Data Bank (PDB ID 3RIY)^[58]. The protein structures were prepared by using the Structure Preparation module in MOE. Hydrogen atoms were added, for titratable amino acids the protonation state was calculated using the Protonate 3D module in MOE. Protein structures were energy minimised using the AMBER99 force field^[184] using a tethering force constant of $(3/2) kT / 2$ ($\sigma = 0.5 \text{ \AA}$) for all atoms during the minimisation. AM1-BCC charges^[185] were used for the studied ligands. All molecules except the zinc ion were removed from the structures. Protein-ligand docking was performed using program GOLD5.6.^[186] Arg105 was used to define the size of the grid box (15 Å radius). 50 docking poses were calculated for each inhibitor. All other options were left at their default values. Using the docking setup the co-crystallised succinyl-lysine based peptide could be correctly docked with RMSD values below 1.5 Å as reported in a previous publication^{[62], [40]}

QM/MM-Simulations^[40]

The docking results using crystal structure 3RIY^[58] [of Sirt5] were applied as starting point for the following reaction path calculations. 3RIY contains a dimeric complex consisting of Sirt5, a histone tail peptide which contains a succinylated lysine (SLL), as well as NAD⁺. This histone tail peptide was used as reference for the natural substrates of Sirt5. For all four substrates, the reaction curve for the first two reaction steps was calculated *via* adiabatic mapping in a QM/MM setting using B3LYP-D3/def2-svp.9. As a first step, force field parameters for the systems were derived. For the protein was used the AMBERFF14SB^[187], for the zinc-finger the ZAFF^[188], and for nicotinamide adenine dinucleotide (NAD⁺) parameters derived by PAVELITIS *et al.*^[189] The substrates *N*-succinyllysine (SLL), balsalazide, **CG_150** and **CG_186** were parametrised with GAFF^[184] and AM1-BCC derived charges^[190] with the tool “antechamber”. The four systems were then solvated in a rectangular box of TIP3P water^[191] with 17.5 Å buffer in each direction. All these tasks were performed using “tleap” of the Amber16^[192] suite. Subsequently, the solvent was minimised for 10,000 steps and then the whole system for an entire 10,000 steps of Newton-Raphson. For those minimisations was used the simulation engine NAMD^[193]. For all following quantum mechanics/molecular mechanics (QM/MM)

calculations was used ChemShell^[194] that called the QM engine FermiONs++^[195-196]. The protein residues that were included in the QM sphere are Phe40, Arg75, His128, and Phe193. The QM sphere also contained all water molecules in the active pocket as well as the substrate/inhibitor, and the nicotinamide part of NAD⁺ up to O1'. All dangling bonds were saturated with hydrogens. This resulted in 164 atoms for SLL, 176 atoms for balsalazide, 175 atoms for **CG_150**, and 176 atoms for **CG_186**. The QM part was described at the B3LYP-D3/def2-svp^[197-203] level of theory. An unconstrained QM/MM minimisation was performed as last step before the adiabatic mapping calculations (constraint optimisations). The convergence criteria for all QM/MM optimisations were: 0.0005 H in the energy, 0.008 a₀ max step, 0.005 a₀ RMS step, 0.002 Ha₀⁻¹ max gradient, RMS 0.0013 Ha₀⁻¹ RMS gradient. The attack of the substrate on the cofactor was steered as a bond difference of the distance between the attacking carbonyl oxygen/thiourea sulfur and C1' and the distance between C1' and the leaving nitrogen atom. The product configuration with the lowest energy was then used to calculate the reverse path to the educts of the reaction. This cycle was repeated until the reaction energy and reaction barriers were very similar. For the second reaction step, the final product configuration was used as a starting point. The second reaction describes the intramolecular attack of O2' on the former carbonyl carbon. Here, His158 acts as a base accepting the hydrogen connected to O2'. The proton transfer is not explicitly included in the reaction coordinate resulting in at least one intermediate along the reaction progress. Again, the forward and backward paths were calculated until convergence was reached.^[40]

MD simulations

For the calculation of the $\Delta\Delta G$ values, the non-equilibrium alchemical approach^[172-173] in conjunction with molecular dynamics simulations was employed. Starting point of the system preparation was the structure of Sirt5 in a complex with NAD⁺ and with balsalazide docked in the active site.^[40] The AMBERFF14SB^[187] force field parameters for the protein, ZAFF^[188] parameters for the zinc finger and NAD⁺ parameters derived by PAVELITES *et al.*^[189] were utilised. The Sirt5 inhibitors were parametrised using the Generalized Amber Force Field (GAFF)^[184] in combination with AM1-BCC charges,^[185] calculated with Antechamber.^[204] The newly proposed inhibitors were paired up for the free energy calculations with balsalazide. For each pair of inhibitors, hybrid structures and topologies were generated with the pmx tool^[205-206] according to the single topology approach. The ligands and ligand–protein complexes were then placed in dodecahedral boxes of TIP3P^[191] water with 1.5 nm distance to the box edge and the systems were neutralised with sodium ions. The hybrid systems were simulated in the end states A (representing one of the ligands, usually balsalazide) and B (representing the second ligand) according to the following protocol.

The systems in state A and B were energy minimised using the steepest descent algorithm, followed by 250 ps of heating to a temperature of 298 K. Then 8 ns production runs were performed in an NPT ensemble with a time step of 2 fs. For temperature and pressure control during the molecular dynamics simulations, the velocity rescaling thermostat^[207] with a time constant of 0.1 ps and the Parrinello–Rahman barostat^[208] with a time constant of 5 ps and a target pressure of 1 bar, respectively, were employed. All bonds were constrained using the LINCS algorithm^[209]. The long-range electrostatics were evaluated using the Particle Mesh Ewald (PME)^[210-211] with a cutoff radius of 1.1 nm and a Fourier grid spacing of 0.12 nm. The van-der-Waals interactions were smoothly switched off between 1.0 and 1.1 nm. During the non-equilibrium transition simulations, the non-bonded interactions were treated with a modified soft-core potential.^[212] All simulations were performed with GROMACS 2019.^[213] The first 2 ns of the production runs were discarded to account for equilibration, then 80 snapshots were extracted from each trajectory as starting structures for the alchemical transitions. For each starting structure a non-equilibrium transition simulation from A to B (or vice versa) was performed for either 50 or 100 ps by gradually increasing the perturbation parameter λ from 0 to 1 (or vice versa). For the ligands **CG_111**, **CG_137**, **CG_48**, **CG_65** and **CG_150**, a transition simulation length of 100 ps was applied to improve the convergence. At each step of the nonequilibrium transition simulations, the derivatives of the Hamiltonian with respect to λ were recorded. The whole procedure, starting from the energy minimisation to the non-equilibrium transitions, was repeated 3 times both for the ligand-water systems and for the ligand-protein complexes.

The free energy difference (ΔG) between the states A and B was estimated from the 240 work values of the forward transitions and the 240 work values of the reverse transitions using Bennet's Acceptance Ratio (BAR)^[214-215] based on the Crooks Fluctuation Theorem.^[216-217] For the error estimation, the ΔG values, resulting from the different trajectories were computed and the standard deviation was calculated. The double free energy differences ($\Delta\Delta G$) were obtained by subtracting the ΔG between inhibitors in water from the ΔG of the enzyme-inhibitor complexes.

6.3. General procedures for compound preparation and characterisation

6.3.1. Solvents and reagents

All solvents were purchased from commercial sources and used without further purification. If needed, solvents were dried according to standard methods *via* distillation over drying agents and stored under N₂ atmosphere with activated molecular sieves. Standard vacuum line techniques were applied, and glassware was flame dried prior to use. Organic solvents were

dried during workup using anhydrous Na₂SO₄, MgSO₄ or by filtration through a phase separation paper (MN 617 WA, Ø125 mm, Macherey-Nagel).

6.3.2. Reactions, purification, and chromatography

Reactions were monitored *via* thin layer silica gel chromatography (TLC) using polyester sheets POLYGRAM SIL G/UV₂₅₄ coated with 0.2 mm silica gel (Macherey-Nagel). Plates were visualised using UV light (254 nm or 365 nm) or staining with KMnO₄, CAM (ceric ammonium molybdate), FeCl₃, bromocresol green, DNPH or ninhydrin reagents. Products were purified by flash column chromatography (normal-phase silica gel chromatography) using SiO₂ 60 (0.040 - 0.063 mm, 230 - 400 mesh ASTM) from Merck. HPLC analytical measurements for determination of the purities of the products were carried out detecting at 210 nm and 254 nm using the following methods:

Method 1:

Agilent Poroshell 120, EC-C18 2.7 µm (3.0 x 100 mm), injection vol. 10 µL, temp. 50 °C

- | | |
|---------------------------------|------------------------------|
| a) MeCN/water/THF | 40:59.9:0.1; flow 1.0 mL/min |
| b) MeCN/phosphate buffer pH 3.0 | 70:30; flow 0.8 mL/min |
| c) MeCN/phosphate buffer pH 3.0 | 50:50; flow 1.0 mL/min |
| d) MeCN/phosphate buffer pH 3.0 | 40:60; flow 1.0 mL/min |
| e) MeCN/phosphate buffer pH 3.0 | 30:70; flow 1.0 mL/min |
| f) MeCN/phosphate buffer pH 3.0 | 25:75; flow 1.0 mL/min |

Method 2:

Zorbax Eclipse Plus, C18 5.0 µm (4.6 x 150 mm), injection vol. 2 µL, temp. 50 °C

- | | |
|---------------------------------|---|
| a) MeOH/phosphate buffer pH 5.0 | gradient: 9/95 → 90/10; flow 1.0 mL/min |
|---------------------------------|---|

Method 3:

Synergi 4u Hydro-RP 80A, 5.0 µm (4.6 x 150 mm), injection vol. 20 µL, max. temp. 60 °C

- | | |
|--|--|
| a) (MeCN+0.05 % TFA)/(H ₂ O+0.05 % TFA) | gradient: 10/90 → 100/0; flow 2.0 mL/min |
|--|--|

Method 4:

Thermo Scientific Hypersil GOLD, 3.0 µm (2.1 x 50 mm), injection vol. 5 – 10 µL, temp. 31 °C

- | | |
|------------------------|---|
| a) MeCN/water+0.1 % FA | gradient: 10/90 → 99/1; flow 0.4 mL/min |
|------------------------|---|

6.3.3. Characterisation

NMR spectra (¹H-NMR, ¹³C-NMR, DEPT, COSY, HSQC, HMBC) were recorded with Avance III HD 400 MHz Bruker BioSpin and Avance III HD 500 MHz Bruker BioSpin (¹H-NMR: 400 MHz and 500 MHz, ¹³C-NMR: 101 MHz and 126 MHz) using the deuterated solvent stated. Chemical shifts (δ) are quoted in parts per million (ppm) and referenced to the residual solvent peak. Multiplicities are denoted as s-singlet, d-doublet, t-triplet, q-quartet and quin-quintet and

derivatives thereof (br denotes a broad resonance peak). Coupling constants J are given in Hz and round to the nearest 0.1 Hz. Infrared spectra were recorded from 4000 to 650 cm^{-1} on a PERKIN ELMER Spectrum BX-59343 FT-IR instrument. A Smiths Detection DuraSamp IR II Diamond ATR sensor was used for detection. The absorption bands are reported in wavenumbers [cm^{-1}]. High resolution mass spectra (HR-MS) were recorded using a Jeol Mstation 700 or JMS GCmate II Jeol instrument for electron impact ionisation (EI). Thermo Finnigan LTQ was used for electrospray ionisation (ESI). Melting points were measured with a Büchi Schmelzpunktapparat B-540 and are reported in $^{\circ}\text{C}$.

6.3.4. Standard synthetic protocols

General Procedure A – Hydrogenation protocol for the reduction of nitro groups and alkenes

The appropriate nitro compound or alkene (1.0 equivalents) was dissolved to a concentration of 0.3 M in dry MeOH and 10 % Pd/C (10 wt% on activated carbon) was added under N_2 atmosphere. Hydrogenation was performed under 1 bar H_2 pressure at room temperature for 3 h. Subsequently, the reaction mixture was filtered through a pad of celite and the solvent was removed *in vacuo*. If necessary, the crude product was purified by flash column chromatography (FCC) using the indicated eluent.

General Procedure B – Azo coupling reaction

The appropriate aniline (1.00 equivalents) was dissolved or suspended in water to a concentration of 0.3 M, before conc. HCl (3.40 equivalents) was added. The mixture was cooled to 0 $^{\circ}\text{C}$ and an ice-cold 2.0 M solution of NaNO_2 (1.03 equivalents) in water was added dropwise. The mixture was stirred for 1 h and then added dropwise to a solution of the corresponding phenol (1.03 equivalents) dissolved in water to a concentration of 0.6 M containing NaOH (2.10 equivalents) and Na_2CO_3 (1.60 equivalents) at 0 $^{\circ}\text{C}$. The reaction mixture was adjusted to pH 8 with 2 N aq. NaOH and stirred for 2 – 3 h (TLC monitoring), then poured into 0.5 M aq. HCl at 0 $^{\circ}\text{C}$. The precipitate obtained was collected by filtration, washed with water and suction dried to give the corresponding azobenzene in sufficient purity if not stated otherwise.

General Procedure C – Protection to acetonides of salicylates

A mixture of the appropriate salicylic acid derivative (1.0 equivalents) in TFA (16 equivalents), TFAA (3.0 equivalents) and acetone (5.0 equivalents) was heated to 90 $^{\circ}\text{C}$ for 16 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with sat. aq. NaHCO_3 (2 x) and brine (2 x).

The organic layer was dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC using the indicated eluent.

General Procedure D – Alkaline deprotection of esters and acetonides

The appropriate protected starting material (1.0 equivalents) was dissolved in THF to a concentration of 0.15 M followed by the addition of a solution of KOH (5.0 equivalents) in water (same volume as THF). The reaction mixture was stirred at the stated temperature for 1 – 3 h (TLC monitoring) and then acidified to pH 1 with 2 N aq. HCl at room temperature. It was proceeded with the indicated work-up procedure.

General Procedure E – Amide formation (I) with HATU and DIPEA

The appropriate carboxylic acid (1.5 equivalents) was dissolved in DMF to a concentration of 0.3 M. Then DIPEA (3.0 equivalents) was added, followed by HATU (1.5 equivalents). The reaction mixture was stirred at room temperature for 40 min. The appropriate amine (1.0 equivalents) was added, and the reaction mixture stirred at room temperature for 18 h. The reaction mixture was diluted with EtOAc and washed sequentially with water (2 x), 10 % aq. K₂CO₃ (1 x), brine (1 x) and 5 % aq. LiCl (1 x). The organic phase was dried using a phase separation paper and the filtrate was concentrated *in vacuo*. The crude product was then purified by FCC using the indicated eluent.

General Procedure F – Amide formation (II) with HOBt, EDC•HCl and DIPEA

The appropriate carboxylic acid (1.0 equivalents) and 1-hydroxybenzotriazole (1.1 equivalents) were dissolved in THF to a concentration of 0.3 M (with respect to the acid). EDC•HCl (1.1 equivalents) was added. If a semi-crystalline solid appeared, DCM (half the volume of THF) was added and a clear solution was obtained. The mixture was stirred for 10 min, then the appropriate amine (1.0 equivalents) and DIPEA (3.0 equivalents) were added. The mixture was stirred for 2 h at room temperature and then heated to reflux for 10 min. The reaction mixture was cooled to room temperature again, then partitioned between water and DCM. The aqueous phase was further extracted with DCM (3 x) and the combined organic layers were dried using a phase separation paper and the solvent removed *in vacuo*. The crude product was purified by FCC using the indicated eluent.

General Procedure G – Amide formation (III) with HOBt, EDC•HCl and Et₃N

The appropriate carboxylic acid/benzoic acid (1.0 equivalents), the corresponding amine (1.2 equivalents), EDC•HCl (1.2 equivalents) and HOBt (1.2 equivalents) were dissolved in dry DCM to a concentration of 0.3 – 0.4 M (with respect to the acid) under N₂ atmosphere. Subsequently, triethylamine (1.8 equivalents) was added, and the mixture was stirred at room

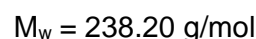
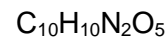
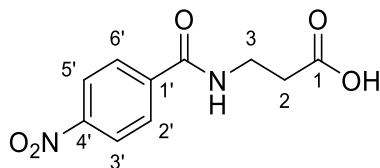
temperature for 24 h. The solution was diluted with ethyl acetate and washed with sat. aq. NH_4Cl (2 x) and water (1 x). The organic phase was dried using a phase separation paper and the solvent was evaporated. The crude product was then purified by FCC using the indicated eluent.

General Procedure H – Sulfonamide formation

The appropriate amine (1.0 equivalents) was dissolved in DCM to a concentration of 0.1 M. Then, pyridine (2.0 equivalents) and 5-chlorosulfonyl-2-hydroxybenzoic acid (**105**, 1.0 equivalents) were added at 0 °C under N_2 atmosphere. The mixture was stirred at 0 °C for 2 h and afterwards at room temperature until completion of the reaction (TLC monitoring). The solvent was removed *in vacuo* and the residue dissolved in CHCl_3 :isopropanol (3:1) and washed with 1 N aq. HCl (3 x). The solvent was then evaporated *in vacuo* and the crude product purified by FCC using the indicated eluent.

6.4. Compound preparation

6.4.1. Balsalazide and analogues thereof

3-(4-Nitrobenzamido)propanoic acid (2)^[78-79]

β -Alanine (2.00 g, 22.4 mmol) was added to a solution of 1 N aq. NaOH (50 mL) at 0 °C. After the addition of finely powdered 4-nitrobenzoyl chloride (**1**, 4.17 g, 22.4 mmol) the solution was stirred for 30 min at 0 °C and was then allowed to warm to room temperature over a period of 2 h. Subsequently, excessive starting material was filtered off and the remaining filtrate was adjusted to pH 2 – 3 through the addition of 1 N aq. HCl at 0 °C. The resulting precipitate was collected by filtration, washed with water (25 mL), and dried under reduced pressure. The crude product was recrystallised from hot acetone to give amide **2** (3.20 g, 13.4 mmol, 60 %) as a white solid.

R_f: 0.53 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 163 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.26 (s, 1H, COOH), 8.87 (t, *J* = 5.4 Hz, 1H, CONH), 8.34 – 8.29 (m, 2H, 3'-H and 5'-H), 8.08 – 8.03 (m, 2H, 2'-H and 6'-H), 3.48 (td, *J* = 7.1, 5.4 Hz, 2H, 3-H), 2.54 (t, *J* = 7.0 Hz, 2H, 2-H).

¹³C-NMR

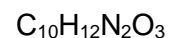
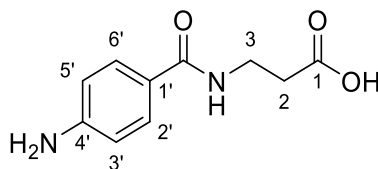
(126 MHz, (CD₃)₂SO): δ (ppm) = 172.8 (C-1), 164.6 (CONH), 149.0 (C-4'), 140.0 (C-1'), 128.7 (C-2' and C-6'), 123.5 (C-3' and C-5'), 35.8 (C-3), 33.5 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3374, 2526, 1714, 1625, 1590, 1545, 1519, 1408, 1348, 1304, 1277, 1207, 878, 851, 727, 608.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₀H₉N₂O₅⁻: 237.0517; found: 237.0519.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1c).

3-(4-Aminobenzamido)propanoic acid (3)^[78-79]



$M_w = 208.22$ g/mol

Prepared according to **General Procedure A** from nitro compound **2** (2.69 g, 11.3 mmol). Product **3** (1.76 g, 8.44 mmol, 75 %) was obtained as a white solid.

R_f: 0.17 (MeOH/DCM 5:95).

m.p.: 153 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.19 (s, 1H, COOH), 8.02 (t, $J = 5.5$ Hz, 1H, CONH), 7.57 – 7.52 (m, 2H, 2'-H and 6'-H), 6.54 – 6.49 (m, 2H, 3'-H and 5'-H), 5.70 – 5.42 (m, 2H, NH₂), 3.39 (td, $J = 7.2, 5.5$ Hz, 2H, 3-H), 2.46 (t, $J = 7.2$ Hz, 2H, 2-H).

¹³C-NMR

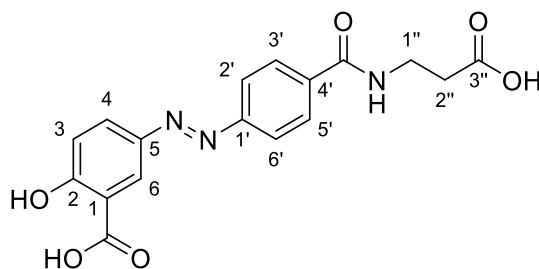
(101 MHz, (CD₃)₂SO): δ (ppm) = 173.0 (C-1), 166.2 (CONH), 151.6 (C-4'), 128.6 (C-2' and C-6'), 121.1 (C-1'), 112.5 (C-3' and C-5'), 35.4 (C-3), 34.1 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1714, 1618, 1595, 1557, 1514, 1340, 1319, 1213, 1197, 1183, 840.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₀H₁₁N₂O₃⁻: 207.0775; found: 207.0775.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1c).

(E)-5-((4-((2-Carboxyethyl)carbamoyl)phenyl)diazenyl)-2-hydroxybenzoic acid
(balsalazide)^[78-79]



$C_{17}H_{15}N_3O_6$

$M_w = 357.32$ g/mol

Prepared according to **General Procedure B** from amine **3** (100 mg, 0.480 mmol) and salicylic acid (68.3 mg, 0.495 mmol). The product balsalazide (146 mg, 0.409 mmol, 85 %) was obtained as a brown solid.

R_f: 0.53 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 239 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.29 (s, 2H, 1-COOH and 3''-COOH), 8.70 (t, $J = 5.4$ Hz, 1H, CONH), 8.36 (d, $J = 2.6$ Hz, 1H, 6-H), 8.09 (dd, $J = 8.9, 2.6$ Hz, 1H, 4-H), 8.02 (d, $J = 8.5$ Hz, 2H, 3'-H and 5'-H), 7.92 (d, $J = 8.5$ Hz, 2H, 2'-H and 6'-H), 7.15 (d, $J = 8.9$ Hz, 1H, 3-H), 3.49 (td, $J = 7.1, 5.4$ Hz, 2H, 1''-H), 2.54 (t, $J = 7.1$ Hz, 2H, 2''-H).

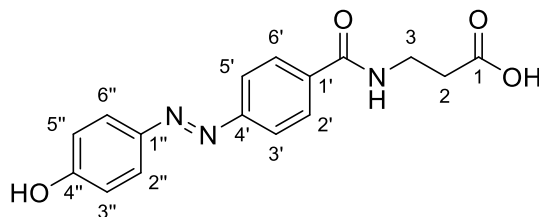
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3''), 171.2 (1-COOH), 165.5 (CONH), 164.3 (C-2), 153.3 (C-1'), 144.4 (C-5), 136.1 (C-4'), 128.9 (C-4), 128.5 (C-3' and C-5'), 126.1 (C-6), 122.1 (C-2' and C-6'), 118.5 (C-3), 114.2 (C-1), 35.7 (C-1''), 33.7 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3067, 1702, 1658, 1634, 1539, 1450, 1302, 1198, 856, 752.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{17}H_{14}N_3O_6^-$: 356.0888; found: 356.0889.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-3-(4-((4-Hydroxyphenyl)diazenyl)benzamido)propanoic acid (CG_21)C₁₆H₁₅N₃O₄M_w = 313.31 g/mol

Prepared according to **General Procedure B** from amine **3** (100 mg, 0.480 mmol) and phenol (**5**, 43.5 μ L, 0.495 mmol). The product **CG_21** (146 mg, 0.466 mmol, 97 %) was obtained as an orange solid.

R_f: 0.41 (EtOAc/hexanes+AcOH 65:35+1).

m.p.: 227 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.10 (s, 1H, COOH), 10.33 (s, 1H, OH), 8.67 (t, *J* = 5.4 Hz, 1H, CONH), 8.03 – 7.98 (m, 2H, 2'-H and 6'-H), 7.88 – 7.81 (m, 4H, 3'-H and 5'-H and 2''-H and 6''-H), 6.99 – 6.93 (m, 2H, 3''-H and 5''-H), 3.49 (td, *J* = 7.1, 5.4 Hz, 2H, 3-H), 2.54 (t, *J* = 7.1 Hz, 2H, 2-H).

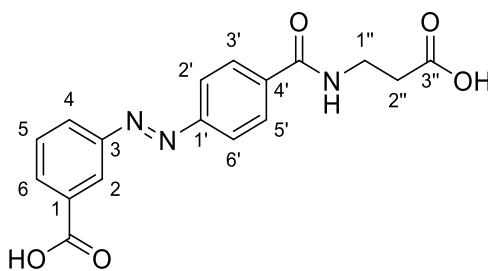
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (COOH), 165.5 (CONH), 161.4 (C-4''), 153.5 (C-4'), 145.3 (C-1''), 135.6 (C-1'), 128.4 (C-2' and C-6''), 125.2 (C-2'' and C-6'), 121.9 (C-3' and C-5'), 116.0 (C-3'' and C-5''), 35.7 (C-3), 33.8 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3330, 2923, 2854, 1703, 1587, 1541, 1203, 1139, 863, 841, 654.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₆H₁₄N₃O₄⁻: 312.0990; found: 312.0991.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1b).

(E)-3-((4-((2-Carboxyethyl)carbamoyl)phenyl)diazenyl)benzoic acid (CG_24)C₁₇H₁₅N₃O₅M_w = 341.32 g/mol

3-Aminobenzoic acid (**7**, 300 mg, 2.19 mmol) was dissolved in DCM (6.0 mL). A solution of Oxone[®] (1.35 g, 4.38 mmol) in water (24 mL) was added and the solution stirred under N₂ atmosphere for 3 h. The precipitate was collected by filtration and washed with water (20 mL) to give nitroso intermediate **8** (163 mg, 1.08 mmol, 49 %) as a beige solid. Without further purification it was dissolved in DMSO (2.0 mL) and a solution of amine **3** (150 mg, 0.720 mmol) in AcOH (0.25 mL) added. The reaction mixture was stirred at room temperature for 72 h. Water (15 mL) was added and the precipitate collected by filtration and washed with water (20 mL). The crude product was purified by FCC (MeOH/DCM+AcOH 2.5:97.5+1) to give azobenzene **CG_24** (37.0 mg, 0.108 mmol, 15 %) in an *E/Z* ratio of 90:10 (measured in DMSO-*d*₆) as an apricot solid. For NMR-spectroscopic analysis the compound was initially heated to 100 °C to generate only *E*-isomer. The probe was then allowed to reach room temperature and all spectra were recorded at this temperature.

R_f: 0.42 (EtOAc/hexanes+AcOH 65:35+1).

m.p.: 234 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 8.77 (t, *J* = 5.4 Hz, 1H, CONH), 8.41 (t, *J* = 1.8 Hz, 1H, 2-H), 8.13 (dt, *J* = 7.7, 1.4 Hz, 1H, 6-H), 8.11 – 8.08 (m, 1H, 4-H), 8.07 – 8.04 (m, 2H, 3'-H and 5'-H), 8.01 – 7.97 (m, 2H, 2'-H and 6'-H), 7.68 (t, *J* = 7.7 Hz, 1H, 5-H), 3.50 (td, *J* = 7.0, 5.4 Hz, 2H, 1''-H), 2.55 (t, *J* = 7.0 Hz, 2H, 2''-H).

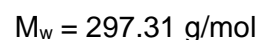
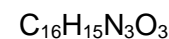
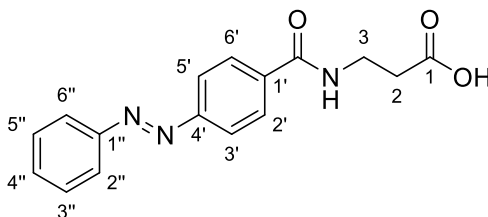
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 173.0 (C-3''), 167.3 (1-COOH), 165.4 (CONH), 153.2 (C-1'), 151.8 (C-3), 136.7 (C-4'), 135.7 (C-1), 132.3 (C-6), 129.5 (C-5), 128.5 (C-3' and C-5'), 126.4 (C-4), 122.5 (C-2' and C-6'), 122.3 (C-2), 35.8 (C-1''), 33.9 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3305, 3285, 2979, 2915, 1689, 1630, 1536, 1421, 1308, 1277, 1212, 856, 676.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₁₇H₁₄N₃O₅⁻: 340.0939; found: 340.0937.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a)

(E)-3-(4-(Phenyldiazenyl)benzamido)propanoic acid (CG_25)


Aniline (**4**, 300 mg, 3.22 mmol) was dissolved in DCM (9.0 mL). A solution of Oxone[®] (1.98 g, 6.44 mmol) in water (35 mL) was added and the solution stirred under N₂ atmosphere for 3 h. After this time, the reaction mixture was extracted with DCM (3 x 35 mL) and the combined organic layers dried over Na₂SO₄, filtered and concentrated *in vacuo* to give nitrosobenzene (**9**, 270 mg, 2.52 mmol, 78 %) as brown crystals. Without further purification nitrosobenzene (**9**, 70.1 mg, 0.655 mmol) and amine **3** (150 mg, 0.720 mmol) were added to AcOH (5.0 mL) and the mixture was stirred at room temperature for 72 h. The solution was diluted with water (10 mL), extracted with DCM (3 x 25 mL), dried over Na₂SO₄ and the solvent evaporated under reduced pressure. The crude product was purified by FCC (EtOAc/hexanes+AcOH 50:50+1) to give azobenzene **CG_25** (112 mg, 0.377 mmol, 58 %) in an *E/Z* ratio of 89:11 (measured in DMSO-*d*₆) as a brown solid. For NMR-spectroscopic analysis the compound was initially heated to 100 °C to generate only *E*-Isomer. The probe was then allowed to reach room temperature and all spectra were recorded at this temperature.

R_f: 0.26 (EtOAc/hexanes+AcOH 50:50+1).

m.p.: 207 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 8.74 (t, *J* = 5.4 Hz, 1H, CONH), 8.07 – 8.02 (m, 2H, 2'-H and 6'-H), 7.97 – 7.94 (m, 2H, 3'-H and 5'-H), 7.96 – 7.90 (m, 2H, 2''-H and 6''-H), 7.65 – 7.57 (m, 3H, 3''-H and 4''-H and 5''-H), 3.49 (td, *J* = 7.0, 5.4 Hz, 2H, 3-H), 2.56 – 2.51 (m, 2H, 2-H, collapses with DMSO).

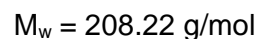
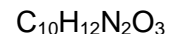
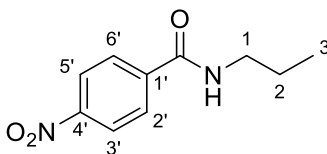
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 173.1 (C-1), 165.4 (CONH), 153.3 (C-4'), 151.9 (C-1'), 136.6 (C-1'), 132.0 (C-4''), 129.6 (C-3'' and C-5''), 128.5 (C-2' and C-6'), 122.8 (C-2'' and C-6''), 122.4 (C-3' and C-5'), 35.9 (C-3), 33.9 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3317, 1694, 1627, 1541, 1439, 1294, 1226, 861, 775, 684.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₆H₁₄N₃O₃⁻: 296.1041; found: 296.1041.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a)

4-Nitro-*N*-propylbenzamide (10*)

Propylamine (1.40 mL, 16.9 mmol) and 4-nitrobenzoyl chloride (**1**, 3.14 g, 16.9 mmol) were dissolved in 1 M aq. NaOH (33.8 mL) under N₂ atmosphere at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and was allowed to reach room temperature over a period of 6 h. The resulting white solid was collected by filtration and washed with water (25 mL). The aqueous phase was further extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (1 x 50 mL), dried over Na₂SO₄ and the solvent was removed *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 40:60) to give amide **10*** (1.42 g, 6.84 mmol, 40 %) as a pale yellow solid.

R_f: 0.64 (EtOAc/hexanes 20:80).

m.p.: 103 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.33 – 8.30 (m, 2H, 3'-H and 5'-H), 8.03 – 7.99 (m, 2H, 2'-H and 6'-H), 3.36 (t, *J* = 7.1 Hz, 2H, 1-H), 1.70 – 1.61 (m, 2H, 2-H), 0.99 (t, *J* = 7.4 Hz, 3H, 3-H).

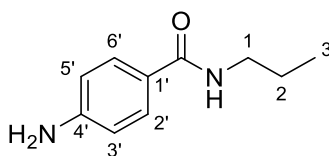
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 168.2 (CONH), 151.0 (C-1'), 141.7 (C-4'), 129.6 (C-2' and C-6'), 124.6 (C-3' and C-5'), 43.0 (C-1), 23.6 (C-2), 11.7 (C-3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3296, 2967, 2933, 1634, 1512, 1313, 1275, 1109, 867, 835, 687, 662.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₀H₁₁N₂O₃⁻: 207.0775; found: 207.0775.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1b)

4-Amino-N-propylbenzamide (11*) $C_{10}H_{14}N_2O$ $M_w = 178.24$ g/mol

Prepared according to **General Procedure A** from amide **10*** (1.00 g, 4.80 mmol). Product **11*** (774 mg, 4.34 mmol, 91 %) was obtained as a white solid.

R_f: 0.14 (EtOAc/hexanes 50:50).

m.p.: 73 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 7.60 – 7.57 (m, 2H, 2'-H and 6'-H), 6.68 – 6.64 (m, 2H, 3'-H and 5'-H), 3.29 (t, $J = 7.2$ Hz, 2H, 1-H), 1.61 (h, $J = 7.4$ Hz, 2H, 2-H), 0.97 (t, $J = 7.4$ Hz, 3H, 3-H).

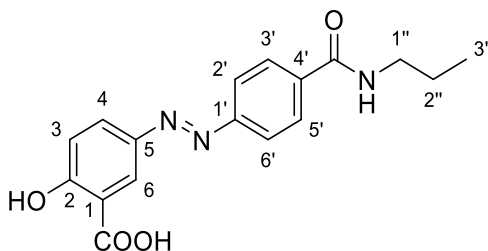
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 169.1 (CONH), 151.6 (C-1'), 128.4 (C-2' and C-6'), 122.1 (C-4'), 113.3 (C-3' and C-5'), 41.2 (C-1), 22.5 (C-2), 10.4 (C-3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3336, 3223, 2957, 2927, 2871, 2349, 1601, 1504, 1279, 1185, 848, 767, 573.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for C₁₀H₁₅N₂O⁺: 179.1179; found: 179.1178.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1c).

(E)-2-Hydroxy-5-((4-(propylcarbamoyl)phenyl)diazenyl)benzoic acid (CGLU_006*)C₁₇H₁₇N₃O₄M_w = 327.34 g/mol

Prepared according to **General Procedure B** from amine **11*** (153 mg, 0.842 mmol) and salicylic acid (121 mg, 0.867 mmol). Azobenzene **CGLU_006*** (242 mg, 0.746 mmol, 89 %) was obtained as a brown solid.

R_f: 0.69 (EtOAc+AcOH 100+1).

m.p.: 249 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 8.59 (t, *J* = 5.6 Hz, 1H, CONH), 8.32 (d, *J* = 2.6 Hz, 1H, 6-H), 8.02 – 7.98 (m, 2H, 3'-H and 5'-H), 7.94 (dd, *J* = 8.8, 2.6 Hz, 1H, 4-H), 7.89 – 7.86 (m, 2H, 2'-H and 6'-H), 6.94 (d, *J* = 8.8 Hz, 1H, 3-H), 3.24 (q, *J* = 6.9, 6.4 Hz, 2H, 1-H), 1.55 (h, *J* = 7.4 Hz, 2H, 2-H), 0.91 (t, *J* = 7.4 Hz, 3H, 3-H).

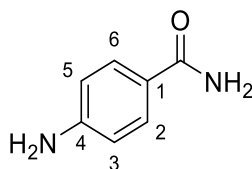
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 171.1 (1-COOH), 165.4 (C-2 and CONH), 153.3 (C-1'), 144.0 (C-5'), 136.2 (C-4'), 128.5 (C-4), 128.4 (C-3 and C-5), 126.1 (C-6), 122.0 (C-2' and C-6'), 118.4 (C-3), 115.2 (C-1), 41.1 (C-1), 22.4 (C-2), 11.5 (C-3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3330, 3068, 2925, 2554, 2342, 1634, 1535, 1293, 1204, 855, 695, 583.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₇H₁₈N₃O₄⁺: 328.1292; found: 328.1292.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1b).

4-Aminobenzamide (13*) $C_7H_8N_2O$ $M_w = 136.15 \text{ g/mol}$

Prepared according to **General Procedure A** from 4-nitrobenzamide (**12**, 1.01 g, 6.02 mmol). The product 4-aminobenzamide (**13***, 817 mg, 5.99 mmol, quant.) was obtained as an off-white solid.

R_f: 0.36 (EtOAc+AcOH 100+1).

m.p.: 179 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 7.66 – 7.62 (m, 2H, 2-H and 6-H), 6.68 – 6.64 (m, 2H, 3-H and 5-H).

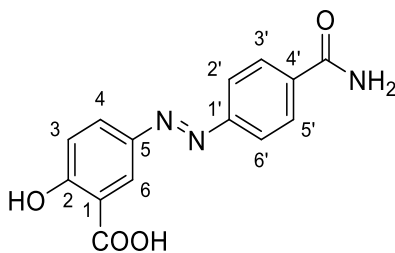
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 172.8 (CONH₂), 153.5 (C-1), 130.5 (C-2 and C-6), 122.2 (C-4), 114.6 (C-3 and C-5).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3463, 3196, 2359, 1592, 1556, 1517, 1391, 1094, 849, 775, 565.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for C₇H₉N₂O⁺: 137.0709; found: 137.0710.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1c).

(E)-5-((4-Carbamoylphenyl)diazenyl)-2-hydroxybenzoic acid (CGLU_005*)C₁₄H₁₁N₃O₄M_w = 285.26 g/mol

Prepared according to **General Procedure B** from amine **13*** (151 mg, 1.10 mmol) and salicylic acid (158 mg, 1.13 mmol). Further purification was carried out by dissolving the crude product in water (5.0 mL) and 1 M aq. NaOH (5.0 mL) and washing with EtOAc (3 x 50 mL). The aq. phase was then adjusted to pH 2 with 2 N aq. HCl and cooled to 0 °C. The precipitate obtained was collected by filtration, washed with water (15 mL) and suction dried to give azobenzene **CGLU_005*** (218 mg, 0.767 mmol, 70 %) as a brown solid.

R_f: 0.42 (EtOAc+AcOH 100+1).

m.p.: 279 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 13.31 (s, 1H, 1-COOH), 8.36 (d, *J* = 2.5 Hz, 1H, 6-H), 8.12 (s, 1H, OH), 8.08 (dd, *J* = 8.9, 2.7 Hz, 1H, 4-H), 8.07 – 8.04 (m, 2H, 3'-H and 5'-H), 7.93 – 7.89 (m, 2H, 2'-H and 6'-H), 7.50 (s, 1H, CONH₂), 7.13 (d, *J* = 8.9 Hz, 1H, 3-H).

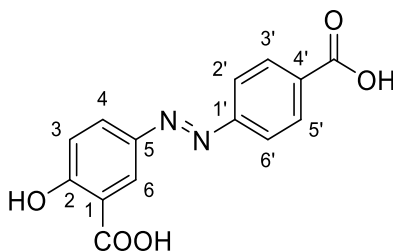
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 171.2 (COOH), 167.2 (CONH₂), 164.6 (C-2), 153.4 (C-1'), 144.3 (C-5), 136.0 (C-4'), 128.9 (C-4), 128.7 (C-3' and C-5'), 126.1 (C-6), 122.1 (C-2' and C-6'), 118.5 (C-3), 114.4 (C-1).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3690, 3649, 2816, 2519, 1677, 1483, 1423, 1232, 1117, 834, 804, 567.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₄H₁₀N₃O₄⁻: 284.0677; found: 284.0678.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1b).

(E)-5-((4-Carboxyphenyl)diazenyl)-2-hydroxybenzoic acid (CGLU_002*)C₁₄H₁₀N₂O₅M_w = 286.24 g/mol

Prepared according to **General Procedure B** from 4-aminobenzoic acid (**14**, 150 mg, 1.09 mmol) and salicylic acid (156 mg, 1.12 mmol). The product **CGLU_002*** (301 mg, 1.05 mmol, 97 %) was obtained as a dark red solid.

R_f: 0.77 (EtOAc+AcOH 100+1).

m.p.: 327 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 13.19 (s, 1H, 1-COOH or 4'-COOH), 8.37 (d, *J* = 2.5 Hz, 1H, 6-H), 8.14 – 8.11 (m, 2H, 3'-H and 5'-H), 8.11 (dd, *J* = 8.8, 2.6 Hz, 1H, 4-H), 7.96 – 7.92 (m, 2H, 2'-H and 6'-H), 7.18 (d, *J* = 8.9 Hz, 1H, 3-H).

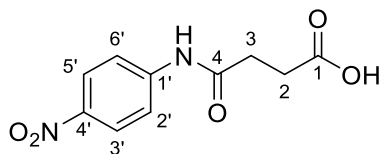
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 171.2 (1-COOH), 166.7 (4'-COOH), 164.2 (C-2), 154.3 (C-1'), 144.5 (C-5), 132.4 (C-4'), 130.6 (C-3' and C-5'), 129.1 (C-4'), 126.3 (C-6'), 122.4 (C-2' and C-6'), 118.5 (C-3), 113.9 (C-1).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3070, 2847, 2338, 2305, 1665, 1308, 1289, 1199, 864, 774, 700, 660, 565.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₄H₉N₂O₅⁻: 285.0517; found: 285.0522.

Purity (HPLC): 254 nm: >95 % (method 1b).

4-((4-Nitrophenyl)amino)-4-oxobutanoic acid (**17**)C₁₀H₁₀N₂O₅M_w = 238.20 g/mol

To 4-nitroaniline (**15**, 1.00 g, 7.24 mmol) dissolved in acetone (10 mL) was added succinic anhydride (**16**, 725 mg, 7.24 mmol) and the mixture heated to reflux for 18 h. The suspension was allowed to cool to room temperature and the precipitate collected by filtration and dried *in vacuo*. Product **17** (1.17 g, 4.89 mmol, 68 %) was obtained as a white solid.

R_f: 0.39 (EtOAc/hexanes 70:30).

m.p.: 206 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.18 (s, 1H, COOH), 10.59 (s, 1H, CONH), 8.24 – 8.18 (m, 2H, 3'-H and 5'-H), 7.85 – 7.79 (m, 2H, 2'-H and 6'-H), 2.63 (td, *J* = 6.2, 5.5, 1.2 Hz, 2H, 2-H), 2.54 (ddd, *J* = 7.2, 6.1, 1.3 Hz, 2H, 3-H).

¹³C-NMR

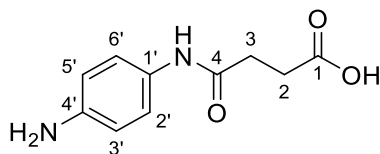
(101 MHz, (CD₃)₂SO): δ (ppm) = 173.7 (C-1), 171.2 (C-4), 145.4 (C-1'), 142.0 (C-4'), 125.0 (C-3' and C-5'), 118.5 (C-2' and C-6'), 31.3 (C-2), 28.5 (C-3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3341, 3127, 1697, 1596, 1542, 1491, 1330, 1146, 1107, 854, 709.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₀H₉N₂O₅: 237.0517; found: 237.0515.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

4-((4-Aminophenyl)amino)-4-oxobutanoic acid (18)



$C_{10}H_{12}N_2O_3$

$M_w = 208.22$ g/mol

Prepared according to **General Procedure A** from nitro compound **17** (600 mg, 2.52 mmol). Amine **18** (363 mg, 1.74 mmol, 69 %) was obtained as pale purple solid.

R_f: 0.29 (MeOH/DCM+AcOH 10:90+1).

m.p.: 188 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 9.50 (s, 1H, CONH), 7.21 – 7.18 (m, 2H, 2'-H and 6'-H), 6.50 – 6.46 (m, 2H, 3'-H and 5'-H), 5.04 (s, 2H, NH₂), 2.48 – 2.46 (m, 4H, 2-H and 3-H).

¹³C-NMR

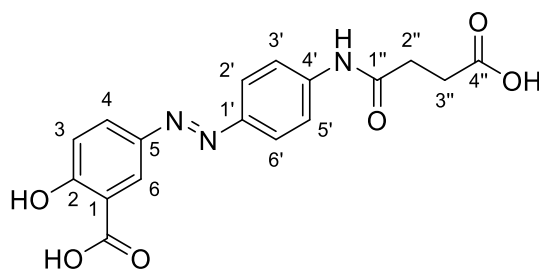
(126 MHz, (CD₃)₂SO): δ (ppm) = 173.9 (C-1), 168.9 (CONH), 144.5 (C-4'), 128.6 (C-1'), 120.7 (C-2' and C-6'), 113.8 (C-3' and C-5'), 30.8 (C-3), 29.0 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3291, 1660, 1601, 1541, 1511, 1408, 1358, 1223, 1174, 1100, 914, 816, 696.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₀H₁₁N₂O₃⁻: 207.0775; found: 207.0774.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

(E)-5-((4-(3-Carboxypropanamido)phenyl)diazenyl)-2-hydroxybenzoic acid (CG_186)



$C_{17}H_{15}N_3O_6$

$M_w = 357.32$ g/mol

Prepared according to **General Procedure B** from amine **18** (100 mg, 0.480 mmol) and salicylic acid (68.3 mg, 0.495 mmol). The crude product was purified by FCC (MeOH/DCM+AcOH 5:95+1) to give azobenzene **CG_186** (52.9 mg, 0.148 mmol, 31 %) as a brown solid.

R_f: 0.18 (MeOH/DCM+AcOH 5:95+1)

m.p.: 262 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.17 (s, 1H, 1-COOH or 3''-COOH), 10.25 (s, 1H, CONH), 8.25 (s, 1H, 6-H), 7.76 (s, 5H, 2', 3', 4', 6'- and 4-H), 6.78 (d, $J = 8.7$ Hz, 1H, 3-H), 2.61 (t, $J = 6.6$ Hz, 2H, 2''-H), 2.55 (d, $J = 6.1$ Hz, 2H, 3''-H).

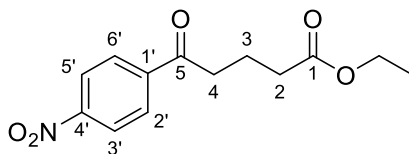
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 173.8 (C-4''), 170.9 (1-COOH), 170.4 (CONH), 167.8 (C-2), 147.7 (C-1'), 142.7 (C-5), 141.0 (C-4'), 126.4 (C-4), 125.5 (C-6), 122.8 (C-2' and C-6'), 119.1 (C-3' and C-5'), 117.5 (C-3), 114.0 (C-1), 31.1 (C-2''), 28.7 (C-3'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3328, 2924, 2340, 1674, 1593, 1527, 1484, 1405, 1322, 1247, 1174, 912, 840, 796.

HR-MS (EI): $m/z = [M-H]^-$ calcd for C₁₇H₁₄N₃O₆⁻: 356.0888; found: 356.0887.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 5-(4-nitrophenyl)-5-oxopentanoate (21)^[88]C₁₃H₁₅NO₅M_w = 265.27 g/mol

4-Nitroacetophenone (**19**, 5.00 g, 30.3 mmol) was dissolved in piperidine (15.0 mL, 151 mmol) and hexane (125 mL) was added. The mixture was stirred at 0 °C for 10 min. Then, TiCl₄ (1.99 mL, 18.2 mmol) was added slowly over 5 min. The reaction was stirred for 30 min at 0 °C and further 24 h at room temperature. Then, solids were filtered off and washed with hexane (50 mL). The filtrate was concentrated *in vacuo*. The resulting enamine **20** was obtained as a brown oil (5.55 g, 23.9 mmol, 79 %). Enamine **20** (5.00 g, 21.5 mmol) was dissolved in dry EtOH (10 mL). The solution was stirred under N₂ atmosphere at room temperature and ethyl acrylate (2.82 mL, 25.8 mmol) was added. Then the reaction was stirred for 10 days. After that, water (25 mL), AcOH (7.0 mL) and EtOAc (10 mL) were added and the reaction was heated to 45 °C for 1 h. More EtOAc (30 mL) was then added and the phases separated. The organic phase was washed with 1 N aq. HCl (2 x 25 mL) and water (25 mL). The organic layer was dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (toluene+AcOH 100+1) to give ketone **21** (1.59 g, 6.01 mmol, 28 %) as a pale yellow solid.

R_f: 0.25 (toluene+AcOH 100+1).

m.p.: 37 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.33 – 8.29 (m, 2H, 3'-H and 5'-H), 8.14 – 8.10 (m, 2H, 2'-H and 6'-H), 4.15 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 3.11 (t, *J* = 7.1 Hz, 2H, 4-H), 2.45 (t, *J* = 7.0 Hz, 2H, 2-H), 2.09 (p, *J* = 7.1 Hz, 2H, 3-H), 1.26 (t, *J* = 7.1 Hz, 3H, CH₃).

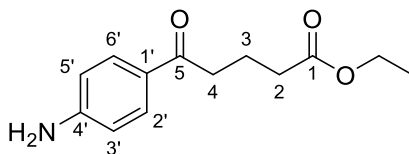
¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 198.0 (C-5), 173.2 (C-1), 150.5 (C-4'), 141.3 (C-1'), 129.2 (C-2' and C-6'), 124.0 (C-3' and C-5'), 60.7 (CH₂CH₃), 38.2 (C-4), 33.2 (C-2), 19.2 (C-3), 14.4 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2911, 1728, 1686, 1605, 1525, 1345, 1171, 855, 737, 687.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₃H₁₄NO₅⁻: 264.0877; found: 264.0879.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Ethyl 5-(4-aminophenyl)-5-oxopentanoate (22a)C₁₃H₁₇NO₃M_w = 235.28 g/mol

Prepared according to **General Procedure A** from ketone **21** (500 mg, 1.88 mmol). The crude product was purified by FCC (EtOAc/hexanes 40:60) to give amine **22a** (188 mg, 0.799 mmol, 42 %) as a white solid.

R_f: 0.38 (EtOAc/hexanes 40:60).

m.p.: 75 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.83 – 7.79 (m, 2H, 2'-H and 6'-H), 6.66 – 6.62 (m, 2H, 3'-H and 5'-H), 4.13 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 2.94 (t, *J* = 7.2 Hz, 2H, 4-H), 2.41 (t, *J* = 7.2 Hz, 2H, 2-H), 2.04 (p, *J* = 7.3 Hz, 2H, 3-H), 1.25 (t, *J* = 7.1 Hz, 3H, CH₃).

¹³C-NMR

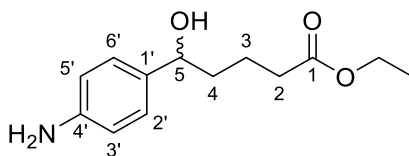
(101 MHz, CDCl₃): δ (ppm) = 197.8 (C-5), 173.6 (C-1), 151.2 (C-1'), 130.7 (C-2' and C-6'), 127.7 (C-4'), 113.9 (C-3' and C-5'), 60.5 (CH₂CH₃), 37.0 (C-4), 33.8 (C-2), 20.0 (C-3), 14.4 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3449, 3358, 3249, 2946, 1713, 1656, 1589, 1560, 1346, 1266, 1206, 1167, 1030, 979, 829, 755.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₃H₁₇NO₃⁺: 235.1203; found: 235.1200.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Ethyl 5-(4-aminophenyl)-5-hydroxypentanoate (22b)



$C_{13}H_{19}NO_3$

$M_w = 237.30$ g/mol

Prepared according to **General Procedure A** from ketone **21** (500 mg, 1.88 mmol). The crude product was purified by FCC (EtOAc/hexanes 50:50) to give product **22b** (291 mg, 1.23 mmol, 65 %) as a yellow oil.

R_f: 0.30 (EtOAc/hexanes 50:50).

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.17 – 7.09 (m, 2H, 2'-H and 6'-H), 6.70 – 6.62 (m, 2H, 3'-H and 5'-H), 4.56 (dd, $J = 7.3, 5.3$ Hz, 1H, 5-H), 4.11 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 2.31 (ddd, $J = 8.5, 5.5, 1.9$ Hz, 2H, 2-H), 1.86 – 1.52 (m, 4H, 3-H and 4-H), 1.24 (t, $J = 7.1$ Hz, 3H, CH₃).

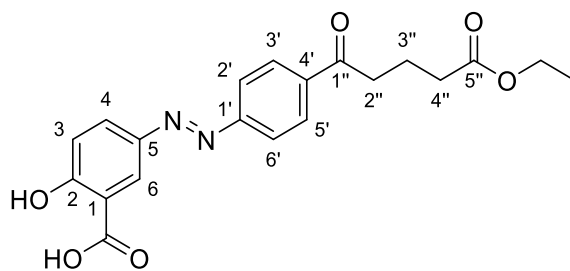
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 173.8 (C-1), 146.1 (C-4'), 134.7 (C-1'), 127.2 (C-2' and C-6'), 115.2 (C-3' and C-5'), 74.1 (C-5), 60.4 (CH₂CH₃), 38.2 (C-4), 34.2 (C-2), 21.5 (C-3) 14.4 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3366, 2926, 2865, 1719, 1614, 1517, 1277, 1177, 1025, 829.

HR-MS (EI): $m/z = [M-H_2]^+$ calcd for C₁₃H₁₇NO₃: 235.1219; found: 235.1203.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-5-((4-(5-Ethoxy-5-oxopentanoyl)phenyl)diazenyl)-2-hydroxybenzoic acid (23)C₂₀H₂₀N₂O₆M_w = 384.39 g/mol

Prepared according to **General Procedure B** from amine **22a** (169 mg, 0.718 mmol) and salicylic acid (102 mg, 0.740 mmol). The crude product was purified by FCC (EtOAc/hexanes+AcOH 30:70+1) to give azobenzene **23** (118 mg, 0.307 mmol, 43 %) as an orange solid.

R_f: 0.30 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 158 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 8.37 (d, *J* = 2.5 Hz, 1H, 6-H), 8.16 – 8.13 (m, 2H, 3'-H and 5'-H), 8.13 – 8.10 (m, 1H, 4-H), 7.97 – 7.93 (m, 2H, 2'-H and 6'-H), 7.17 (d, *J* = 8.9 Hz, 1H, 3-H), 4.06 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 3.13 (t, *J* = 7.2 Hz, 2H, 2''-H), 2.39 (t, *J* = 7.5 Hz, 2H, 4''-H), 1.89 (p, *J* = 7.3 Hz, 2H, 3''-H), 1.18 (t, *J* = 7.1 Hz, 3H, CH₃).

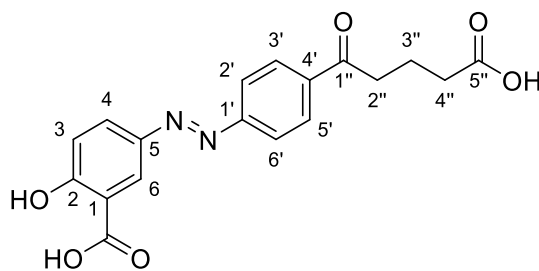
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 198.9 (C-1''), 172.7 (C-5''), 171.2 (1-COOH), 164.3 (C-2), 154.2 (C-1'), 144.5 (C-5), 137.8 (C-4'), 129.2 (C-3' and C-5'), 129.1 (C-4), 126.4 (C-6), 122.5 (C-2' and C-6'), 118.6 (C-3), 114.0 (C-1), 59.8 (CH₂CH₃), 37.3 (C-2''), 32.7 (C-4''), 19.1 (C-3''), 14.1 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1676, 1580, 1289, 1183, 1167, 986, 805, 758, 685, 577.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₂₀H₁₉N₂O₆⁻: 383.1249; found: 383.1251.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-5-((4-(4-Carboxybutanoyl)phenyl)diazenyl)-2-hydroxybenzoic acid (CG_64)C₁₈H₁₆N₂O₆M_w = 356.33 g/mol

Azobenzene **23** (80.0 mg, 0.208 mmol) was dissolved in MeOH (2.1 mL). KOH (117 mg, 2.08 mmol) was added, and the reaction mixture was stirred at room temperature for 10 min. The solvent was removed *in vacuo* and the resulting crude product was resuspended in EtOAc (20 mL) and treated with 1 N aq. HCl (10 mL). The organic phase was dried over Na₂SO₄ and concentrated *in vacuo* to give product **CG_64** (74.0 mg, 0.208 mmol, quant.) as an orange solid.

R_f: 0.30 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 207 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.08 (s, 2H, 1-COOH and 5''-COOH), 8.37 (d, *J* = 2.5 Hz, 1H, 6-H), 8.16 – 8.13 (m, 2H, 3'-H and 5'-H), 8.12 (dd, *J* = 8.9, 2.6 Hz, 1H, 4-H), 7.98 – 7.92 (m, 2H, 2'-H and 6'-H), 7.17 (d, *J* = 8.9 Hz, 1H, 3-H), 3.13 (t, *J* = 7.2 Hz, 2H, 2''-H), 2.33 (t, *J* = 7.4 Hz, 2H, 4''-H), 1.86 (p, *J* = 7.3 Hz, 2H, 3''-H).

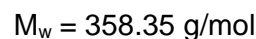
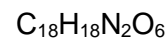
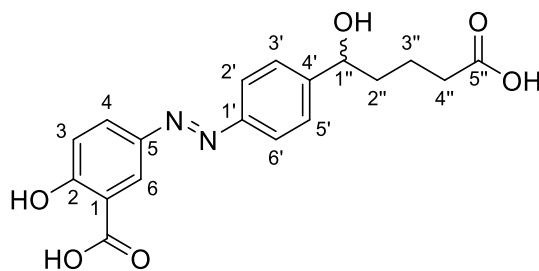
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 199.1 (C-1''), 174.3 (C-5''), 171.2 (1-COOH), 164.2 (C-2), 154.2 (C-1'), 144.5 (C-5), 137.8 (C-4'), 129.3 (C-3' and C-5'), 129.1 (C-4), 126.4 (C-6), 122.5 (C-2' and C-6'), 118.6 (C-3), 114.0 (C-1), 37.4 (C-2''), 32.8 (C-4''), 19.2 (C-3'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2846, 1676, 1654, 1450, 1407, 1309, 1201, 1153, 973, 844, 681, 576.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₈H₁₅N₂O₆⁻: 355.0936; found: 355.0939.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-5-((4-(4-Carboxy-1-hydroxybutyl)phenyl)diazenyl)-2-hydroxybenzoic acid (CG_65)


Prepared according to **General Procedure B** from amine **22b** (151 mg, 0.638 mmol) and salicylic acid (90.7 mg, 0.657 mmol). The crude product was purified by FCC (EtOAc/hexanes+AcOH 40:60+1) to give azobenzene **CG_65** (82.6 mg, 0.231 mmol, 36 %) as a reddish oil.

R_f: 0.18 (EtOAc/hexanes+AcOH 40:60+1).

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.03 (s, 2H, 1-COOH and 5''-COOH), 8.32 (d, $J = 2.5$ Hz, 1H, 6-H), 8.05 (dd, $J = 8.9, 2.5$ Hz, 1H, 4-H), 7.85 – 7.81 (m, 2H, 2'-H and 6'-H), 7.53 – 7.49 (m, 2H, 3'-H and 5'-H), 7.12 (d, $J = 8.9$ Hz, 1H, 3-H), 4.62 (dd, $J = 7.3, 4.6$ Hz, 1H, 1''-H), 2.23 (q, $J = 7.3$ Hz, 2H, 4''-H), 1.71 – 1.43 (m, 4H, 2''-H and 3''-H).

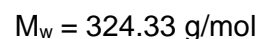
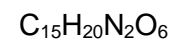
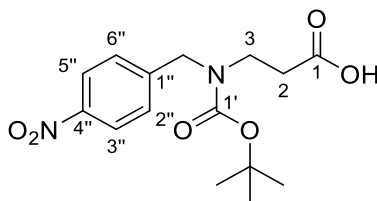
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 174.5 (C-5''), 171.3 (1-COOH), 163.8 (C-2), 150.8 (C-1'), 149.6 (C-4'), 144.4 (C-5), 128.7 (C-4), 126.7 (C-3' and C-5'), 125.5 (C-6), 122.1 (C-2' and C-6'), 118.3 (C-3), 114.1 (C-1), 71.6 (C-1''), 38.5 (C-2''), 33.6 (C-4''), 21.0 (C-3'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2865, 1731, 1660, 1589, 1483, 1288, 1237, 1205, 1174, 1023, 985, 843, 683, 576.

HR-MS (ESI): $m/z = [\text{M-H}]^-$ calcd for C₁₈H₁₇N₂O₆⁻: 357.1092; found: 357.1097.

Purity (HPLC): could not be determined due to instability of product.

3-((*tert*-Butoxycarbonyl)(4-nitrobenzyl)amino)propanoic acid (25***)^[89]**

4-Nitrobenzaldehyde (**24**, 800 mg, 5.29 mmol) and β-alanine (470 mg, 5.27 mmol) were dissolved in MeOH (50 mL) under N₂ atmosphere. AcOH (605 μL, 10.6 mmol) was added dropwise and the solution was stirred for 3 h. NaBH₃CN (366 mg, 5.82 mmol) was added in one portion and the reaction mixture was stirred for another 2 h. The reaction was quenched with 2 M aq. HCl until the pH of the solution reached 1 – 2. Afterwards, the pH was adjusted to pH 7 with triethylamine and the solvent was removed *in vacuo*. The crude product, di-*tert*-butyl dicarbonate (2.17 g, 9.92 mmol) and DMAP (69.0 mg, 0.564 mmol) were then dissolved in MeCN (50 mL) under N₂ atmosphere. Triethylamine (2.10 mL, 15.9 mmol) was added dropwise, and the reaction mixture was stirred for 24 h at room temperature. The solvent was removed *in vacuo* and the obtained precipitate was suspended in 2 M aq. HCl (20 mL) and extracted with EtOAc (3 x 50 mL). The organic phase was washed with 0.1 M aq. HCl (25 mL), water (25 mL) and brine (25 mL), dried over Na₂SO₄ and the solvent removed *in vacuo*. The obtained crude product was purified by FCC (acetone/hexanes+AcOH 10:90+1) to give product **25*** (1.21 g, 3.37 mmol, 71 %) as an orange oil.

R_f: 0.32 (acetone/hexanes+AcOH 10:90+1).

¹H-NMR

(500 MHz, (CD₃)₂SO): mixture of rotamers in a 1:1.25 ratio

δ (ppm) = 12.12 (s, 1H, 1-COOH), 8.22 (d, *J* = 8.5 Hz, 2H, 3-H and 5-H), 7.49 – 7.45 (m, 2H, 2-H and 6-H), 4.53 (s, 2H, Ar-CH₂), 3.45 – 3.38 (m, 2H, 3-H, collapses with H₂O), 2.48 – 2.43 (m, 2H, 2-H), 1.47 – 1.25 (m, 9H, C(CH₃)₃).

¹³C-NMR

(126 MHz, (CD₃)₂SO): mixture of rotamers in a 1:1.25 ratio

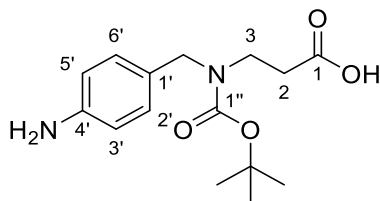
δ (ppm) = 172.8 (C-1), 154.9 (C-1'), 150.8 (C-4), 146.6 (C-1), 128.2 (C-2 and C-6), 123.7 (C-3 and C-5), 79.4 (C(CH₃)₃), 49.4 (Ar-CH₂), 43.3 (C-3), 33.4 (C-2), 27.9 (C(CH₃)₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2981, 2933, 1722, 1648, 1516, 1346, 1278, 1214, 1164, 990, 848, 733.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{15}H_{19}N_2O_6^-$: 323.1249; found: 323.1250.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1a)

3-((4-Aminobenzyl)(*tert*-butoxycarbonyl)amino)propanoic acid (26*)



$C_{15}H_{22}N_2O_2$

$M_w = 294.35 \text{ g/mol}$

Prepared according to **General Procedure A** from nitro compound **25*** (806 mg, 2.49 mmol). Amine **26*** (671 mg, 2.27 mmol, 92 %) was obtained as a dark brown oil.

R_f: 0.23 (EtOAc/hexanes 50:50).

¹H-NMR

(500 MHz, (CD₃)₂SO): mixture of rotamers

δ (ppm) = 6.89 (d, $J = 8.0 \text{ Hz}$, 2H, 2'-H and 6'-H), 6.51 (d, $J = 8.0 \text{ Hz}$, 2H, 3'-H and 5'-H), 5.00 (br s, 2H, NH₂), 4.19 (s, 2H, Ar-CH₂), 3.28-3.20 (m, 2H, 3-H), 2.37 – 2.31 (m, 2H, 2-H), 1.40 (s, 9H, C(CH₃)₃).

¹³C-NMR

(126 MHz, (CD₃)₂SO): mixture of rotamers

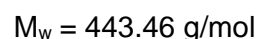
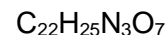
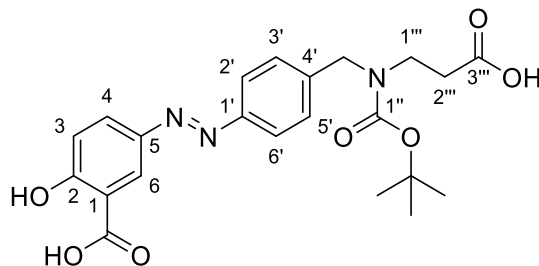
δ (ppm) = 173.0 (C-1), 154.8 (C-1), 147.8 (C-4'), 128.5 (C-2' and C-6'), 125.1 (C-1'), 113.8 (C-3' and C-5'), 78.7 (C(CH₃)₃), 48.9 (Ar-CH₂), 42.0 (C-3), 33.3 (C-2), 28.1 (C(CH₃)₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2976, 2938, 1671, 1517, 1477, 1415, 1366, 1248, 1159, 1119, 1024, 872, 815, 778.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₅H₂₁N₂O₄⁻: 293.1507; found: 293.1508.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1a).

(E)-5-((4-(((*tert*-Butoxycarbonyl)(2-carboxyethyl)amino)methyl)phenyl)diazenyl)-2-hydroxybenzoic acid (27*)



Prepared according to **General Procedure B** from amine **26*** (300 mg, 1.02 mmol) and salicylic acid (144 mg, 1.04 mmol). Further purification was carried out by dissolving the crude product in water (5.0 mL) and 1 M aq. NaOH (5.0 mL) and washing with EtOAc (3 x 50 mL). The aq. phase was then adjusted to pH 2 with 2 N aq. HCl and cooled to 0 °C. The obtained precipitate was collected by filtration, washed with water (15 mL), suction dried and purified by FCC (EtOAc/hexanes+AcOH 30:70+1) to give azobenzene **27*** (113 mg, 0.254 mmol, 25 %) as a red solid.

R_f: 0.77 (EtOAc+AcOH 100+1).

m.p.: 159 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.16 (s, 2H, 1-COOH and 3'''-COOH), 8.33 (d, *J* = 2.6 Hz, 1H, 6-H), 8.07 (dd, *J* = 8.9, 2.6 Hz, 1H, 4-H), 7.86 (d, *J* = 8.1 Hz, 2H, 2'-H and 6'-H), 7.44 – 7.39 (m, 2H, 3'-H and 5'-H), 7.15 (d, *J* = 8.9 Hz, 1H, 3-H), 4.50 (s, 2H, Ar-CH₂), 3.40 (s, 2H, 1'''-H, collapses with H₂O), 2.49 – 2.43 (m, 1H, 2'''-H, collapses with DMSO), 1.41 (d, *J* = 34.2 Hz, 9H, C(CH₃)₃).

¹³C-NMR

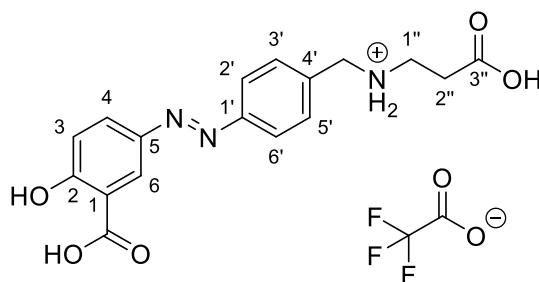
(101 MHz, (CD₃)₂SO): δ (ppm) = 172.8 (C-3'''), 171.30 (1-COOH), 163.8 (C-2), 151.0 (C-1'), 144.4 (C-5), 128.8 (C-4), 128.1 (C-3' and C-5'), 125.6 (C-6), 122.6 (C-2' and C-6'), 118.4 (C-3), 114.0 (C-1), 79.3 (C(CH₃)₃), 49.3 (Ar-CH₂), 34.5 (C-1'''), 33.3 (C-2'''), 28.0 (C(CH₃)₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2930, 2359, 2301, 1699, 1672, 1649, 1433, 1412, 1202, 1159, 858, 837, 676.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₂₂H₂₄N₃O₇⁻: 442.1620; found: 442.1642.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-5-((4-(((2-Carboxyethyl)amino)methyl)phenyl)diazenyl)-2-hydroxybenzoic acid trifluoroacetic acid salt (CG_48)



$C_{17}H_{17}N_3O_5 \cdot CF_3CO_2H$

$M_w = 457.36 \text{ g/mol}$

Azobenzene **27*** (91.0 mg, 0.205 mmol) was dissolved in DCM (1.2 mL) and TFA (0.80 mL) was added dropwise. The reaction mixture was stirred for 2 h at room temperature. After this time, the product was concentrated *in vacuo* to give TFA-salt **CG_48** (93.5 mg, 0.204 mmol, 99 %) as a reddish oil which solidified upon standing in the cold (2 – 8 °C).

R_f: 0.37 (MeOH/hexanes 20:80).

m.p.: 85 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.73 (s, 2H, 1-COOH and 3''-COOH), 8.99 (s, 2H, NH₂), 8.35 (d, $J = 2.6$ Hz, 1H, 6-H), 8.07 (dd, $J = 8.9, 2.6$ Hz, 1H, 4-H), 7.96 – 7.90 (m, 2H, 2'-H and 6'-H), 7.72 – 7.66 (m, 2H, 3'-H and 5'-H), 7.14 (d, $J = 8.9$ Hz, 1H, 3-H), 4.29 (s, 2H, Ar-CH₂), 3.18 (t, $J = 7.3, 6.6$ Hz, 2H, 1''-H), 2.69 (t, $J = 7.3$ Hz, 2H, 2''-H).

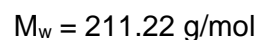
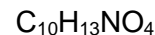
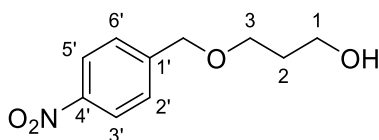
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 171.6 (C-3''), 171.2 (1-COOH), 164.4 (C-2), 158.1 (q, CF₃COO⁻), 152.1 (C-1'), 144.2 (C-5), 134.5 (C-4'), 131.1 (C-3' and C-5'), 128.8 (C-4), 125.9 (C-6), 122.5 (C-2' and C-6'), 118.4 (C-3), 116.4 (q, CF₃COO⁻) 114.4 (C-1), 49.7 (Ar-CH₂), 42.3 (C-1''), 30.4 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3041, 2846, 1666, 1609, 1589, 1428, 1173, 1139, 838, 797, 721, 680.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₇H₁₆N₃O₅⁻: 342.1095; found: 342.1095.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

3-((4-Nitrobenzyl)oxy)propan-1-ol (29^{})**^[90]

4-Nitrobenzyl bromide (**28**, 1.81 g, 8.39 mmol) and KOH (525 mg, 9.36 mmol) were dissolved in 1,3-propanediol (20 mL) under N₂ atmosphere. The reaction mixture was stirred for 22 h at 80 °C. After cooling to room temperature, the mixture was diluted with water (50 mL), extracted with DCM (3 x 75 mL), dried over Na₂SO₄ and the solvent was removed *in vacuo*. The obtained crude product was purified by FCC (EtOAc/hexanes 20:80) to give alcohol **29^{**}** (1.15 g, 5.45 mmol, 65 %) as a yellow oil.

R_f: 0.28 (EtOAc/hexanes 50:50).

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 8.23 – 8.19 (m, 2H, 3'-H and 5'-H), 7.61 – 7.57 (m, 2H, 2'-H and 6'-H), 4.60 (s, 2H, Ar-CH₂), 4.43 (t, *J* = 5.2 Hz, 1H, OH), 3.54 (t, *J* = 6.5 Hz, 2H, 3-H), 3.49 (td, *J* = 6.3, 5.0 Hz, 2H, 1-H), 1.72 (p, *J* = 6.4 Hz, 2H, 2-H).

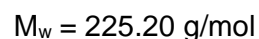
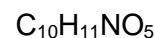
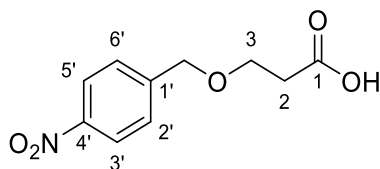
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 146.9 (C-4'), 146.7 (C-1'), 127.9 (C-2' and C-6'), 123.4 (C-3' and C-5'), 70.7 (Ar-CH₂), 67.4 (C-3), 57.7 (C-1), 32.7 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2939, 2867, 1740, 1605, 1518, 1343, 1091, 1050, 1014, 844, 800, 738.

HR-MS (EI): *m/z* = [M-H]⁺ calcd for C₁₀H₁₂NO₄⁺: 210.0772; found: 210.0762.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1a).

3-((4-Nitrobenzyl)oxy)propanoic acid (30^{})**

For the preparation of the JONES reagent: chromium(VI) oxide (1.85 g, 18.4 mmol) was dissolved in conc. H₂SO₄ (1.6 mL) under N₂ atmosphere at 0 °C. Over a period of 20 min water (5.3 mL) was added dropwise to the solution.

Alcohol **29^{**}** (1.05 g, 4.97 mmol) was dissolved in acetone (12 mL) under N₂ atmosphere at 0 °C. Over a period of 80 min JONES reagent (approx. 3.30 mL) was added dropwise to the solution. The reaction mixture was stirred for another 15 min and the chromium salts were filtered off. The solvent was removed from the filtrate *in vacuo* and the precipitate was dissolved in EtOAc (50 mL). The organic phase was washed with water (2 x 25 mL) and brine (25 mL), dried over Na₂SO₄ and the solvent was removed *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes+AcOH 30:70+1) to give carboxylic acid **30^{**}** (590 mg, 2.62 mmol, 53 %) as an off-white solid.

R_f: 0.25 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 73 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.23 (s, 1H, 1-COOH), 8.24 – 8.20 (m, 2H, 3'-H and 5'-H), 7.62 – 7.57 (m, 2H, 2'-H and 6'-H), 4.64 (s, 2H, Ar-CH₂), 3.70 (t, J = 6.2 Hz, 2H, 3-H), 2.54 (t, J = 6.2 Hz, 2H, 2-H).

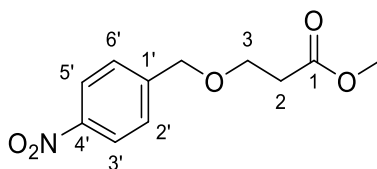
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 172.6 (C-1), 146.7 (C-1' and C-4'), 146.6 (C-1' and C-4'), 127.9 (C-2' and C-6'), 123.4 (C-3' and C-5'), 70.7 (Ar-CH₂), 66.1 (C-3), 34.7 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2875, 1703, 1603, 1510, 1339, 1226, 1105, 852, 844, 739.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₁₀H₁₁N₂O₃⁻: 224.0564; found: 224.0565.

Purity (HPLC): 210 nm: 85 %; 254 nm: 85 % (method 2a).

Methyl 3-((4-nitrobenzyl)oxy)propanoate (31)C₁₁H₁₃NO₅M_w = 239.23 g/mol

Carboxylic acid **30**** (1.15 g, 5.11 mmol) was dissolved in MeOH (13 mL) at room temperature. Then SOCl₂ (1.5 mL) was added dropwise to the fast-stirred mixture. After completion of the reaction, the volatiles were evaporated *in vacuo*. The residue was dissolved in EtOAc (15 mL) and washed with sat. aq. NaHCO₃ (2 x 10 mL). The organic phase was concentrated *in vacuo* to give product **31** (1.11 g, 4.65 mmol, 91 %) as a brown solid.

R_f: 0.48 (EtOAc/hexanes 30:70).

m.p.: 68 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.24 – 8.17 (m, 2H, 3'-H and 5'-H), 7.49 (d, *J* = 8.3 Hz, 2H, 2'-H and 6'-H), 4.63 (s, 2H, Ar-CH₂), 3.80 (t, *J* = 6.2 Hz, 2H, 3-H), 3.72 (s, 3H, CH₃), 2.66 (t, *J* = 6.2 Hz, 2H, 2-H).

¹³C-NMR

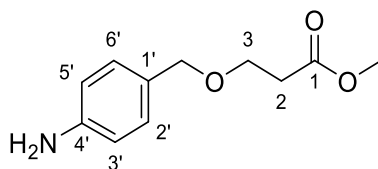
(101 MHz, CDCl₃): δ (ppm) = 172.0 (C-1), 147.6 (C-4'), 145.9 (C-1'), 127.8 (C-2' and C-6'), 123.8 (C-3' and C-5'), 72.0 (Ar-CH₂), 66.4 (C-3), 52.0 (CH₃), 35.0 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3116, 3077, 1730, 1603, 1510, 1340, 1199, 1107, 858, 843, 738.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₁H₁₃NO₅⁺: 239.0788; found: 239.0785.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Methyl 3-((4-aminobenzyl)oxy)propanoate (32)



$C_{11}H_{15}NO_3$

$M_w = 209.25 \text{ g/mol}$

Prepared according to **General Procedure A** from nitro compound **31** (500 mg, 2.09 mmol). Amine **32** (364 mg, 1.74 mmol, 83 %) was obtained as a pale yellow oil.

R_f: 0.23 (EtOAc/hexanes 40:60).

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 6.98 – 6.90 (m, 2H, 2'-H and 6'-H), 6.53 – 6.47 (m, 2H, 3'-H and 5'-H), 5.03 (s, 2H, NH₂), 4.24 (s, 2H, Ar-CH₂), 3.58 (s, 2H, CH₃), 3.56 (t, $J = 6.3 \text{ Hz}$, 2H, 3-H), 2.53 (t, $J = 6.3 \text{ Hz}$, 2H, 2-H).

¹³C-NMR

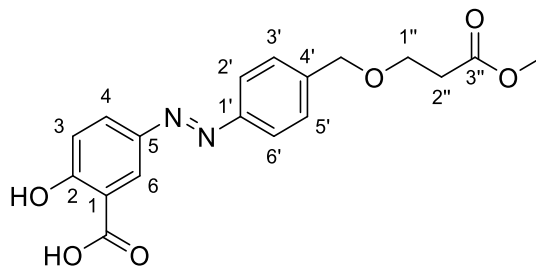
(101 MHz, (CD₃)₂SO): δ (ppm) = 171.6 (C-1), 148.2 (C-4'), 129.2 (C-2' and C-6'), 124.9 (C-1'), 113.5 (C-3' and C-5'), 72.2 (Ar-CH₂), 64.5 (C-3), 51.3 (CH₃), 34.5 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3445, 3367, 2862, 1731, 1625, 1519, 1437, 1363, 1276, 1174, 1068, 822.

HR-MS (EI): $m/z = [M]^{+}$ calcd for C₁₁H₁₅NO₃⁺: 209.1046; found: 209.1045.

Purity (HPLC): 210 nm: >95 % (method 3a).

(E)-2-Hydroxy-5-((4-((3-methoxy-3-oxopropoxy)methyl)phenyl)diazenyl)benzoic acid
(33)


 $C_{18}H_{18}N_2O_6$
 $M_w = 358.35 \text{ g/mol}$

Prepared according to **General Procedure B** from amine **32** (150 mg, 0.717 mmol) and salicylic acid (102 mg, 0.738 mmol). Azobenzene **33** (183 mg, 0.512 mmol, 71 %) was obtained as a light brown solid.

R_f: 0.41 (EtOAc/hexanes+AcOH 40:60+1).

m.p.: 136 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 8.33 (d, $J = 2.5$ Hz, 1H, 6-H), 8.08 (dd, $J = 8.9, 2.5$ Hz, 1H, 4-H), 7.88 – 7.83 (m, 2H, 2'-H and 6'-H), 7.52 – 7.47 (m, 2H, 3'-H and 5'-H), 7.16 (d, $J = 8.9$ Hz, 1H, 3-H), 4.57 (s, 2H, Ar-CH₂), 3.71 (t, $J = 6.1$ Hz, 2H, 1''-H), 3.62 (s, 3H, CH₃), 2.63 (t, $J = 6.1$ Hz, 2H, 2''-H).

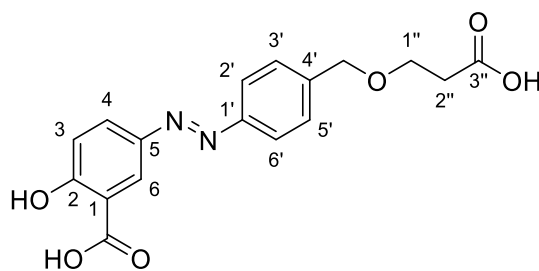
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 171.6 (C-3''), 171.3 (1-COOH), 163.6 (C-2), 151.2 (C-1'), 144.5 (C-5), 141.6 (C-4'), 128.9 (C-4), 128.2 (C-3' and C-5'), 125.7 (C-6), 122.4 (C-2' and C-6'), 118.4 (C-3), 113.8 (C-1), 71.3 (Ar-CH₂), 65.5 (C-1''), 51.4 (CH₃), 34.4 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3065, 2873, 1727, 1655, 1454, 1198, 1086, 844, 713, 570.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₈H₁₇N₂O₆⁻: 357.1092; found: 357.1096.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-5-((4-((2-Carboxyethoxy)methyl)phenyl)diazenyl)-2-hydroxybenzoic acid (CG_57)

 $C_{17}H_{16}N_2O_6$
 $M_w = 344.32 \text{ g/mol}$

Azobenzene **33** (130 mg, 0.363 mmol) was dissolved in MeOH (3.60 mL). KOH (204 mg, 3.63 mmol) was added, and the reaction mixture was heated to reflux for 30 min. The solvent was removed *in vacuo* and the resulting crude product was resuspended in EtOAc (20 mL) and treated with 1 N aq. HCl (10 mL). Phases were separated and the organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes+AcOH 20:80+1) to give product **CG_57** (42.0 mg, 0.122 mmol, 34 %) as a yellow solid.

R_f: 0.27 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 200 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 8.44 (d, $J = 2.5$ Hz, 1H, 6-H), 8.08 (dd, $J = 8.9, 2.5$ Hz, 1H 4-H), 7.88 – 7.84 (m, 2H, 2'-H and 6'-H), 7.52 – 7.49 (m, 2H, 3'-H and 5'-H), 7.08 (d, $J = 8.9$ Hz, 1H, 3-H), 4.61 (s, 2H, Ar-CH₂), 3.79 (t, $J = 6.2$ Hz, 2H, 1''-H), 2.61 (t, $J = 6.2$ Hz, 2H, 2''-H).

¹³C-NMR

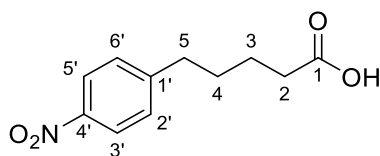
(126 MHz, CD₃OD): δ (ppm) = 175.4 (C-3''), 173.2 (1-COOH), 165.7 (C-2), 153.3 (C-1'), 146.6 (C-5), 142.8 (C-4'), 129.5 (C-4), 129.3 (C-3' and C-5'), 128.0 (C-6), 123.6 (C-2' and C-6'), 119.2 (C-3), 114.3 (C-1), 73.4 (Ar-CH₂), 67.2 (C-1''), 35.9 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2867, 1698, 1650, 1453, 1301, 1201, 1090, 843, 682.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{17}H_{15}N_2O_6^-$: 343.0936; found: 343.0938.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

5-(4-Nitrophenyl)pentanoic acid (35*)



$C_{11}H_{13}NO_4$

$M_w = 223.23$ g/mol

5-Phenylpentanoic acid (**34**, 4.00 g, 22.4 mmol) was dissolved in a mixture of AcOH (15 mL) and conc. H_2SO_4 (8.5 mL) under N_2 atmosphere at 0 °C. Over a period of 2 h a solution of aq. HNO_3 (65 %, 1.7 mL) in conc. H_2SO_4 (2.8 mL) was added dropwise to the solution. Crushed ice (30 g) and water (20 mL) were added, and the obtained precipitate was collected by filtration, washed with cold water (25 mL) and dried *in vacuo*. The crude product was recrystallised from hot toluene to give product **35*** (2.80 g, 12.6 mmol, 56 %) as a pale yellow solid.

R_f: 0.61 (EtOAc/hexanes 50:50).

m.p.: 80 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 8.17 – 8.13 (m, 2H, 3'-H and 5'-H), 7.51 – 7.46 (m, 2H, 2'-H and 6'-H), 2.72 (t, $J = 7.5$ Hz, 2H, 5-H), 2.24 (t, $J = 7.3$ Hz, 2H, 2-H), 1.66 – 1.56 (m, 2H, 4-H), 1.56 – 1.46 (m, 2H, 3-H).

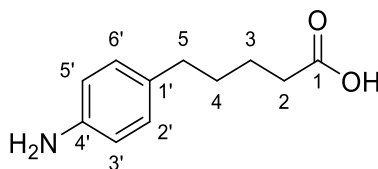
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 174.3 (C-1), 150.6 (C-1'), 145.8 (C-4'), 129.6 (C-2' and C-6'), 123.4 (C-3' and C-5'), 34.5 (C-5), 33.4 (C-2), 29.8 (C-4), 24.0 (C-3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2947, 2871, 1700, 1598, 1510, 1466, 1429, 1401, 1339, 1207, 947, 841, 741.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{11}H_{12}NO_4^-$: 222.0772; found: 222.0772.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1a).

5-(4-Aminophenyl)pentanoic acid (36*)C₁₁H₁₅NO₂M_w = 193.25 g/mol

Prepared according to **General Procedure A** from nitro compound **35*** (501 mg, 2.24 mmol). Amine **36*** (426 mg, 2.21 mmol, 99 %) was obtained as a pale brown solid.

R_f: 0.35 (EtOAc/hexanes 50:50).

m.p.: 101 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 6.83 – 6.79 (m, 2H, 2'-H and 6'-H), 6.49 – 6.45 (m, 2H, 3'-H and 5'-H), 2.42 – 2.35 (m, 2H, 5-H), 2.22 – 2.16 (m, 2H, 2-H), 1.51 – 1.44 (m, 4H, 3-H and 4-H).

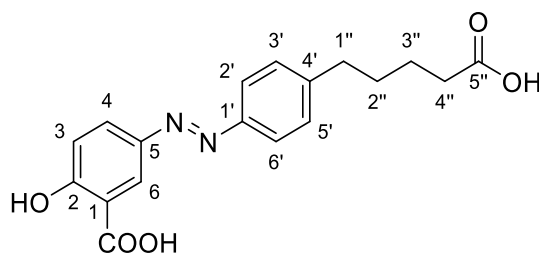
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 174.5 (C-1), 146.3 (C-1'), 129.0 (C-4'), 128.6 (C-2' and C-6'), 114.0 (C-3' and C-5'), 34.1 (C-5), 33.6 (C-2), 30.9 (C-3 or C-4), 24.1 (C-3 or C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3393, 3310, 2926, 2862, 1698, 1630, 1580, 1512, 1382, 1258, 1067, 814, 773.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₀H₁₄N₂O₂⁻: 192.1030; found: 192.1030.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-5-((4-(4-Carboxybutyl)phenyl)diazenyl)-2-hydroxybenzoic acid (CGLU_046)**C₁₈H₁₈N₂O₅M_w = 342.35 g/mol

Prepared according to **General Procedure B** from amine **36*** (150 mg, 0.776 mmol) and salicylic acid (111 mg, 0.802 mmol). Further purification was carried out by dissolving the crude product in water (5.0 mL) and 1 M aq. NaOH (5.0 mL) and washing with EtOAc (3 x 50 mL). The aq. solution was then adjusted to pH 2 with 2 N aq. HCl and cooled to 0 °C. The obtained precipitate was collected by filtration, washed with water (15 mL) and suction dried to give azobenzene **CGLU_046**** (47.6 mg, 0.139 mmol, 18 %) as a brown solid.

R_f: 0.75 (EtOAc+AcOH 100+1).

m.p.: 225 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.07 (s, 2H, 1-COOH and 5''-COOH), 8.31 (d, *J* = 2.5 Hz, 1H, 6-H), 8.06 (dd, *J* = 8.9, 2.6 Hz, 1H, 4-H), 7.82 – 7.77 (m, 2H, 2'-H and 6'-H), 7.42 – 7.37 (m, 2H, 3'-H and 5'-H), 7.15 (d, *J* = 8.9 Hz, 1H, 3-H), 2.67 (t, *J* = 7.4 Hz, 2H, 1''-H), 2.25 (t, *J* = 7.2 Hz, 2H, 4''-H), 1.63 (tt, *J* = 7.9, 5.9 Hz, 2H, 2''-H), 1.58 – 1.49 (m, 2H, 3''-H).

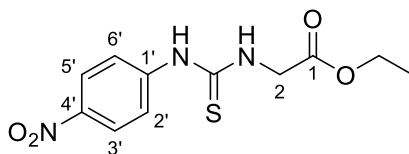
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 174.4 (5''-COOH), 171.4 (1-COOH), 163.4 (C-2), 150.2 (C-1'), 145.8 (C-4'), 144.5 (C-5), 129.3 (C-3' and C-5'), 128.9 (C-4), 125.5 (C-6), 122.4 (C-2' and C-6'), 118.3 (C-3), 113.7 (C-1), 34.6 (C-1''), 33.5 (C-4''), 30.1 (C-2''), 24.1 (C-3'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3090, 2821, 2362, 2331, 1702, 1588, 1488, 1412, 1249, 1103, 912, 842, 686, 579.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₈H₁₇N₃O₅⁻: 341.1143; found: 341.1143.

Purity (HPLC): 254 nm: >95 % (method 4a).

Ethyl ((4-nitrophenyl)carbamoithioyl)glycinate (38)

 $C_{11}H_{13}N_3O_4S$
 $M_w = 283.30 \text{ g/mol}$

Ethyl isothiocyanatoacetate (**37**, 2.00 g, 13.8 mmol) and 4-nitroaniline (**15**, 1.90 g, 13.8 mmol) in EtOH (50 mL) were mixed and heated to reflux for 6 h. The precipitate was collected by filtration, washed with cold EtOH (2 x 30 mL) and dried *in vacuo* to afford thiourea derivative **38** (1.55 g, 5.46 mmol, 40 %) as an off-white solid.

R_f: 0.40 (EtOAc/hexanes 10:90).

m.p.: 199 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 10.51 (s, 1H, 1'-NH), 8.45 (t, $J = 5.5$ Hz, 1H, 2-NH), 8.23 – 8.17 (m, 2H, 3'-H and 5'-H), 7.90 – 7.84 (m, 2H, 2'-H and 6'-H), 4.30 (d, $J = 5.2$ Hz, 2H, 2-H), 4.14 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 1.22 (t, $J = 7.1$ Hz, 3H, CH₃).

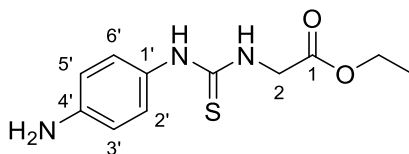
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 181.0 (C=S), 169.2 (C-1), 146.0 (C-1'), 142.2 (C-4'), 124.6 (C-3' and C-5'), 120.7 (C-2' and C-6'), 60.6 (CH₂CH₃), 45.6 (C-2), 14.1 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3332, 2984, 1725, 1513, 1345, 1322, 1300, 1212, 1109, 979, 848, 703.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₁H₁₂N₃O₄S⁻: 282.0554; found: 292.1190.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Ethyl ((4-aminophenyl)carbamothioyl)glycinate (39)C₁₁H₁₅N₃O₂SM_w = 253.32 g/mol

To a mixture of thiourea derivative **38** (100 mg, 0.358 mmol) and AcOH (1.0 mL), iron powder (78.9 mg, 1.41 mmol) was added, and the resulting reaction mixture was stirred at 30 °C for 16 h. Subsequently, the reaction mixture was filtered through a pad of celite and the solvent removed *in vacuo*. To the residue was added EtOAc (40 mL) and sat. aq. NaHCO₃ (40 mL). Phases were separated and the aq. phase extracted with EtOAc (3 x 40 mL). The combined organic extracts were washed with brine (20 mL), dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 70:30) to give product **39** (59.0 mg, 0.233 mmol, 66 %) as an off-white solid.

R_f: 0.43 (EtOAc/hexanes 70:30).

m.p.: 263 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 7.05 – 7.00 (m, 2H, 2'-H and 6'-H), 6.76 – 6.71 (m, 2H, 3'-H and 5'-H), 4.29 (s, 2H, 2-H), 4.18 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 1.28 (t, *J* = 7.1 Hz, 3H, CH₃).

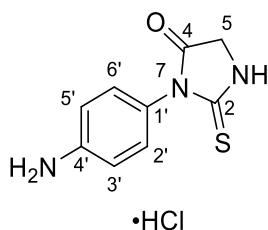
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 183.3 (C=S), 171.7 (C-1), 148.4 (C-4'), 128.3 (C-1'), 128.1 (C-2' and C-6'), 116.9 (C-3' and C-5'), 62.2 (CH₂CH₃), 47.2 (C-2), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3347, 1722, 1552, 1514, 1406, 1384, 1226, 1014, 972, 840.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₁H₁₄N₃O₂S⁻: 252.0812; found: 252.0811.

Purity (HPLC): 210 nm: 77 %; 254 nm: 86 % (method 3a).

3-(4-Aminophenyl)-2-thioximidazolidin-4-one hydrochloride (40b)C₉H₉N₃OS•HClM_w = 243.71 g/mol

Amine **39** (47 mg, 0.186 mmol) was dissolved in 1,4-dioxane (1.3 mL) followed by the dropwise addition of conc. HCl (87 μ L). The reaction mixture was heated to 60 °C for 2 h, cooled to room temperature and the solid collected by filtration and dried *in vacuo*. 2-Thiohydantoin **40b** (34.0 mg, 0.140 mmol, 75 %) was obtained as a white solid.

R_f: 0.14 (EtOAc/hexanes 50:50).

m.p.: 248 °C (decomposition).

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 10.43 (s, 1H, NH), 7.36 – 7.33 (m, 2H, 3'-H and 5'-H), 7.33 – 7.30 (m, 2H, 2'-H and 6'-H), 4.29 (d, *J* = 1.3 Hz, 2H, 5-H).

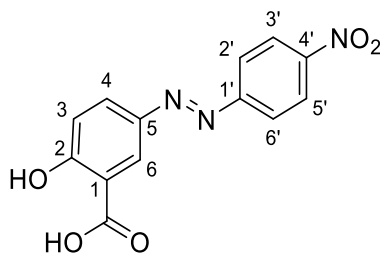
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 183.2 (C-2), 172.2 (C-4), 134.9 (C-4'), 131.1 (C-1'), 130.1 (C-2' and C-6'), 122.0 (C-3' and C-5'), 49.2 (C-5).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3156, 2796, 2563, 1758, 1520, 1414, 1328, 1278, 1181, 825, 717, 696.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₉H₈N₃OS⁻: 206.0394; found: 206.0393.

Purity (HPLC): 254 nm: 95 % (method 4a).

(E)-2-Hydroxy-5-((4-nitrophenyl)diazenyl)benzoic acid (41)C₁₃H₉N₃O₅M_w = 287.23 g/mol

Prepared according to **General Procedure B** from 4-nitroaniline (**15**, 2.00 g, 14.5 mmol) and salicylic acid (2.06 g, 14.9 mmol). The crude product was of sufficient purity without further purification. Azobenzene **41** (4.02 g, 14.0 mmol, 97 %) was obtained as an orange solid.

R_f: 0.67 (EtOAc/hexanes+AcOH 50:50+1).

m.p.: 256 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 8.43 – 8.40 (m, 2H, 3'-H and 5'-H), 8.39 (d, *J* = 2.6 Hz, 1H, 6-H), 8.13 (dd, *J* = 8.9, 2.5 Hz, 1H, 4-H), 8.07 – 8.03 (m, 2H, 2'-H and 6'-H), 7.18 (d, *J* = 8.9 Hz, 1H, 3-H).

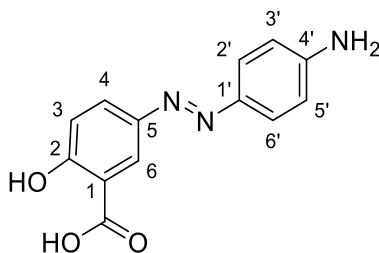
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 171.1 (1-COOH), 164.7 (C-2), 155.2 (C-1'), 148.2 (C-4'), 144.5 (C-5), 129.2 (C-4), 126.9 (C-6), 125.1 (C-3' and C-5'), 123.3 (C-2' and C-6'), 118.7 (C-3), 114.1 (C-1).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3087, 1666, 1520, 1343, 1218, 1198, 865, 852, 844, 707, 686.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₃H₈N₃O₅⁻: 286.0469; found: 286.0468.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1e).

(E)-5-((4-Aminophenyl)diazenyl)-2-hydroxybenzoic acid (42)C₁₃H₁₁N₃O₃M_w = 257.25 g/mol

Nitro compound **41** (1.00 g, 3.48 mmol) was dissolved in EtOH/1,4-dioxane (1:1, 38 mL). A solution of Na₂S (35 %, 2.58 g, 33.1 mmol) in water (28 mL) was added. After the reaction mixture was heated to reflux for 3 h, it was cooled to room temperature and the organic solvents evaporated *in vacuo*. Water (30 mL) was added, and the mixture adjusted to pH 3 with 1 N aq. HCl. The precipitate was collected by filtration and dried *in vacuo* to give product **42** (892 mg, 3.47 mmol, quant.) as a dark purple solid.

R_f: 0.53 (EtOAc/hexanes+AcOH 50:50+1).

m.p.: 225 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 11.53 (s, 1H, COOH), 8.17 (d, *J* = 2.5 Hz, 1H, 6-H), 7.94 (dd, *J* = 8.8, 2.5 Hz, 1H, 4-H), 7.66 – 7.61 (m, 2H, 2'-H and 6'-H), 7.09 (d, *J* = 8.8 Hz, 1H, 3-H), 6.69 – 6.64 (m, 2H, 3'-H and 5'-H).

¹³C-NMR

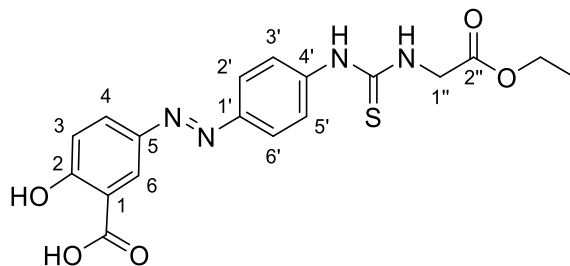
(126 MHz, (CD₃)₂SO): δ (ppm) = 171.6 (COOH), 162.0 (C-2), 152.3 (C-4'), 145.0 (C-5), 142.7 (C-1'), 128.6 (C-4), 124.9 (C-2' and C-6'), 124.0 (C-6), 118.1 (C-3), 113.6 (C-3' and C-5'), 113.4 (C-1).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3087, 2596, 1662, 1520, 1448, 1342, 1217, 1198, 864, 851, 843, 704, 686.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₃H₁₀N₃O₃⁻: 256.0728; found: 256.0727.

Purity (HPLC): 254 nm: >95 % (method 3a).

**(E)-5-((4-(3-(2-Ethoxy-2-oxoethyl)thioureido)phenyl)diazenyl)-2-hydroxybenzoic acid
(43)**



$C_{18}H_{18}N_4O_5S$

$M_w = 402.43$ g/mol

Ethyl isothiocyanatoacetate (**37**, 169 mg, 1.17 mmol) and azobenzene **42** (200 mg, 0.777 mmol) in EtOH (3.0 mL) were dissolved and stirred at 80 °C for 3 h and at room temperature for 16 h. The precipitate was then collected by filtration, washed with EtOH (3 x 10 mL), and dried *in vacuo* to give thiourea derivative **43** (146 mg, 0.362 mmol, 47 %) as a greenish-yellow solid.

R_f: 0.48 (EtOAc/hexanes+AcOH 50:50+1).

m.p.: 205 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 10.23 (s, 1H, 4'-NH), 8.30 (d, $J = 2.6$ Hz, 1H, 6-H), 8.23 (t, $J = 5.6$ Hz, 1H, 1''-NH), 8.06 (dd, $J = 8.8, 2.6$ Hz, 1H, 4-H), 7.89 – 7.83 (m, 2H, 2'-H and 6'-H), 7.77 – 7.71 (m, 2H, 3'-H and 5'-H), 7.15 (d, $J = 8.9$ Hz, 1H, 3-H), 4.31 (d, $J = 5.6$ Hz, 2H, 1''-H), 4.14 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 1.22 (t, $J = 7.1$ Hz, 3H, CH₃).

¹³C-NMR

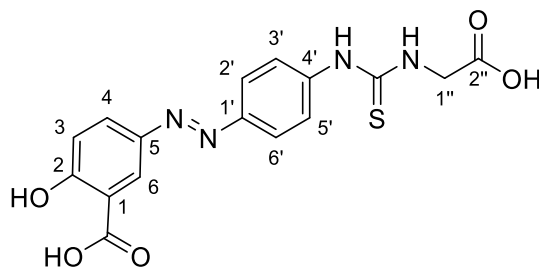
(126 MHz, (CD₃)₂SO): δ (ppm) = 181.0 (C=S), 171.4 (1-COOH), 169.5 (C-2''), 163.3 (C-2), 147.9 (C-1'), 144.6 (C-5), 142.0 (C-4'), 128.9 (C-4), 125.4 (C-6), 123.1 (C-2' and C-6'), 122.3 (C-3' and C-5'), 118.4 (C-3), 113.8 (C-1), 60.6 (CH₂CH₃), 45.64 (C-1''), 14.1 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3319, 3050, 1746, 1678, 1548, 1455, 1346, 1200, 1131, 977, 837, 731, 690.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₈H₁₇N₄O₅S⁻: 401.0925; found: 401.0925.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1e).

**(E)-5-((4-(3-(Carboxymethyl)thioureido)phenyl)diazenyl)-2-hydroxybenzoic acid
(CG_150)**



$C_{16}H_{14}N_4O_5S$

$M_w = 374.37$ g/mol

Prepared according to **General Procedure D** at room temperature from azobenzene **43** (100 mg, 0.248 mmol). The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes+AcOH 50:50+1 → EtOAc/MeOH+AcOH 50:50+1) to give product **CG_150** (29.3 mg, 0.0729 mmol, 29 %) as a dark orange solid.

R_f: 0.33 (EtOAc/hexanes+AcOH 50:50+1).

m.p.: 195 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.72 (s, 1H, 1-COOH or 1''-COOH), 10.24 (s, 1H, 4'-NH), 8.30 (d, $J = 2.5$ Hz, 1H, 6-H), 8.16 (t, $J = 5.4$ Hz, 1H, 1''-NH), 8.05 (dd, $J = 8.9, 2.6$ Hz, 1H, 4-H), 7.88 – 7.83 (m, 2H, 2'-H and 6'-H), 7.79 – 7.74 (m, 2H, 3'-H and 5'-H), 7.14 (d, $J = 8.9$ Hz, 1H, 3-H), 4.24 (d, $J = 5.4$ Hz, 2H, 1''-H).

¹³C-NMR

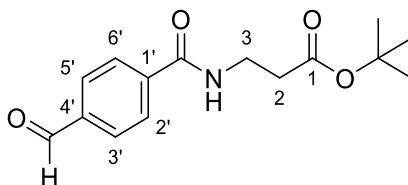
(126 MHz, (CD₃)₂SO): δ (ppm) = 180.6 (C=S), 171.4 (1-COOH), 170.9 (C-2''), 163.4 (C-2), 147.8 (C-1'), 144.5 (C-5), 142.1 (C-4'), 128.8 (C-4), 125.3 (C-6), 123.1 (C-2' and C-6'), 122.1 (C-3' and C-5'), 118.3 (C-3), 113.8 (C-1), 45.6 (C-1'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3054, 1722, 1679, 1547, 1454, 1344, 1207, 1176, 1074, 974, 836, 686.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{16}H_{13}N_4O_5S^-$: 373.0612; found: 373.0614.

Purity (HPLC): 254 nm: >95 % (method 4a).

***tert*-Butyl 3-(4-formylbenzamido)propanoate (46)^[101]**



$C_{15}H_{19}NO_4$

$M_w = 277.32$ g/mol

Prepared according to **General Procedure E** from 4-carboxybenzaldehyde (**44**, 1.50 g, 9.99 mmol) and *tert*-butyl 3-aminopropanoate hydrochloride (**45**, 1.21 g, 6.66 mmol). The crude product was purified by FCC (EtOAc/hexanes 35:65) to give aldehyde **46** (1.76 g, 6.35 mmol, 95 %) as a white solid.

R_f: 0.26 (EtOAc/hexanes 40:60).

m.p.: 103 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 10.08 (s, 1H, CHO), 7.97 – 7.93 (m, 2H, 3'-H and 5'-H), 7.93 – 7.88 (m, 2H, 2'-H and 6'-H), 7.01 (t, J = 6.2 Hz, 1H, CONH), 3.74 – 3.68 (m, 2H, 3-H), 2.59 – 2.55 (m, 2H, 2-H), 1.47 (s, 9H, C(CH₃)₃).

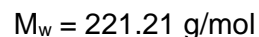
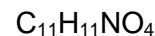
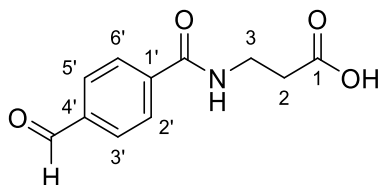
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 191.7 (CHO), 172.5 (C-1), 166.2 (CONH), 139.8 (C-1'), 138.4 (C-4'), 130.0 (C-3' and C-5'), 127.8 (C-2' and C-6'), 81.6 (C(CH₃)₃), 35.8 (C-3), 35.0 (C-2), 28.3 (C(CH₃)₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3299, 2724, 1708, 1634, 1552, 1330, 1146, 846, 826, 754, 653.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₅H₁₈NO₄⁻: 276.1241; found: 276.1243.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

3-(4-Formylbenzamido)propanoic acid (47)^[101]

A solution of 4 M HCl in 1,4-dioxane (6.5 mL) was added to aldehyde **46** (900 mg, 3.25 mmol) and the reaction mixture was stirred at room temperature for 3 h. The resulting precipitate was collected by filtration, washed with ether (20 mL), and dried *in vacuo* to afford product **47** (517 mg, 2.34 mmol, 72 %) as a white solid.

R_f: 0.33 (EtOAc/hexanes+AcOH 70:30+1).

m.p.: 162 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.25 (s, 1H, COOH), 10.07 (s, 1H, CHO), 8.75 (t, $J = 5.5$ Hz, 1H, CONH), 8.03 – 8.00 (m, 2H, 2'-H and 6'-H), 8.00 – 7.97 (m, 2H, 3'-H and 5'-H), 3.48 (td, $J = 7.1, 5.4$ Hz, 2H, 3-H), 2.53 (t, $J = 7.1$ Hz, 2H, 2-H).

¹³C-NMR

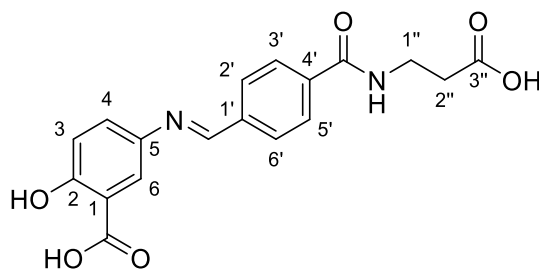
(126 MHz, (CD₃)₂SO): δ (ppm) = 192.9 (CHO), 172.8 (C-1), 165.4 (CONH), 139.4 (C-1'), 137.8 (C-4'), 129.4 (C-3' and C-5'), 127.9 (C-2' and C-6'), 35.7 (C-3), 33.6 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3297, 1698, 1633, 1546, 1436, 1334, 1226, 928, 854, 821.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₁H₁₀NO₄⁻: 220.0615; found: 220.0616.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-5-((4-((2-Carboxyethyl)carbamoyl)benzylidene)amino)-2-hydroxybenzoic acid
(CG_71)



$C_{18}H_{16}N_2O_6$

$M_w = 356.33$ g/mol

Aldehyde **47** (235 mg, 1.06 mmol) and 5-aminosalicylic acid (5-ASA; 54.2 mg, 0.354 mmol) were added to MeOH (6.0 mL). AcOH (4.0 μ L) was then added to the reaction mixture, which turned into a clear orange solution after 5 min and was stirred at room temperature for 24 h. Precipitates were collected by centrifugation, washed with MeOH (3 x 8.0 mL), and dried *in vacuo* to provide imine **CG_71** (88.3 mg, 0.248 mmol, 70 %) as an orange solid.

R_f: 0.33 (EtOAc/hexanes+AcOH 70:30+1).

m.p.: 240 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.26 (s, 2H, 1-COOH and 3''-COOH), 10.07 (s, 1H, OH), 8.76 (s, 1H, Ar-CH), 8.65 (t, $J = 5.5$ Hz, 1H, CONH), 8.03 – 7.98 (m, 2H, 2'-H and 6'-H), 7.98 – 7.91 (m, 2H, 3'-H and 5'-H), 7.77 (d, $J = 2.7$ Hz, 1H, 6-H), 7.59 (dd, $J = 8.8, 2.7$ Hz, 1H, 4-H), 7.03 (d, $J = 8.8$ Hz, 1H, 3-H), 3.48 (td, $J = 7.1, 5.5$ Hz, 2H, 1''-H), 2.54 (t, $J = 7.1$ Hz, 2H, 2''-H).

¹³C-NMR

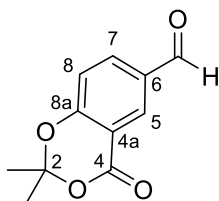
(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3''), 171.6 (1-COOH), 165.7 (CONH), 160.0 (C-2), 158.3 (Ar-CH), 142.2 (C-5), 138.4 (C-1'), 136.4 (C-4'), 129.0 (C-4), 128.3 (C-2' and C-6'), 127.6 (C-3' and C-5'), 122.4 (C-6), 118.0 (C-3), 113.4 (C-1), 35.7 (C-1''), 33.7 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1709, 1553, 1489, 1293, 1193, 1088, 1013, 838, 569.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{18}H_{15}N_2O_6^-$: 355.0936; found: 355.0938.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

2,2-Dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxine-6-carbaldehyde (50)^[103]



$C_{11}H_{10}O_4$

$M_w = 206.20$ g/mol

Prepared according to **General Procedure C** from 5-formylsalicylic acid (**48**, 1.00 g, 6.02 mmol). The crude product was purified by FCC (EtOAc/hexanes 12:88) to give acetonide **50** (618 mg, 3.00 mmol, 50 %) as a pale yellow solid.

R_f: 0.43 (EtOAc/hexanes 30:70).

m.p.: 117 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 9.97 (d, $J = 0.8$ Hz, 1H, CHO), 8.47 (d, $J = 2.1$ Hz, 1H, 5-H), 8.12 (dd, $J = 8.6, 2.1$ Hz, 1H, 7-H), 7.12 (dd, $J = 8.6, 0.8$ Hz, 1H, 8-H), 1.78 (s, 6H, C(CH₃)₂).

¹³C-NMR

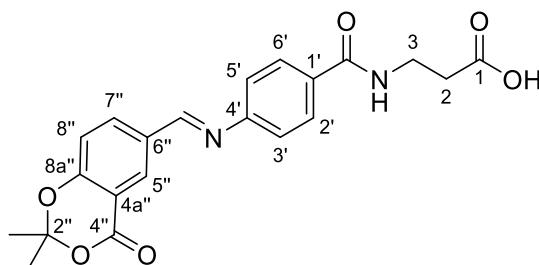
(126 MHz, CDCl₃): δ (ppm) = 189.7 (CHO), 160.6 (C-4), 160.0 (C-8a), 135.9 (C-7), 133.6 (C-5), 131.6 (C-6), 118.6 (C-8), 113.7 (C-4a), 107.4 (C-2), 26.1 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1737, 1686, 1611, 1580, 1265, 1196, 1108, 841, 779, 754, 581.

HR-MS (EI): $m/z = [M]^{+}$ calcd for C₁₁H₁₀O₄⁺: 206.0574; found: 206.0577.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-3-(4-(((2,2-Dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)methylene)amino)benzamido)propanoic acid (CG_103)



$$\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_6$$

$$M_w = 396.40 \text{ g/mol}$$

Aldehyde **50** (250 mg, 1.21 mmol) was dissolved in MeOH (6.0 mL). Amine **3** (278 mg, 1.33 mmol) and AcOH (4.0 μL) were added and the reaction mixture was stirred at room temperature for 16 h. Precipitates were then collected by centrifugation, washed with MeOH (3 x 6.0 mL), and dried *in vacuo* to provide imine **CG_103** (255 mg, 0.634 mmol, 53 %) as a white solid.

R_f: 0.46 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 203 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.23 (s, 1H, COOH), 8.71 (s, 1H, Ar-CH), 8.53 (t, $J = 5.5$ Hz, 1H, CONH), 8.44 (d, $J = 2.1$ Hz, 1H, 5''-H), 8.27 (dd, $J = 8.5, 2.1$ Hz, 1H, 7''-H), 7.92 – 7.88 (m, 2H, 2'-H and 6'-H), 7.36 – 7.31 (m, 2H, 3'-H and 5'-H), 7.29 (d, $J = 8.6$ Hz, 1H, 8''-H), 3.47 (td, $J = 7.1, 5.3$ Hz, 2H, 3-H), 2.54 – 2.51 (m, 2H, 2-H), 1.74 (s, 6H, C(CH₃)₂).

¹³C-NMR

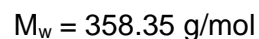
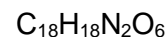
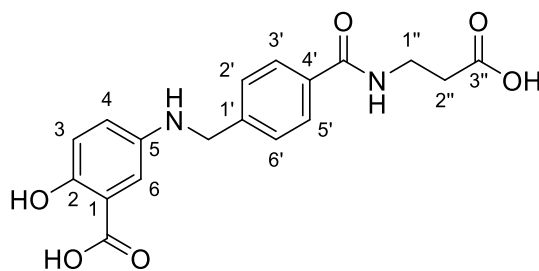
(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (COOH), 165.7 (CONH), 160.1 (Ar-CH), 159.8 (C-4''), 157.7 (C-8a''), 153.5 (C-4'), 136.4 (C-7''), 131.8 (C-1'), 130.9 (C-6''), 130.3 (C-5''), 128.4 (C-2' and C-6'), 120.8 (C-3' and C-5'), 118.2 (C-8''), 113.2 (C-4a''), 107.0 (C-2), 35.6 (C-3), 33.8 (C-2), 25.3 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3357, 2359, 1728, 1707, 1648, 1591, 1498, 1296, 1231, 1174, 947, 854, 771, 672.

HR-MS (ESI): $m/z = [\text{M}-\text{H}]^-$ calcd for C₂₁H₁₉N₂O₆⁻: 395.1249; found: 395.1249.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

5-((4-((2-Carboxyethyl)carbamoyl)benzyl)amino)-2-hydroxybenzoic acid (CG_79)



Aldehyde **47** (190 mg, 0.859 mmol) and 5-aminosalicylic acid (5-ASA, 65.8 mg, 0.429 mmol) were added to MeOH (4.6 mL). AcOH (3.0 μL) was then added to the reaction mixture, which turned into a clear orange solution after 5 min and was stirred at room temperature for 24 h. Precipitates were collected by centrifugation and washed with MeOH (3 x 8.0 mL). The crude precipitates were then resuspended in MeOH (5.0 mL) and NaBH_3CN (85.2 mg, 1.290 mmol) was added in portions at room temperature to the stirred mixture. After stirring for additional 4 h, water (5.0 mL) was added. The solvents were removed *in vacuo* and the crude product purified by FCC (100 % MeOH) to give amine **CG_79** (37.3 mg, 0.104 mmol, 24 %) as a yellow solid.

R_f: 0.22 (EtOAc/hexanes+AcOH 80:20+1), streaks.

m.p.: 330 °C.

¹H-NMR

(400 MHz, D₂O): δ (ppm) = 7.71 – 7.65 (m, 2H, 3'-H and 5'-H), 7.45 (d, J = 8.1 Hz, 2H, 2'-H and 6'-H), 7.35 (d, J = 2.9 Hz, 1H, 6-H), 7.00 (dd, J = 8.8, 2.9 Hz, 1H, 4-H), 6.82 (d, J = 8.7 Hz, 1H, 3-H), 4.36 (s, 2H, Ar-CH₂), 3.58 (t, J = 7.0 Hz, 2H, 1''-H), 2.51 (t, J = 7.0 Hz, 2H, 2''-H).

¹³C-NMR

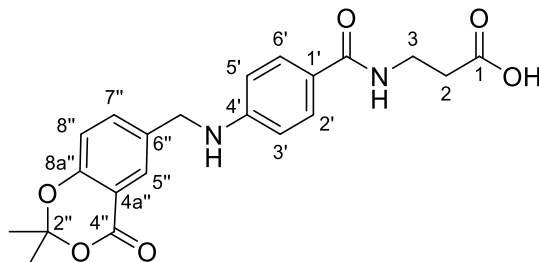
(101 MHz, D₂O): δ (ppm) = 180.2 (C-3''), 175.0 (1-COOH), 170.4 (CONH), 154.2 (C-2), 141.7 (C-1'), 132.8 (C-4'), 128.4 (C-2' and C-6'), 127.3 (C-3' and C-5'), 123.0 (C-4), 118.4 (C-1), 117.8 (C-6), 117.0 (C-3), 49.5 (Ar-CH₂), 37.1 (C-1''), 36.5 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3335, 2000, 1659, 1631, 1566, 1494, 1484, 1413, 1300, 1234, 1018, 822.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₁₈H₁₇N₂O₆⁻: 357.1092; found: 357.1094.

Purity (HPLC): 210 nm: 95 %; 254 nm: 87 % (method 3a).

3-(4-(((2,2-Dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)methyl)amino)benzamido)propanoic acid (51)



$$\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_6$$

$$M_w = 398.42 \text{ g/mol}$$

Imine **CG_103** (100 mg, 0.252 mmol) was dissolved in MeOH (2.5 mL) under N_2 atmosphere and NaBH_3CN (50.1 mg, 0.757 mmol) was added in portions. After stirring for 4 h, the reaction was quenched with sat. aq. NaHCO_3 (10 mL). The mixture was concentrated *in vacuo* and directly purified by FCC (EtOAc/hexanes+AcOH 80:20+1) to give amine **51** (99.5 mg, 0.250 mmol, 99 %) as a white solid.

R_f: 0.31 (EtOAc/hexanes+AcOH 80:20+1).

m.p.: 172 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 8.08 (t, J = 5.6 Hz, 1H, CONH), 7.84 (d, J = 2.2 Hz, 1H, 5''-H), 7.66 (dd, J = 8.5, 2.3 Hz, 1H, 7''-H), 7.59 – 7.55 (m, 2H, 2'-H and 6'-H), 7.09 (d, J = 8.4 Hz, 1H, 8''-H), 6.82 (t, J = 6.2 Hz, 1H, NH), 6.61 – 6.55 (m, 2H, 3'-H and 5'-H), 4.34 (d, J = 6.1 Hz, 2H, Ar-CH₂), 3.38 (td, J = 7.1, 5.4 Hz, 2H, 3-H), 2.42 (t, J = 7.2 Hz, 2H, 2-H), 1.67 (s, 6H, C(CH₃)₂).

¹³C-NMR

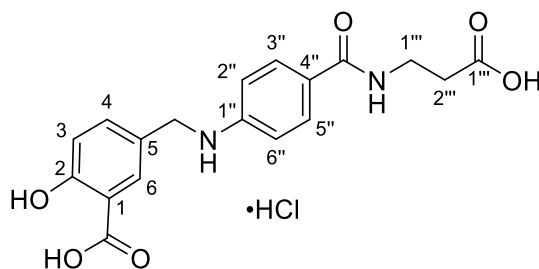
(126 MHz, (CD₃)₂SO): δ (ppm) = 173.3 (C-1), 166.1 (CONH), 160.3 (C-4''), 154.3 (C-8a''), 150.7 (C-4'), 136.0 (C-7''), 134.7 (C-6''), 128.6 (C-2' and C-6'), 127.4 (C-5''), 121.6 (C-1'), 117.3 (C-8''), 112.8 (C-4a''), 111.1 (C-3' and C-5'), 106.3 (C-2''), 44.9 (Ar-CH₂), 35.5 (C-3), 34.5 (C-2), 25.2 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3340, 2828, 1736, 1694, 1620, 1599, 1509, 1501, 1301, 1261, 1214, 1185, 915, 835, 766.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₂₁H₂₁N₂O₆⁻: 397.1405; found: 397.1406.

Purity (HPLC): 210 nm: 90 %; 254 nm: 93 % (method 3a).

5-(((4-((2-Carboxyethyl)carbamoyl)phenyl)amino)methyl)-2-hydroxybenzoic acid hydrochloride (CG_112)



$C_{18}H_{19}ClN_2O_6$

$M_w = 394.81$ g/mol

Amine **51** (35.0 mg, 0.0880 mmol) was dissolved in 1,4-dioxane (0.60 mL) followed by the dropwise addition of conc. HCl (45 μ L). The reaction mixture was heated to 60 °C for 2 h, cooled to room temperature and the precipitate collected by filtration and dried *in vacuo* to give HCl-salt **CG_112** (26.2 mg, 0.0664 mmol, 76 %) as a white solid.

R_f: not determinable.

m.p.: 170 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 7.94 (d, $J = 2.4$ Hz, 1H, 6-H), 7.90 – 7.87 (m, 2H, 3''-H and 5''-H), 7.50 (dd, $J = 8.6, 2.4$ Hz, 1H, 4-H), 7.33 – 7.30 (m, 2H, 2''-H and 6''-H), 6.96 (d, $J = 8.5$ Hz, 1H, 3-H), 4.55 (s, 2H, Ar-CH₂), 3.63 (t, $J = 6.8$ Hz, 2H, 1'''-H), 2.66 (t, $J = 6.8$ Hz, 2H, 2'''-H).

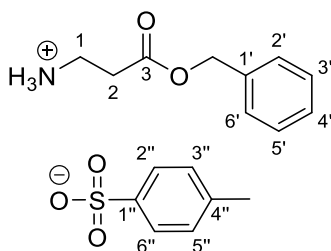
¹³C-NMR

(126 MHz, CD₃OD): δ (ppm) = 173.9 (C-1'''), 172.9 (1-COOH), 168.9 (CONH), 163.9 (C-2), 141.7 (C-1''), 138.0 (C-4), 133.9 (C-4''), 133.3 (C-6), 130.3 (C-3'' and C-5''), 124.1 (C-5), 122.1 (C-2'' and C-6''), 119.0 (C-3), 114.2 (C-1), 54.2 (Ar-CH₂), 37.1 (C-1'''), 34.6 (C-2''').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3321, 2544, 1666, 1641, 1596, 1278, 1185, 1112, 862, 848, 754.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{18}H_{17}N_2O_6^-$: 357.1092; found: 357.1092.

Purity (HPLC): 210 nm: 91 %; 254 nm: 91 % (method 2a).

3-(Benzyloxy)-3-oxopropan-1-aminium 4-methylbenzenesulfonate (53)^[108]

 $C_{17}H_{21}NO_5S$
 $M_w = 351.42 \text{ g/mol}$

β -Alanine (445 mg, 5.00 mmol) and *p*-toluenesulfonic acid hydrate (**52**, 1.14 g, 6.00 mmol) were suspended in benzyl alcohol (1.55 mL, 15.0 mmol) and toluene (15 mL). The mixture was heated to reflux with a Dean-Stark equipment for 5 h and then cooled to room temperature. The resulting precipitate was collected by filtration and washed with diethyl ether (3 x 20 mL). It was then resuspended in EtOAc (300 mL) and heated to reflux. The hot suspension was repeatedly filtered, and the precipitate collected and dried *in vacuo* to give aminoester salt **53** (1.45 g, 3.33 mmol, 83 %) as a white crystalline solid.

R_f: not determinable.

m.p.: 140 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 7.75 (s, 3H, NH₃⁺), 7.50 – 7.46 (m, 2H, 2''-H and 6''-H), 7.41 – 7.32 (m, 5H, Ar-CH₂), 7.13 – 7.09 (m, 2H, 3''-H and 5''-H), 5.14 (s, 2H, Ar-CH₂), 3.06 (t, $J = 6.9 \text{ Hz}$, 2H, 1-H), 2.72 (t, $J = 6.9 \text{ Hz}$, 2H, 2-H), 2.29 (s, 3H, CH₃).

¹³C-NMR

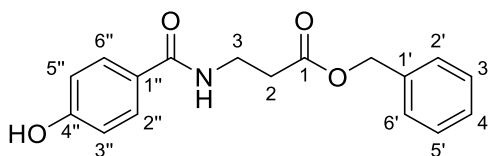
(101 MHz, (CD₃)₂SO): δ (ppm) = 170.2 (C-3), 145.6 (C-1''), 137.7 (C-4''), 135.8 (C-1'), 128.5 (C-3' and C-5'), 128.2 (C-4'), 128.09 (C-3'' and C-5''), 128.07 (C-2' and C-6'), 125.5 (C-2'' and C-6''), 66.0 (Ar-CH₂), 34.7 (C-1), 31.4 (C-2), 20.8 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3051, 1732, 1518, 1400, 1228, 1201, 1154, 1120, 1031, 1007, 815, 742, 865, 568.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for C₁₀H₁₄NO₂⁺: 180.1019; found: 180.1018.

Purity (HPLC): 254 nm: >95 % (method 4a).

Benzyl 3-(4-hydroxybenzamido)propanoate (55)^[108]



$C_{17}H_{17}NO_4$

$M_w = 299.33$ g/mol

Prepared according to **General Procedure G** from 4-hydroxybenzoic acid (**54**, 276 mg, 2.00 mmol) and aminoester salt **53** (843 mg, 2.40 mmol). The crude product was purified by FCC (EtOAc/hexanes 50:50) to give product **55** (532 mg, 1.78 mmol, 89 %) as a white solid.

R_f: 0.24 (EtOAc/hexanes 50:50).

m.p.: 112 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.61 – 7.54 (m, 2H, 2''-H and 6''-H), 7.38 – 7.29 (m, 5H, Ar-CH₂), 6.86 – 6.82 (m, 2H, 3''-H and 5''-H), 6.79 (d, $J = 6.0$ Hz, 1H, CONH), 5.15 (s, 2H, Ar-CH₂), 3.73 (qd, $J = 6.6, 6.1, 3.1$ Hz, 2H, 3-H), 2.70 (t, $J = 5.8$ Hz, 2H, 2-H).

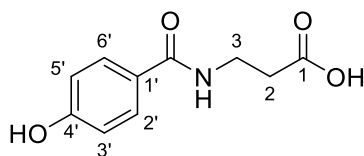
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 172.9 (C-1), 167.8 (CONH), 159.9 (C-4''), 135.6 (C-1'), 129.1 (C-2'' and C-6''), 128.8 (C-3' and C-5'), 128.6 (C-4'), 128.5 (C-2' and C-6'), 125.7 (C-1''), 115.7 (C-3'' and C-5''), 66.9 (Ar-CH₂), 35.6 (C-3), 34.2 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3303, 1708, 1635, 1510, 1273, 1205, 1174, 960, 846, 757, 697.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₇H₁₆NO₄⁻: 298.1085; found: 298.1087.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

3-(4-Hydroxybenzamido)propanoic acid (56)^[108]C₁₀H₁₁NO₄M_w = 209.20 g/mol

Phenol **55** (500 mg, 1.67 mmol) was dissolved in dry MeOH (10 mL) and 10 % Pd/C (10 wt% on activated carbon, 50.0 mg) was added under N₂ atmosphere. Hydrogenation was performed under 1 bar H₂ pressure at room temperature for 1.5 h. Subsequently, the reaction mixture was filtered through a pad of celite and the solvent was removed *in vacuo* to give carboxylic acid **56** (336 mg, 1.61 mmol, 96 %) as a white solid.

R_f: 0.27 (EtOAc/hexanes+AcOH 70:30+1).

m.p.: 180 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 8.25 (t, *J* = 5.5 Hz, 1H, CONH), 7.73 – 7.64 (m, 2H, 2'-H and 6'-H), 6.83 – 6.74 (m, 2H, 3'-H and 5'-H), 3.41 (td, *J* = 7.2, 5.4 Hz, 2H, 3-H), 2.52 – 2.44 (m, 2H, 2-H).

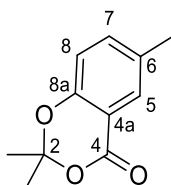
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 173.0 (C-1), 165.9 (CONH), 160.1 (C-4'), 129.0 (C-2' and C-6'), 125.1 (C-1'), 114.7 (C-3' and C-5'), 35.5 (C-3), 34.0 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3439, 3247, 1703, 1637, 1503, 1331, 1205, 923, 851, 726.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₀H₁₀NO₄: 208.0615; found: 208.0616.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

2,2,6-Trimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (58)C₁₁H₁₂O₃M_w = 192.21 g/mol**Method A:**^[109]

Under N₂ atmosphere, first acetone (4.83 mL, 65.7 mmol) and then dropwise SOCl₂ (3.36 mL, 46.0 mmol) were added to a solution of 5-methylsalicylic acid (**57**, 5.00 g, 32.9 mmol) and DMAP (2.01 g, 16.4 mmol) in 1,2-dimethoxyethane (25 mL) at 0 °C and stirred for 1 h at this temperature. The reaction mixture was then allowed to reach room temperature and stirred for further 19 h. Sat. aq. NaHCO₃ (25 mL) was added to the mixture and the aq. phase was extracted with diethyl ether (3 x 35 mL). The combined organic layers were washed with brine (2 x 20 mL), dried using a phase separation paper and the solution concentrated under reduced pressure. The crude product was purified by FCC (EtOAc/hexanes 5:95) to give acetonide **58** (1.61 g, 8.39 mmol, 26 %) as an off-white solid.

Method B:

Prepared according to **General Procedure C** from 5-methylsalicylic acid (**57**, 6.70 g, 44.0 mmol). The crude product was purified by FCC (EtOAc/hexanes 7:93) to give acetonide **58** (4.26 g, 22.2 mmol, 50 %) as an off-white solid.

R_f: 0.33 (EtOAc/hexanes 7:93).

m.p.: 87 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.75 (dp, *J* = 2.0, 0.8 Hz, 1H, 5-H), 7.37 – 7.32 (m, 1H, 7-H), 6.86 (d, *J* = 8.3 Hz, 1H, 8-H), 2.34 (d, *J* = 0.8 Hz, 3H, 6-CH₃), 1.72 (s, 6H, C(CH₃)₂).

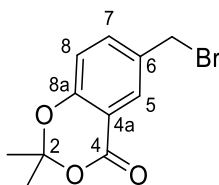
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 161.5 (C-4), 154.1 (C-8a), 137.4 (C-7), 132.4 (C-6), 129.6 (C-5), 117.1 (C-8), 113.4 (C-4a), 106.4 (C-2), 25.9 (C(CH₃)₂), 20.7 (6-CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1729, 1618, 1489, 1380, 1292, 1200, 1046, 932, 832, 779.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₁H₁₃O₃⁺: 193.0859; found: 193.0859.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

6-(Bromomethyl)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (59)^[109]C₁₁H₁₁BrO₃M_w = 271.11 g/mol

Acetonide **58** (1.00 g, 5.20 mmol) was dissolved in CCl₄ (13 mL) in a pressure tube. NBS (1.02 g, 5.72 mmol) and AIBN (87.0 mg, 0.520 mmol) were added and the reaction mixture heated to 80 °C for 16 h. Then, it was cooled to room temperature, filtered and the solvent of the filtrate removed *in vacuo*. The resulting residue was dissolved in DCM (15 mL), washed with brine (2 x 15 mL) and the solvent removed *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 5:95) to give benzyl bromide **59** (645 mg, 2.38 mmol, 46 %) as a white solid.

R_f: 0.20 (EtOAc/hexanes 5:95).

m.p.: 149 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.99 – 7.96 (m, 1H, 5-H), 7.59 (dd, *J* = 8.5, 2.3 Hz, 1H, 7-H), 6.96 (d, *J* = 8.5 Hz, 1H, 8-H), 4.48 (s, 2H, CH₂), 1.74 (s, 6H, C(CH₃)₂).

¹³C-NMR

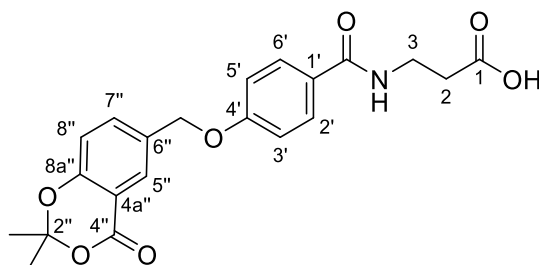
(101 MHz, CDCl₃): δ (ppm) = 160.7 (C-4), 156.1 (C-8a), 137.3 (C-7), 132.6 (C-6), 130.0 (C-5), 118.1 (C-8), 113.7 (C-4a), 106.8 (C-2), 32.2 (CH₂), 26.0 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1736, 1615, 1492, 1302, 1201, 1053, 936, 847, 779, 705, 607.

HR-MS (EI): *m/z* = [M]⁺⁺ calcd for C₁₁H₁₁BrO₃⁺⁺: 269.9886; found: 269.9881.

Purity (NMR): >95 %.

3-(4-((2,2-Dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)methoxy)benzamido)propanoic acid (CG_94)



$C_{21}H_{21}NO_7$

$M_w = 399.40$ g/mol

To phenol **56** (100 mg, 0.478 mmol), bromide **59** (130 mg, 0.478 mmol) and Cs_2CO_3 (389 mg, 1.20 mmol) was added dry DMF (5.0 mL) and the mixture heated to 60 °C for 20 min. Water (50 mL) was added and stirring continued for 30 min. The aq. phase was acidified with 2 N aq. HCl and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with 1 N aq. HCl (20 mL), dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/toluene+AcOH 50:50+1) to give ether **CG_94** (117 mg; 0.292 mmol, 61 %) as a colourless to white oil.

R_f: 0.29 (EtOAc/toluene+AcOH 50:50+1).

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 9.97 (s, 1H, COOH), 8.28 (t, $J = 5.5$ Hz, 1H, CONH), 7.89 (d, $J = 2.2$ Hz, 1H, 5''-H), 7.70 – 7.63 (m, 3H, 2'-H and 6'-H and 7''-H), 7.08 (d, $J = 8.5$ Hz, 1H, 8''-H), 6.79 – 6.74 (m, 2H, 3'-H and 5'-H), 5.10 (s, 2H, Ar-CH₂), 3.47 (td, $J = 6.9, 5.4$ Hz, 2H, 3-H), 2.62 (t, $J = 6.9$ Hz, 2H, 2-H), 1.68 (s, 6H, C(CH₃)₂).

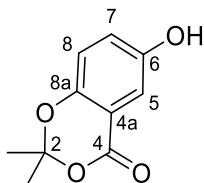
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 171.3 (C-1), 166.0 (CONH), 160.1 (C-4'), 160.0 (C-4''), 155.2 (C-8a''), 137.0 (C-7''), 131.0 (C-6''), 129.0 (C-2' and C-6'), 128.9 (C-5''), 125.0 (C-1'), 117.4 (C-8''), 114.7 (C-3' and C-5'), 112.8 (C-4a''), 106.5 (C-2''), 64.6 (Ar-CH₂), 35.4 (C-3), 33.9 (C-2), 25.2 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2956, 2924, 2855, 2360, 1732, 1458, 1259, 1172, 1029, 799.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{21}H_{20}NO_7^-$: 398.1245; found: 398.1246.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

6-Hydroxy-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (62)^[110]C₁₀H₁₀O₄M_w = 194.19 g/mol

TFAA (2.71 mL, 19.5 mmol) and acetone (2.39 mL, 32.4 mmol) were added to a stirred mixture of 2,5-dihydroxybenzoic acid (**61**, 1.00 g, 6.49 mmol) and TFA (8.03 mL, 107 mmol) at 0 °C. The mixture was slowly warmed to room temperature and after 14 h the solvent was concentrated to 1/3 of its volume. EtOAc (5.0 mL) and sat. aq. NaHCO₃ (50 mL) were added and the mixture was stirred for 2 h. The layers were separated, and the aq. phase was extracted with EtOAc (3 x 50 mL). The combined extracts were dried using a phase separation paper, concentrated *in vacuo* and purified by FCC (EtOAc/hexanes 25:75) to give acetonide **62** (388 mg, 2.00 mmol, 31 %) as an off-white solid.

R_f: 0.32 (EtOAc/hexanes 25:75).

m.p.: 145 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.48 (d, *J* = 3.0 Hz, 1H, 5-H), 7.11 (dd, *J* = 8.8, 3.0 Hz, 1H, 7-H), 6.87 (d, *J* = 8.8 Hz, 1H, 8-H), 5.94 (s, 1H, OH), 1.72 (s, 6H, C(CH₃)₂).

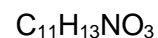
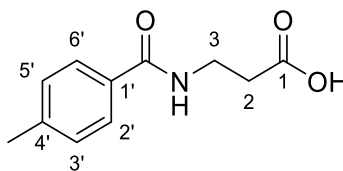
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 162.0 (C-4), 151.4 (C-6), 150.1 (C-8a), 124.9 (C-7), 118.6 (C-8), 114.6 (C-5), 113.9 (C-4a), 106.8 (C-2), 25.8 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3313, 1710, 1623, 1500, 1468, 1381, 1328, 1283, 1197, 1060, 985, 887, 780, 640.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₀H₉O₄⁻: 193.0506; found: 193.0507.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

3-(4-Methylbenzamido)propanoic acid (64)M_w = 207.23 g/mol

β -Alanine (535 mg, 6.00 mmol) was added to a solution of 1 N aq. NaOH (14 mL) at 0 °C. After the addition of *p*-toluoyl chloride (**63**, 800 μ L, 6.00 mmol) the solution was stirred for 30 min at 0 °C and was allowed to warm to room temperature over a period of 2 h. Subsequently, excessive starting material was filtered off and the remaining filtrate was adjusted to pH 2 – 3 through the addition of 1 N aq. HCl. The resulting precipitate was collected by filtration, washed with 1 N aq. HCl (2 x 20 mL) and water (20 mL) and dried under reduced pressure. The crude product was purified by FCC (acetone/hexanes+AcOH 30:70+1) to give amide **64** (912 mg, 4.40 mmol, 73 %) as a white solid.

R_f: 0.20 (acetone/hexanes+AcOH 30:70+1).

m.p.: 134 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 7.71 – 7.67 (m, 2H, 2'-H and 6'-H), 7.28 – 7.24 (m, 2H, 3'-H and 5'-H), 3.62 (t, *J* = 6.9 Hz, 2H, 3-H), 2.63 (t, *J* = 6.9 Hz, 2H, 2-H), 2.39 (s, 3H, CH₃).

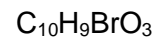
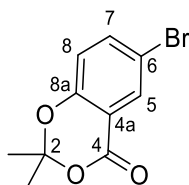
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 175.2 (C-1), 170.3 (CONH), 143.4 (C-4'), 132.7 (C-1'), 130.1 (C-3' and C-5'), 128.3 (C-2' and C-6'), 37.0 (C-3), 34.7 (C-2), 21.4 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3369, 1710, 1608, 1548, 1507, 1424, 1200, 643, 633, 623.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₁H₁₂NO₃⁻: 206.0823; found: 206.0823.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

6-Bromo-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (72)^[114]

M_w = 257.08 g/mol

5-Bromosalicylic acid (**71**, 2.00 g, 9.22 mmol) in TFA (13.8 mL, 184 mmol) was cooled to 0 °C. TFAA (12.8 mL, 92.2 mmol) and acetone (2.03 mL, 27.6 mmol) were added, and the mixture stirred at room temperature for 48 h. The crude reaction mixture was concentrated *in vacuo*, quenched with sat. aq. NaHCO₃ (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with water (2 x 25 mL) and brine (2 x 25 mL), dried using a phase separation paper and the solvent removed *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 7:93) to give acetonide **72** (480 mg, 1.87 mmol, 20 %) as a yellow solid.

R_f: 0.33 (EtOAc/hexanes 7:93).

m.p.: 74 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.08 (d, *J* = 2.4 Hz, 1H, 5-H), 7.64 (dd, *J* = 8.7, 2.4 Hz, 1H, 7-H), 6.87 (d, *J* = 8.7 Hz, 1H, 8-H), 1.73 (s, 6H, C(CH₃)₂).

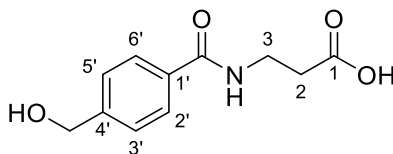
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 160.0 (C-4), 155.1 (C-8a), 139.3 (C-7), 132.3 (C-5), 119.3 (C-8), 115.2 (C-4a), 115.1 (C-6), 107.0 (C-2), 25.9 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1733, 1601, 1468, 1419, 1379, 1279, 1199, 1048, 929, 824, 779, 581.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₀H₉BrO₃⁺: 255.9730; found: 255.9727.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

3-(4-(Hydroxymethyl)benzamido)propanoic acid (74)C₁₁H₁₃NO₄M_w = 223.23 g/mol

4-(Hydroxymethyl)benzoic acid (**73**, 500 mg, 3.29 mmol) was dissolved in dry DCM (20 mL). At 0 °C oxalyl dichloride (417 μL, 4.93 mmol) was added dropwise, followed by the addition of a catalytic amount of DMF (2 drops). The resulting mixture was stirred at room temperature for 2 h. After this time, the solvent was removed *in vacuo* to afford crude acid chloride.

To a solution of K₂CO₃ (1.09 g, 6.57 mmol) in a mixture of EtOAc/water (2:1, 20 mL) was added β-alanine (351 mg, 3.94 mmol). The resulting solution was cooled to 0 °C, followed by dropwise addition of the crude substituted benzoyl chloride dissolved in DCM (3.0 mL). The reaction mixture was warmed to room temperature and stirred for 16 h. Phases were separated, and the aq. phase further extracted with EtOAc (20 mL). The combined organic layers were discarded, and the aq. phase was adjusted to pH 1 – 2 by the addition of 2 N aq. HCl and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried using a phase separation paper and the solvent removed *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes+AcOH 70:30+1) to give product **74** (374 mg, 1.68 mmol, 51 %) as a white solid.

R_f: 0.17 (EtOAc/hexanes+AcOH 70:30+1).

m.p.: 102 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.13 (s, 1H, COOH), 8.70 (t, *J* = 6.0 Hz, 1H, OH), 8.47 (t, *J* = 5.5 Hz, 1H, CONH), 7.81 – 7.77 (m, 2H, 2'-H and 6'-H), 7.40 – 7.36 (m, 2H, 3'-H and 5'-H), 4.54 (s, 2H, Ar-CH₂), 3.45 (td, *J* = 7.1, 5.4 Hz, 2H, 3-H), 2.52 (d, *J* = 1.8 Hz, 1H, 2-H).

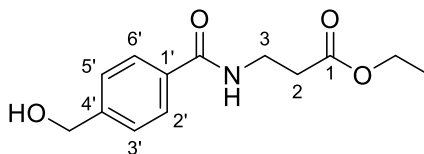
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (COOH), 166.1 (CONH), 145.8 (C-4'), 132.7 (C-1'), 127.0 (C-2' and C-6'), 125.9 (C-3' and C-6'), 62.4 (Ar-CH₂), 35.6 (C-3), 33.9 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3308, 2930, 2602, 1711, 1641, 1548, 1269, 1202, 1005, 850, 673.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₁H₁₂NO₄: 222.0772; found: 222.0774.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Ethyl 3-(4-(hydroxymethyl)benzamido)propanoate (77)C₁₃H₁₇NO₄M_w = 251.28 g/mol

Prepared according to **General Procedure E** from 4-(hydroxymethyl)benzoic acid (**73**, 500 mg, 3.29 mmol) and β-alanine ethyl ester hydrochloride (**76**, 337 mg, 2.19 mmol). The crude product was purified by FCC (DCM/EtOAc 50:50) to give amide **77** (315 mg, 1.25 mmol, 57 %) as a colourless oil.

R_f: 0.23 (DCM/EtOAc 50:50).

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.68 (d, *J* = 7.9 Hz, 2H, 2'-H and 6'-H), 7.35 (d, *J* = 7.8 Hz, 2H, 3'-H and 5'-H), 6.96 (t, *J* = 6.1 Hz, 1H, CONH), 4.71 (s, 2H, Ar-CH₂), 4.15 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 3.69 (q, *J* = 5.8 Hz, 2H, 3-H), 2.62 (t, *J* = 5.9 Hz, 2H, 2-H), 1.26 (t, *J* = 7.1 Hz, 3H, CH₃).

¹³C-NMR

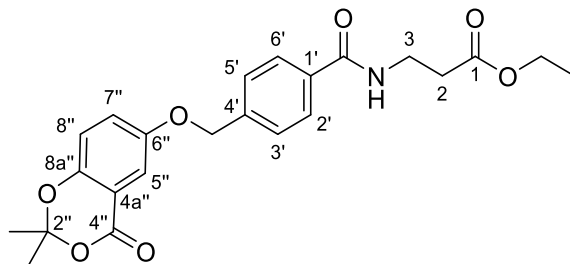
(101 MHz, CDCl₃): δ (ppm) = 173.1 (C-1), 167.4 (CONH), 144.9 (C-4'), 133.4 (C-1'), 127.2 (C-2' and C-6'), 126.8 (C-3' and C-5'), 64.6 (Ar-CH₂), 61.0 (CH₂CH₃), 35.5 (C-3), 34.0 (C-2), 14.3 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3321, 1937, 1727, 1714, 1633, 1537, 1307, 1270, 1181, 1017, 853, 752.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₃H₁₈NO₄⁺: 252.1230; found: 252.1231.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Ethyl 3-(4-(((2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)oxy)methyl)benzamido)propanoate (78)



$C_{23}H_{25}NO_7$

$M_w = 427.45$ g/mol

To a solution of primary alcohol **77** (50.0 mg, 0.199 mmol) and phenol **62** (38.6 mg, 0.199 mmol) in dry THF (1.0 mL), triphenylphosphine (52.7 mg, 0.199 mmol) was added at room temperature. The reaction mixture was cooled to 0 °C and DEAD (40 % in toluene, 90.6 μ L, 0.199 mmol) was added dropwise and the reaction mixture was allowed to reach room temperature and was stirred for 16 h. The solvent was evaporated under reduced pressure and the crude product purified by FCC (EtOAc/hexanes 50:50) to give ether **78** (56.4 mg, 0.132 mmol, 66 %) as a white solid.

R_f: 0.40 (EtOAc/hexanes 50:50).

m.p.: 112 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.81 – 7.77 (m, 2H, 2'-H and 6'-H), 7.51 – 7.46 (m, 3H, 3'-H, 5'-H and 5''-H), 7.20 (dd, $J = 9.0, 3.1$ Hz, 1H, 7''-H), 6.91 (d, $J = 9.0$ Hz, 1H, 8''-H), 6.87 (d, $J = 5.9$ Hz, 1H, CONH), 5.10 (s, 2H, 4'-CH₂), 4.17 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 3.73 (q, $J = 6.1$ Hz, 2H, 3-H), 2.68 – 2.62 (m, 2H, 2-H), 1.72 (s, 6H, C(CH₃)₂), 1.27 (t, $J = 7.1$ Hz, 3H, CH₂CH₃).

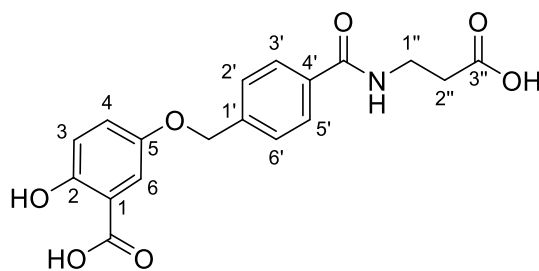
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 173.1 (C-1), 167.0 (CONH), 161.3 (C-4''), 153.8 (C-6''), 150.7 (C-8a''), 140.1 (C-4'), 134.3 (C-1'), 127.5 (C-3' and C-5'), 127.4 (C-2' and C-6'), 125.5 (C-7''), 118.7 (C-8''), 113.9 (C-4a''), 112.5 (C-5''), 106.6 (C-2''), 70.1 (4'-CH₂), 61.0 (CH₂CH₃), 35.4 (C-3), 34.1 (C-2), 25.8 (C(CH₃)₂), 14.3 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3340, 2958, 1726, 1629, 1494, 1291, 1193, 1178, 1135, 1058, 1015, 846.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for C₂₃H₂₆NO₇⁺: 428.1704; found: 428.1705.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

5-((4-((2-Carboxyethyl)carbamoyl)benzyl)oxy)-2-hydroxybenzoic acid (CG_129)C₁₈H₁₇NO₇M_w = 359.33 g/mol

Prepared according to **General Procedure D** from compound **78** (40.0 mg, 0.0936 mmol) and heating the reaction mixture to reflux. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give pure ether **CG_129** (27.4 mg, 0.0763 mmol, 82 %) as a white solid.

R_f: 0.34 (EtOAc/hexanes+AcOH 60:40+1).

m.p.: 209 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.59 – 8.47 (m, 1H, CONH), 7.84 – 7.79 (m, 2H, 3'-H and 5'-H), 7.55 – 7.50 (m, 2H, 2'-H and 6'-H), 7.42 (d, *J* = 3.1 Hz, 1H, 6-H), 7.18 (dd, *J* = 9.0, 3.2 Hz, 1H, 4-H), 6.86 (d, *J* = 9.1 Hz, 1H, 3-H), 5.11 (s, 2H, 1'-CH₂), 3.67 – 3.60 (m, 2H, 1''-H), 2.64 (t, *J* = 6.9 Hz, 2H, 2''-H).

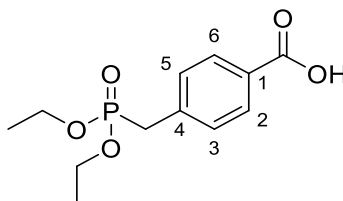
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 175.4 (C-3''), 173.1 (1-COOH), 170.0 (CONH), 157.8 (C-2), 152.2 (C-5), 142.6 (C-1'), 135.1 (C-4'), 128.5 (C-3' and C-5' or C-2' and C-6'), 128.4 (C-3' and C-5' or C-2' and C-6'), 125.4 (C-4), 119.2 (C-3), 115.5 (C-6), 113.7 (C-1), 71.1 (1'-CH₂), 37.1 (C-1''), 34.6 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3311, 3921, 1692, 1676, 1624, 1486, 1441, 1292, 1209, 1182, 1084, 1034, 828.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₈H₁₆NO₇⁻: 358.0932; found: 358.0933.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

4-((Diethoxyphosphoryl)methyl)benzoic acid (**79**)^[115]C₁₂H₁₇O₅PM_w = 272.24 g/mol

A suspension of 4-(bromomethyl)benzoic acid (**66**, 1.50 g, 6.98 mmol) in triethyl phosphite (1.32 mL, 7.67 mmol) was heated to reflux for 20 h. The reaction was allowed to cool to room temperature. The slowly formed precipitate was collected by filtration, washed intensively with hexanes (50 mL) and dried *in vacuo* to give phosphonate **79** (1.74 g, 6.40 mmol, 92 %) as a white solid.

R_f: 0.29 (EtOAc/hexanes+AcOH 70:30+1).

m.p.: 107 - 111 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.04 – 8.01 (m, 2H, 2-H and 6-H), 7.40 (dd, *J* = 8.4, 2.4 Hz, 2H, 3-H and 5-H), 4.12 – 4.00 (m, 4H, (CH₂CH₃)₂), 3.25 (d, *J* = 22.3 Hz, 2H, Ar-CH₂), 1.27 (t, *J* = 7.0 Hz, 6H, (CH₂CH₃)₂).

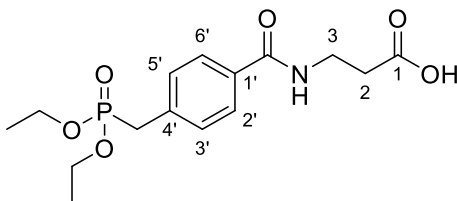
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 170.4 (COOH), 137.7 (C-4), 130.5 (C-3 and C-5), 130.1 (C-2 and C-6), 128.6 (C-1), 62.6 ((CH₂CH₃)₂), 34.9 (Ar-CH₂), 16.5 ((CH₂CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2983, 1700, 1609, 1418, 1249, 1206, 1172, 1020, 978, 952, 867, 746.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₂H₁₆O₅P⁻: 271.0741; found: 271.0742.

Purity (HPLC): 210 nm: 77 %; 254 nm: 72 % (method 3a).

3-(4-((Diethoxyphosphoryl)methyl)benzamido)propanoic acid (80)C₁₅H₂₂NO₆PM_w = 343.32 g/mol

To a solution of benzoic acid **79** (1.50 g, 5.51 mmol) in cyclohexane (65 mL), thionyl dichloride (0.804 mL, 11.0 mmol) was added. The reaction mixture was heated to reflux for 3.5 h. The solvent and excess of reagent were then removed under reduced pressure. The resulting crude oil was added to β-alanine (491 mg, 5.51 mmol) dissolved in 1 N aq. NaOH (12 mL) at 0 °C and the mixture stirred for 30 min at 0 °C. After this time, it was allowed to warm to room temperature over a period of 2 h. Subsequently, excessive starting material was filtered off and the remaining filtrate was adjusted to a pH 2 – 3 through the addition of 1 N aq. HCl and diluted with EtOAc (20 mL). Phases were separated and the aqueous phase was further extracted with EtOAc (2 x 20 mL), DCM (20 mL) and diethyl ether (20 mL). The combined organic extracts were dried using a phase separation paper and the solvent removed *in vacuo*. The crude product was purified by FCC (MeOH/DCM 4:96) to give phosphonate **80** (954 mg, 2.78 mmol, 50 %) as a pale yellow oil.

R_f: 0.21 (MeOH/DCM 4:96).

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.10 (s, 2H, COOH), 8.49 (t, J = 5.5 Hz, 1H, CONH), 7.79 – 7.74 (m, 2H, 2'-H and 6'-H), 7.37 – 7.32 (m, 2H, 3'-H and 5'-H), 3.98 – 3.91 (m, 4H, (CH₂CH₃)₂), 3.44 (td, J = 7.1, 5.5 Hz, 2H, 3-H), 3.29 (d, J = 21.9 Hz, 2H, Ar-CH₂), 2.52 (m, 1H, 2-H, collapses with DMSO), 1.16 (t, J = 7.0 Hz, 6H, (CH₂CH₃)₂).

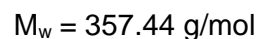
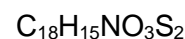
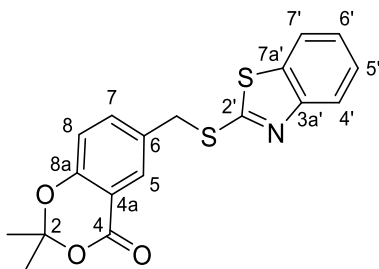
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (COOH), 166.0 (CONH), 135.8 (C-4'), 132.6 (C-1'), 129.6 (C-2' and C-6'), 127.1 (C-3' and C-5'), 61.4 ((CH₂CH₃)₂), 35.6 (C-3), 33.8 (C-2), 32.2 (d, Ar-CH₂), 16.2 ((CH₂CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2915, 1724, 1637, 1546, 1504, 1186, 1020, 968, 863, 754

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₅H₂₁NO₆P⁻: 342.1112; found: 342.1116.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

6-((Benzo[d]thiazol-2-ylthio)methyl)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (83)^[109]

To a solution of bromide **59** (500 mg, 1.84 mmol) and 2-mercaptobenzothiazole (**82**; 370 mg, 2.21 mmol) in DCM (5.0 mL), triethylamine (514 μL, 3.69 mmol) was added at 0 °C. Then the mixture was stirred at room temperature for 2.5 h. Water (50 mL) was added and the aq. phase extracted with DCM (3 x 30 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL) and dried using a phase separation paper. The filtrate was concentrated under reduced pressure. The resulting crude product was purified by FCC (EtOAc/hexanes 10:90) to give thioether **83** (540 mg, 1.51 mmol, 82 %) as a white solid.

R_f: 0.20 (EtOAc/hexanes 10:90).

m.p.: 102 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.05 (d, *J* = 2.3 Hz, 1H, 5-H), 7.92 (dd, *J* = 8.1, 1.0 Hz, 1H, 4'-H), 7.76 (ddd, *J* = 8.1, 1.3, 0.7 Hz, 1H, 7'-H), 7.69 (dd, *J* = 8.5, 2.3 Hz, 1H, 7-H), 7.44 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H, 5'-H), 7.31 (ddd, *J* = 8.4, 7.3, 1.2 Hz, 1H, 6'-H), 6.92 (d, *J* = 8.5 Hz, 1H, 8-H), 4.60 (s, 2H, Ar-CH₂), 1.72 (s, 7H, C(CH₃)₂).

¹³C-NMR

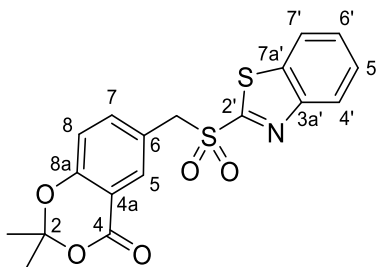
(126 MHz, CDCl₃): δ (ppm) = 165.9 (C-2'), 160.9 (C-4), 155.6 (C-8a), 152.9 (C-3a'), 137.4 (C-7), 135.4 (C-7a'), 131.3 (C-6), 130.3 (C-5), 126.4 (C-5'), 124.7 (C-6'), 121.7 (C-4'), 121.2 (C-7'), 117.8 (C-8), 113.6 (C-4a), 106.7 (C-2), 36.7 (Ar-CH₂), 26.0 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1733, 1618, 1494, 1457, 1427, 1298, 1202, 1050, 993, 843, 754, 724.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₈H₁₆NO₃S₂⁺: 358.0566; found: 358.0568.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

6-((Benzo[d]thiazol-2-ylsulfonyl)methyl)-2,2-dimethyl-4*H*-benzo[d][1,3]dioxin-4-one
(84)^[109]



C₁₈H₁₅NO₅S₂

M_w = 389.44 g/mol

To a solution of thioether **83** (357 mg, 1.00 mmol) in MeOH (5.0 mL) was added Na₂WO₄ dihydrate (330 mg, 1.00 mmol) and 30 % H₂O₂ (1.70 mL, 15.0 mmol). The mixture was stirred at room temperature for 12 h. The solvent was then evaporated under reduced pressure and the residue resuspended in water (50 mL) and extracted with DCM (3 x 40 mL). The combined organic layers were dried using a phase separation paper and concentrated under reduced pressure to give product **84** (375 mg, 0.963 mmol, 96 %) as a white solid.

R_f: 0.39 (EtOAc/hexanes 35:65).

m.p.: 187 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.26 (ddd, *J* = 8.3, 1.3, 0.7 Hz, 1H, 4'-H), 7.96 (ddd, *J* = 8.2, 1.3, 0.7 Hz, 1H, 7'-H), 7.84 (d, *J* = 2.2 Hz, 1H, 5-H), 7.66 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H, 5'-H), 7.62 – 7.56 (m, 2H, 7-H and 6'-H), 6.94 (d, *J* = 8.5 Hz, 1H, 8-H), 4.74 (s, 2H, Ar-CH₂), 1.69 (s, 6H, C(CH₃)₂).

¹³C-NMR

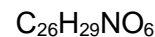
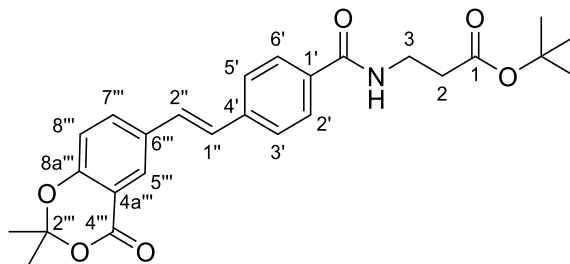
(101 MHz, CDCl₃): δ (ppm) = 164.9 (C-2'), 160.2 (C-4), 156.8 (C-8a), 152.7 (C-3a'), 139.0 (C-7), 137.0 (C-7a'), 132.6 (C-5), 128.4 (C-6'), 128.0 (C-5'), 125.8 (C-4'), 122.4 (C-7'), 121.0 (C-6), 118.3 (C-8), 113.9 (C-4a), 106.9 (C-2), 60.1 (Ar-CH₂), 25.9 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 1729, 1620, 1494, 1302, 1202, 1147, 1056, 767, 619.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₈H₁₆NO₅S₂⁺: 390.0464; found: 390.0467.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

tert*-Butyl (*E/Z*)-3-(4-(2-(2,2-dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxin-6-yl)vinyl)benzamido)propanoate (**85-*E/Z)**



$$M_w = 451.52 \text{ g/mol}$$

To a solution of sulfone **84** (850 mg, 2.18 mmol) in dry DMF (21 mL), sodium hydride (131 mg, 3.27 mmol) was added at 0 °C under N₂ atmosphere. The solution turned reddish indicating the formation of carbanion. After 5 min of stirring, aldehyde **46** (605 mg, 2.18 mmol) was added. The temperature of the reaction was gradually raised from 0 °C to room temperature and stirring continued at this temperature for 16 h. The reaction mixture was quenched with sat. aq. NH₄Cl (15 mL). The aqueous phase was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with 10 % aq. NaOH (3 x 15 mL) to remove by-product hydroxybenzothiazole, followed by water (20 mL) and brine (20 mL). The solvent was then removed *in vacuo* and the crude product dissolved in MeOH (10 mL). To remove unreacted aldehyde, sat. NaHSO₃ (50 mL) was added to the solution, shaken for approx. 30 s, diluted with water (50 mL) and extracted with a solvent mixture of 10 % EtOAc in hexanes (3 x 50 mL). The obtained crude product was purified by FCC (EtOAc/hexanes 40:60) to give the *E*-isomer **85-*E*** (349 mg, 0.774 mmol, 35 %) as a white solid, the *Z*-isomer **85-*Z*** (24.9 mg, 0.0552 mmol, 3 %) as a pale yellow oil and a mixture of *E*- and *Z*-isomer **85-*E/Z*** as a pale yellow oil (154 mg, 0.341 mmol, 16 %).

E-Isomer:

R_f: 0.27 (EtOAc/hexanes 40:60).

m.p.: 166 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.12 (d, *J* = 2.2 Hz, 1H, 5'''-H), 7.79 – 7.75 (m, 2H, 2'-H and 6'-H), 7.70 (dd, *J* = 8.5, 2.3 Hz, 1H, 7'''-H), 7.56 – 7.52 (m, 2H, 3'-H and 5'-H), 7.13 (d, *J* = 16.4 Hz, 1H, 2''-H), 7.08 (d, *J* = 16.4 Hz, 1H, 1''-H), 6.98 (d, *J* = 8.5 Hz, 1H, 8'''-H), 6.89 (t, *J* = 6.0 Hz, 1H, CONH), 3.70 (q, *J* = 6.0 Hz, 2H, 3-H), 2.60 – 2.53 (m, 3H, 2-H), 1.76 (s, 6H, C(CH₃)₂), 1.47 (s, 9H, C(CH₃)₃).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 172.5 (C-1), 166.8 (CONH), 161.2 (C-4'''), 155.7 (C-8a'''), 140.1 (C-4'), 134.6 (C-7'''), 133.6 (C-1'), 132.1 (C-6'''), 128.6 (C-2''), 128.2 (C-1''), 127.6 (C-2' and C-6'), 127.5 (C-5'''), 126.7 (C-3' and C-5'), 117.8 (C-8'''), 113.9 (C-4a'''), 106.8 (C-2'''), 81.4 (C(CH₃)₃), 35.6 (C-3), 35.2 (C-2), 28.3 (C(CH₃)₂), 26.0 (C(CH₃)₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3384, 2931, 1727, 1712, 1657, 1602, 1537, 1503, 1316, 1269, 1206, 1156, 1138, 1062, 945, 838.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₂₆H₃₀NO₆⁺: 452.2068; found: 452.2069.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Z-Isomer:

R_f: 0.34 (EtOAc/hexanes 40:60).

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.84 (d, J = 2.4 Hz, 1H, 5'''-H), 7.66 – 7.62 (m, 2H, 2'-H and 6'-H), 7.35 (dd, J = 8.6, 2.2 Hz, 1H, 7'''-H), 7.30 – 7.26 (m, 2H, 3' and 5'-H), 6.84 (t, J = 6.1 Hz, 1H, CONH), 6.76 (d, J = 8.5 Hz, 1H, 8'''-H), 6.64 (d, J = 12.3 Hz, 1H, 1''-H or 2''-H), 6.60 (d, J = 12.3 Hz, 1H, 1''-H or 2''-H), 3.68 (q, J = 6.0 Hz, 2H, 3-H), 2.59 – 2.51 (m, 2H, 2-H), 1.73 (s, 6H, C(CH₃)₂), 1.46 (s, 9H, C(CH₃)₃).

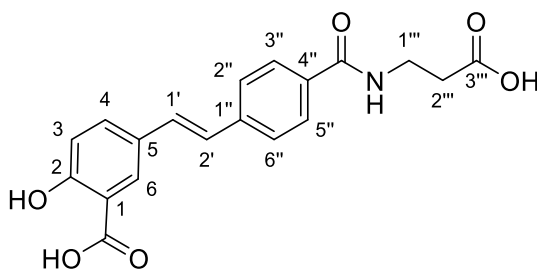
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 172.5 (C-1), 166.9 (CONH), 161.0 (C-4'''), 155.3 (C-8a'''), 140.2 (C-4'), 136.8 (C-7'''), 133.4 (C-1'), 131.6 (C-6'''), 130.3 (C-5'''), 130.0 (C-1''), 129.8 (C-2''), 129.1 (C-3' and C-5'), 127.2 (C-2' and C-6'), 117.2 (C-8'''), 113.6 (C-4a'''), 106.6 (C-2'''), 81.4 (C(CH₃)₃), 35.6 (C-3), 35.2 (C-2), 28.3 (C(CH₃)₂), 26.0 (C(CH₃)₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3336, 2979, 1726, 1642, 1614, 1493, 1276, 1203, 1154, 1049, 843, 779.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₂₆H₃₀NO₆⁺: 452.2068; found: 452.2069.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

(E)-5-(4-((2-Carboxyethyl)carbamoyl)styryl)-2-hydroxybenzoic acid (CG_111)C₁₉H₁₇NO₆M_w = 355.35 g/mol

Prepared according to **General Procedure D** from compound **85-E** (80.0 mg, 0.177 mmol) and heating the reaction mixture to 60 °C. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo*. The crude product was resuspended in 1 N aq. NaOH and 2 N aq. HCl was added until no more product precipitated. The crude product was collected by filtration and purified by FCC (MeOH/DCM+AcOH 5:95+1) to give stilbene **CG_111** (37.0 mg, 0.104 mmol, 59 %) as a white solid.

R_f: 0.19 (DCM/hexanes+AcOH 50:50+1).

m.p.: 245 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 8.51 (s, 1H, CONH), 7.94 (d, *J* = 2.4 Hz, 1H, 6-H), 7.81 (d, *J* = 8.0 Hz, 2H, 3''-H and 5''-H), 7.59 (d, *J* = 8.0 Hz, 2H, 2''-H and 6''-H), 7.45 (dd, *J* = 8.4, 2.4 Hz, 1H, 4-H), 7.24 (d, *J* = 16.4 Hz, 1H, 1'-H), 6.98 (d, *J* = 16.3 Hz, 1H, 2'-H), 6.65 (d, *J* = 8.3 Hz, 1H, 3-H), 3.52 – 3.38 (m, 1H, 1'''-H), 2.35 – 2.23 (m, 3H, 2'''-H).

¹³C-NMR

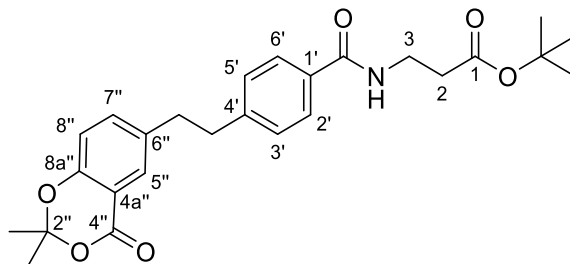
(101 MHz, (CD₃)₂SO): δ (ppm) = 177.0 (C-3'''), 171.2 (1-COOH), 165.6 (CONH), 161.6 (C-2), 140.5 (C-1''), 132.4 (C-4''), 130.8 (C-1'), 130.0 (C-4), 128.9 (C-6), 127.6 (C-3'' and C-5''), 125.5 (C-2'' and C-6''), 123.0 (C-2'), 120.2 (C-1), 116.6 (C-3), 36.6 (C-1'''), 36.5 (C-2''').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3296, 3021, 1678, 1634, 1440, 1330, 1291, 1203, 1085, 954, 838, 681.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₉H₁₆NO₆: 354.0983; found: 354.0984.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1f).

***tert*-Butyl 3-(4-(2-(2,2-dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxin-6-yl)ethyl)benzamido)propanoate (**86**)**



$C_{26}H_{31}NO_6$

$M_w = 453.54$ g/mol

Prepared according to **General Procedure A** from stilbene **85-*E/Z*** (100 mg, 0.221 mmol). Product **86** (72.2 mg, 0.159 mmol, 72 %) was obtained as a white solid.

R_f: 0.32 (DCM/hexanes+AcOH 50:50+1).

m.p.: 102 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 7.78 (d, $J = 2.3$ Hz, 1H, 5''-H), 7.69 – 7.66 (m, 2H, 2'-H and 6'-H), 7.28 – 7.25 (m, 1H, 7''-H), 7.21 – 7.17 (m, 2H, 3'-H and 5'-H), 6.85 (d, $J = 8.4$ Hz, 1H, 8''-H), 6.84 – 6.81 (m, 1H, CONH), 3.68 (q, $J = 6.0$ Hz, 2H, 3-H), 2.98 – 2.93 (m, 2H, 6''-CH₂), 2.93 – 2.89 (m, 2H, 4'-CH₂), 2.58 – 2.53 (m, 2H, 2-H), 1.72 (s, 6H, C(CH₃)₂), 1.46 (s, 9H, C(CH₃)₃).

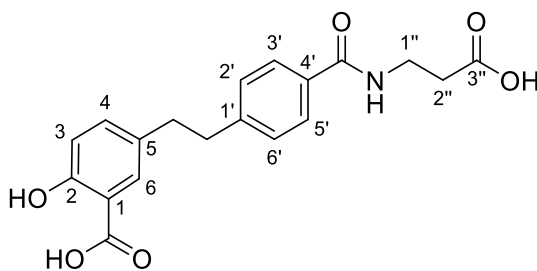
¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 172.5 (C-1), 167.2 (CONH), 161.5 (C-4''), 154.5 (C-8a''), 144.9 (C-4'), 136.9 (C-7''), 135.8 (C-6''), 132.6 (C-1'), 129.1 (C-5''), 128.8 (C-3' and C-5'), 127.2 (C-2' and C-6'), 117.2 (C-8''), 113.5 (C-4a''), 106.5 (C-2''), 81.4 (C(CH₃)₃), 37.6 (4'-CH₂), 36.7 (6''-CH₂), 35.6 (C-3), 35.2 (C-2), 28.3 (C(CH₃)₃), 25.9 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3396, 2929, 1712, 1657, 1494, 1308, 1255, 1210, 1160, 1058, 944, 848, 782.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for C₂₆H₃₂NO₆⁺: 454.2224; found: 454.2224.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

5-(4-((2-Carboxyethyl)carbamoyl)phenethyl)-2-hydroxybenzoic acid (**CG_142**)C₁₉H₁₉NO₆M_w = 357.36 g/mol

Prepared according to **General Procedure D** from compound **86** (60.0 mg, 0.132 mmol) and heating the reaction mixture to reflux. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give the pure product **CG_142** (43.3 mg, 0.121 mmol, 92 %) as a white solid.

R_f: 0.34 (EtOAc/DCM+AcOH 50:50+1).

m.p.: 200 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.21 (s, 1H, 1-COOH or 2''-COOH), 8.43 (t, *J* = 5.5 Hz, 1H, CONH), 7.76 – 7.70 (m, 2H, 3'-H and 5'-H), 7.62 (d, *J* = 2.3 Hz, 1H, 6-H), 7.35 (dd, *J* = 8.5, 2.4 Hz, 1H, 4-H), 7.31 – 7.26 (m, 2H, 2'-H and 6'-H), 6.85 (d, *J* = 8.4 Hz, 1H, 3-H), 3.44 (td, *J* = 7.1, 5.4 Hz, 2H, 1''-H), 2.92 – 2.86 (m, 2H, 1'-CH₂), 2.88 – 2.79 (m, 2H, 5-CH₂), 2.54 – 2.51 (m, 2H, 2''-H, collapses with DMSO).

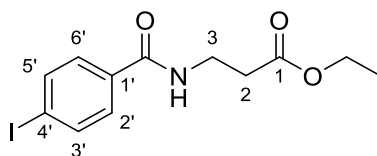
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3''), 171.9 (1-COOH), 166.1 (CONH), 159.4 (C-2), 144.7 (C-1'), 135.9 (C-4), 132.0 (C-5 or C-4'), 131.8 (C-5 or C-4'), 129.6 (C-6), 128.3 (C-2' and C-6'), 127.1 (C-3' and C-5'), 116.9 (C-3), 112.6 (C-1), 36.9 (1'-CH₂), 35.6 (C-1''), 35.5 (5-CH₂), 33.8 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2924, 2855, 1722, 1666, 1630, 1442, 1331, 1291, 1196, 836, 674.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₉H₁₈NO₆⁻: 356.1140; found: 356.1142.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Ethyl 3-(4-iodobenzamido)propanoate (**88**)^[121]C₁₂H₁₄INO₃M_w = 347.15 g/mol

A solution of β -alanine ethyl ester hydrochloride (**76**, 1.56 g, 10.1 mmol) in dry pyridine (10 mL) was added to a suspension of 4-iodo-benzoylchloride (**87**, 2.00 g, 7.51 mmol) in dry pyridine (7.0 mL). The reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated *in vacuo* and the residue was taken up in EtOAc (20 mL). The suspension was washed with 1 N aq. HCl (2 x 10 mL), then with 1 N aq. NaOH (2 x 10 mL) and dried using a phase separation paper. The solvent was evaporated *in vacuo* and product **88** (1.84 g, 5.31 mmol, 71 %) was obtained as an off-white solid.

R_f: 0.35 (EtOAc/hexanes 40:60).

m.p.: 95 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.80 – 7.76 (m, 2H, 3'-H and 5'-H), 7.51 – 7.47 (m, 2H, 2'-H and 6'-H), 6.86 (t, J = 5.7 Hz, 1H, CONH), 4.17 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.71 (q, J = 6.1 Hz, 2H, 3-H), 2.63 (t, J = 5.9 Hz, 2H, 2-H), 1.27 (t, J = 7.1 Hz, 3H, CH₃).

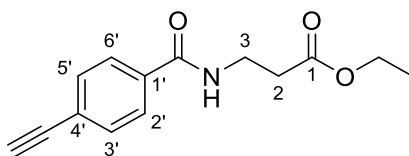
¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 173.2 (C-1), 166.6 (CONH), 137.9 (C-3' and C-5'), 133.9 (C-1'), 128.7 (C-2' and C-6'), 98.6 (C-4'), 61.1 (CH₂CH₃), 35.5 (C-3), 33.9 (C-2), 14.3 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3298, 2935, 1723, 1634, 1585, 1538, 1323, 1180, 1151, 1077, 1006, 853, 838.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₁₂H₁₅INO₃⁺: 348.0091; found: 348.0090.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-ethynylbenzamido)propanoate (**91**)C₁₄H₁₅NO₃M_w = 245.28 g/mol

Iodo compound **88** (868 mg, 2.50 mmol) was added to Et₃N (25 mL) under N₂ atmosphere. The mixture was degassed with N₂ for 10 min. Copper(I) iodide (47.6 mg, 0.250 mmol) and Pd(PPh₃)₂Cl₂ (175 mg, 0.250 mmol) were added. Then, trimethylsilylacetylene (**89**, 707 μL, 5.00 mmol) was added dropwise over 3 min. The reaction mixture was stirred at 60 °C for 16 h. Then, the mixture was diluted with EtOAc (20 mL) and filtered through a pad of celite. The filtrate was washed with 1 N aq. HCl (10 mL), sat. aq. NaHCO₃ (10 mL) and brine (10 mL). The organic phase was dried using a phase separation paper and concentrated *in vacuo*. The crude TMS-protected product (**90**) was used without further purification in the next step (identification *via* ¹H-NMR and HRMS: [M+H]⁺ calcd for C₁₇H₂₄NO₃Si⁺: 318.1520; found: 318.1522).

To a solution of crude alkyne **90** (2.50 mmol) in THF (25 mL), TBAF (1.0 M in THF; 2.75 mL, 2.75 mmol) was added. The solution was stirred at room temperature for 15 min. After removal of the solvent *in vacuo*, the crude product was purified by FCC (EtOAc/hexanes 40:60) to give alkyne **91** (577 mg, 2.35 mmol, 94 % over two steps) as a light brown solid.

R_f: 0.31 (EtOAc/hexanes 40:60).

m.p.: 55 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 7.74 – 7.70 (m, 2H, 2'-H and 6'-H), 7.56 – 7.53 (m, 2H, 3'-H and 5'-H), 6.88 (t, *J* = 5.4 Hz, 1H, CONH), 4.18 (q, *J* = 7.2 Hz, 2H, CH₂CH₃), 3.72 (q, *J* = 6.0 Hz, 2H, 3-H), 3.19 (s, 1H, C≡CH), 2.64 (t, *J* = 5.9 Hz, 2H, 2-H), 1.28 (t, *J* = 7.2 Hz, 3H, CH₃).

¹³C-NMR

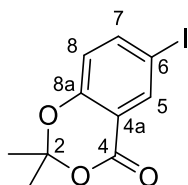
(101 MHz, CDCl₃): δ (ppm) = 173.1 (C-1), 166.6 (CONH), 134.5 (C-1'), 132.4 (C-3' and C-5'), 127.1 (C-2' and C-6'), 125.5 (C-4'), 82.9 (C≡CH), 79.6 (C≡CH), 61.0 (CH₂CH₃), 35.5 (C-3), 34.0 (C-2), 14.3 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3283, 3250, 2981, 2929, 1723, 1635, 1607, 1543, 1497, 1320, 1291, 1181, 1151, 1025, 852, 765, 664.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₄H₁₄NO₃⁻: 244.0979; found: 244.0978.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

6-Iodo-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (93)^[123]



$C_{10}H_9IO_3$

$M_w = 304.08$ g/mol

5-Iodosalicylic acid (**92**, 2.00 g, 7.58 mmol) in TFA (15.9 mL, 212 mmol) was cooled to 0 °C under N_2 atmosphere. TFAA (10.0 mL, 72.0 mmol) and acetone (4.46 mL, 60.6 mmol) were added, and the solution was allowed to warm to room temperature and stirred for 48 h. The solution was concentrated *in vacuo* and the residue purified by FCC (EtOAc/hexanes 5:95) to give product **93** (895 mg, 2.94 mmol, 39 %) as a pale yellow solid.

R_f: 0.28 (EtOAc/hexanes 5:95).

m.p.: 52 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.26 (d, $J = 2.2$ Hz, 1H, 5-H), 7.81 (dd, $J = 8.6, 2.2$ Hz, 1H, 7-H), 6.75 (d, $J = 8.7$ Hz, 1H, 8-H), 1.73 (s, 6H, C(CH₃)₂).

¹³C-NMR

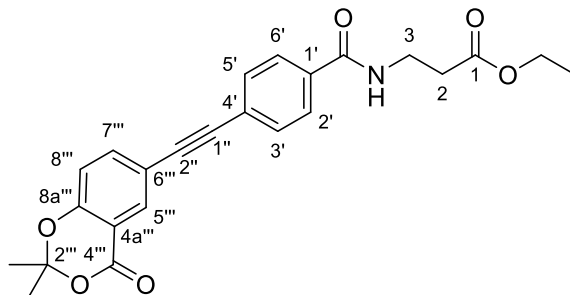
(101 MHz, CDCl₃): δ (ppm) = 159.8 (C-4), 155.8 (C-8a), 145.0 (C-7), 138.3 (C-5), 119.6 (C-8), 115.7 (C-4a), 106.9 (C-2), 84.6 (C-6), 25.9 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3346, 2984, 2938, 1733, 1688, 1634, 1535, 1275, 1246, 1169, 1143, 856, 834, 662.

HR-MS (EI): $m/z = [M]^{+}$ calcd for $C_{10}H_9IO_3^{+}$: 303.9591; found: 303.9591.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-((2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)ethynyl)benzamido)propanoate (94)



$C_{24}H_{23}NO_6$

$M_w = 421.45$ g/mol

Iodo compound **93** (76.0 mg, 0.250 mmol) was added to triethylamine (2.5 mL) under N_2 atmosphere. The mixture was degassed with N_2 for 10 min. Copper(I) iodide (4.76 mg, 0.0250 mmol), $Pd(PPh_3)_2Cl_2$ (17.5 mg, 0.0250 mmol), and alkyne **91** (92.0 μ L, 0.375 mmol) were added sequentially and the reaction mixture was stirred at 60 °C for 1 h. Then, the mixture was diluted with EtOAc (20 mL) and filtered through a pad of celite. The filtrate was washed with 1 N aq. HCl (10 mL), sat. aq. $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 60:40) to give alkyne **94** (84.5 mg, 0.200 mmol, 80 %) as an off-white solid.

R_f: 0.24 (EtOAc/hexanes 40:60).

m.p.: 132 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.15 (d, $J = 2.1$ Hz, 1H, 5'''-H), 7.77 – 7.74 (m, 2H, 2'-H and 6'-H), 7.69 (dd, $J = 8.5, 2.1$ Hz, 1H, 7'''-H), 7.58 – 7.55 (m, 2H, 3'-H and 5'-H), 6.97 (d, $J = 8.5$ Hz, 1H, 8'''-H), 6.90 (t, $J = 6.0$ Hz, 1H, CONH), 4.18 (q, $J = 7.1$ Hz, 2H, CH_2CH_3), 3.74 (q, $J = 6.0$ Hz, 2H, 3-H), 2.65 (t, $J = 5.9$ Hz, 2H, 2-H), 1.75 (s, 6H, $C(CH_3)_2$), 1.28 (t, $J = 7.1$ Hz, 4H, CH_2CH_3).

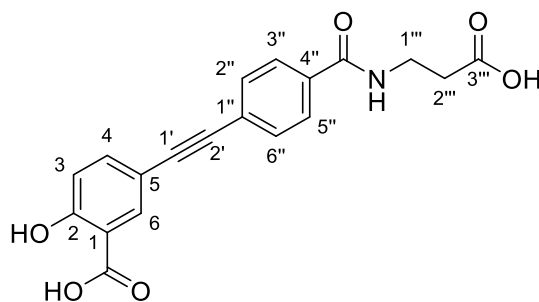
¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 173.2 (C-1), 166.6 (CONH), 160.4 (C-4'''), 156.1 (C-8a'''), 139.4 (C-7'''), 134.1 (C-1'), 133.2 (C-5'''), 131.9 (C-3' and C-5'), 127.2 (C-2' and C-6'), 126.3 (C-4'), 117.8 (C-8'''), 117.7 (C-6'''), 113.9 (C-4a'''), 107.0 (C-2'''), 89.9 (C-2''), 89.0 (C-1''), 61.0 (CH_2CH_3), 35.5 (C-3), 34.0 (C-2), 26.0 ($C(CH_3)_2$), 14.3 (CH_2CH_3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3390, 1725, 1641, 1537, 1505, 1290, 1180, 1159, 1142, 1058, 1031, 941, 849, 762, 688.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₂₄H₂₄NO₆⁺: 422.1598; found: 422.1594.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

5-((4-((2-Carboxyethyl)carbamoyl)phenyl)ethynyl)-2-hydroxybenzoic acid (**CG_219**)C₁₉H₁₅NO₆M_w = 353.33 g/mol

Prepared according to **General Procedure D** at room temperature from alkyne **94** (50.0 mg, 0.119 mmol). The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give the pure product **CG_219** (30.3 mg, 0.0858 mmol, 72 %) as a white solid.

R_f: 0.45 (EtOAc/hexanes+AcOH 80:20+1).

m.p.: 216 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.27 (s, 2H, 1-COOH and 2'''-COOH), 8.62 (t, *J* = 5.5 Hz, 1H, CONH), 7.96 (d, *J* = 2.3 Hz, 1H, 6-H), 7.89 – 7.84 (m, 2H, 3''-H and 5''-H), 7.68 (dd, *J* = 8.6, 2.2 Hz, 1H, 4-H), 7.65 – 7.60 (m, 2H, 2''-H and 6''-H), 7.01 (d, *J* = 8.6 Hz, 1H, 3-H), 3.46 (td, *J* = 7.1, 5.4 Hz, 2H, 1'''-H), 2.55 – 2.51 (m, 2H, 2'''-H).

¹³C-NMR

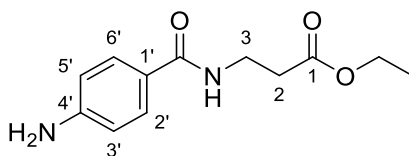
(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3'''), 170.9 (1-COOH), 165.5 (CONH), 161.5 (C-2), 138.0 (C-4), 133.9 (C-4''), 133.7 (C-6), 131.1 (C-2'' and C-6''), 127.5 (C-3'' and C-5''), 125.1 (C-1''), 118.0 (C-3), 114.1 (C-5), 112.5 (C-1), 90.6 (C-1'), 87.5 (C-2'), 35.6 (C-1'''), 33.7 (C-2''').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3292, 2921, 2207, 1694, 1667, 1628, 1538, 1505, 1421, 1325, 1294, 1200, 1162, 1086, 848, 833, 765, 672.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₉H₁₄NO₆⁻: 352.0827; found: 352.0825.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-aminobenzamido)propanoate (95)^[124]



$C_{12}H_{16}N_2O_3$

$M_w = 236.27$ g/mol

Prepared according to **General Procedure F** from 4-aminobenzoic acid (**14**, 500 mg, 3.65 mmol) and β -alanine ethyl ester hydrochloride (**76**, 560 mg, 3.65 mmol). The crude product was purified by FCC (EtOAc/hexanes 70:30) to give amide **95** (857 mg, 3.63 mmol, 99 %) as a beige solid.

R_f: 0.25 (EtOAc/hexanes 70:30).

m.p.: 114 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 8.05 (t, $J = 5.5$ Hz, 1H, CONH), 7.57 – 7.51 (m, 2H, 2'-H and 6'-H), 6.55 – 6.51 (m, 2H, 3'-H and 5'-H), 5.63 (s, 2H, NH₂), 4.05 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 3.42 (td, $J = 7.1, 5.5$ Hz, 2H, 3-H), 2.55 – 2.51 (m, 3H, 2-H), 1.17 (t, $J = 7.1$ Hz, 3H, CH₃).

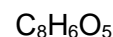
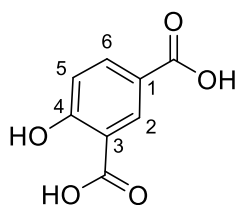
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 171.4 (C-1), 166.3 (CONH), 151.4 (C-4'), 128.7 (C-2' and C-6'), 121.2 (C-1'), 112.6 (C-3' and C-5'), 59.9 (CH₂CH₃), 35.3 (C-3), 34.1 (C-2), 14.1 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3454, 3345, 3240, 1720, 1594, 1512, 1308, 1258, 1181, 1034, 842, 690.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for C₁₂H₁₇N₂O₃⁺: 237.1234; found: 237.1236.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

4-Hydroxyisophthalic acid (97)^[125]M_w = 182.13 g/mol

4-Bromoisophthalic acid (**96**, 1.00 g, 4.10 mmol) was dissolved in water (4.0 mL) and Na₂CO₃ (1.20 g, 11.0 mmol) was added. The resulting solution was stirred for 1.5 h at 85 °C.

Meanwhile TMEDA (31.0 mg, 270 μmol) and copper(I) bromide (18.0 mg, 126 μmol) were dissolved in water (0.50 mL) and stirred for 1 h.

The two solutions were then mixed and stirred for 18 h at 85 °C, cooled to room temperature and acidified with 1 N aq. HCl. The resulting precipitate was collected by filtration and dried *in vacuo* to give 4-hydroxyisophthalic acid (**97**, 695 mg, 3.81 mmol, 93 %) as a white solid.

R_f: 0.31 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 303 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.95 (s, 1H, 1-COOH or 3-COOH), 11.86 (s, 1H, 1-COOH or 3-COOH), 10.23 (s, 1H, OH), 8.38 (d, *J* = 2.3 Hz, 1H, 2-H), 8.03 (dd, *J* = 8.7, 2.3 Hz, 1H, 6-H), 7.04 (d, *J* = 8.7 Hz, 1H, 5-H).

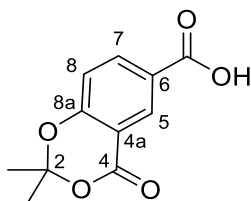
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 171.2 (3-COOH), 166.3 (1-COOH), 164.4 (C-4), 136.2 (C-6), 132.3 (C-2), 121.7 (C-1), 117.5 (C-5), 113.1 (C-3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2993, 1666, 1584, 1444, 1414, 1290, 1198, 897, 847, 770, 691.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₈H₅O₅⁻: 181.0142; found: 181.0141.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

2,2-Dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxine-6-carboxylic acid (98**)^[103]**C₁₁H₁₀O₅M_w = 222.20 g/mol

A suspension of 4-hydroxyisophthalic acid (**97**, 500 mg, 2.75 mmol) in acetone (1.01 mL, 13.7 mmol), TFA (3.09 mL, 41.2 mmol) and TFAA (1.03 mL, 7.41 mmol) was heated to 100 °C for 24 h. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The residue was taken up with 1 N aq. HCl (15 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried using a phase separation paper and the solvents were removed under reduced pressure. The crude product was purified by FCC (EtOAc/hexanes 20:80 → 40:60) to give acetonide **98** (404 mg, 1.82 mmol, 66 %) as a yellow solid.

R_f: 0.35 (EtOAc/hexanes 25:75).

m.p.: 228 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 13.23 (s, 1H, COOH), 8.38 (d, *J* = 2.1 Hz, 1H, 5-H), 8.19 (dd, *J* = 8.6, 2.2 Hz, 1H, 7-H), 7.23 (d, *J* = 8.6 Hz, 1H, 8-H), 1.72 (s, 6H, C(CH₃)₂).

¹³C-NMR

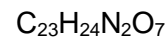
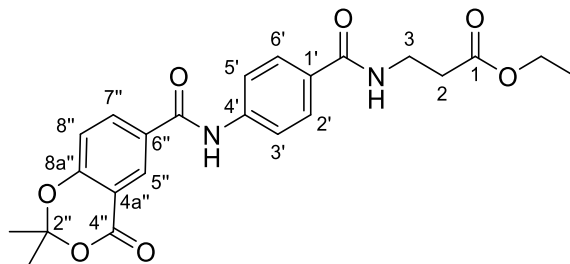
(101 MHz, (CD₃)₂SO): δ (ppm) = 165.8 (COOH), 159.6 (C-4), 158.6 (C-8a), 137.6 (C-7), 130.6 (C-5), 125.6 (C-6), 117.9 (C-8), 112.8 (C-4a), 107.1 (C-2), 25.3 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2538, 1743, 1680, 1613, 1422, 1381, 1276, 1198, 1132, 927, 769.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₁H₉O₅⁻: 221.0455; found: 221.0454.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Ethyl 3-(4-(2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxine-6-carboxamido)benzamido)propanoate (99)



$$M_w = 440.45 \text{ g/mol}$$

To a solution of benzoic acid **98** (100 mg, 0.450 mmol) in toluene (2.0 mL), SOCl_2 (164 μL , 2.25 mmol) was added. The reaction mixture was heated to reflux for 3 h. The solvent and excess of reagent were then removed under reduced pressure and the resulting crude acid chloride was dissolved in DCM (2.5 mL) and added to a flask containing amine **95** (106 mg, 0.45 mmol) in DCM (14 mL) and 10 % aq. NaOH (4.5 mL). The mixture was stirred for 1.5 h. Then the layers were separated, and the aqueous layer extracted with DCM (3 x 40 mL). The combined organic layers were washed with water (15 mL) and dried using a phase separation paper. The solvent was removed *in vacuo* and the crude product purified by FCC (EtOAc/hexanes 40:60 \rightarrow 80:20) to give amide **99** (75.2 mg, 0.171 mmol, 38 %) as a yellow solid.

R_f: 0.31 (EtOAc/hexanes 60:40).

m.p.: 227 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 10.60 (s, 1H, 6''-CONH), 8.57 (d, J = 2.3 Hz, 1H, 5''-H), 8.48 (t, J = 5.5 Hz, 1H, 1'-CONH), 8.29 (dd, J = 8.7, 2.3 Hz, 1H, 7''-H), 7.89 – 7.81 (m, 4H, 2'-, 3'-, 5'- and 6'-H), 7.30 (d, J = 8.7 Hz, 1H, 8''-H), 4.07 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.49 (q, J = 6.8 Hz, 2H, 3'-H), 2.58 (t, J = 7.0 Hz, 2H, 2-H), 1.74 (s, 6H, C(CH₃)₂), 1.18 (t, J = 7.1 Hz, 3H, CH₂CH₃).

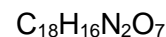
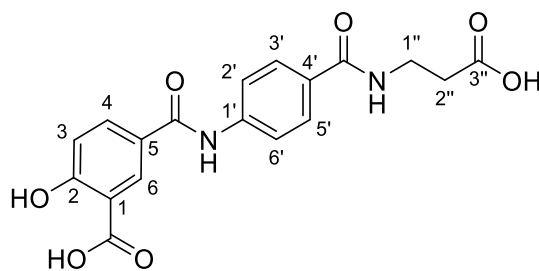
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 171.3 (C-1), 165.8 (1'-CONH), 163.9 (6''-CONH), 159.8 (C-4''), 157.9 (C-8a''), 141.6 (C-1'), 136.6 (C-7''), 129.4 (C-6''), 129.1 (C-4'), 129.0 (C-5''), 127.9 (C-2' and C-6'), 119.5 (C-3' and C-5'), 117.7 (C-8''), 112.6 (C-4a''), 107.0 (C-2''), 59.9 (CH₂CH₃), 35.5 (C-3), 33.9 (C-2), 25.3 (C(CH₃)₂), 14.1 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3403, 3332, 2924, 1736, 1721, 1640, 1536, 1504, 1258, 1182, 844, 762.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₂₃H₂₅N₂O₇⁺: 441.1656; found: 441.1513.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

5-((4-((2-Carboxyethyl)carbamoyl)phenyl)carbamoyl)-2-hydroxybenzoic acid (CG_128)M_w = 372.33 g/mol

Prepared according to **General Procedure D** from compound **99** (50.0 mg, 0.114 mmol) and heating the reaction mixture to reflux. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo*. The crude product was recrystallised from MeOH to give amide **CG_128** (42.0 mg, 0.113 mmol, 99 %) as a white solid.

R_f: 0.22 (EtOAc/hexanes+AcOH 80:20+1).

m.p.: 280 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.22 (s, 2H, 1-COOH and 2''-COOH), 10.43 (s, 1H, 5-CONH), 8.50 (d, *J* = 2.4 Hz, 1H, 6-H), 8.44 (t, *J* = 5.5 Hz, 1H, 4'-CONH), 8.14 (dd, *J* = 8.7, 2.4 Hz, 1H, 4-H), 7.88 – 7.80 (m, 4H, 2', 3', 5'- and 6'-H), 7.10 (d, *J* = 8.7 Hz, 1H, 3-H), 3.45 (td, *J* = 7.1, 5.4 Hz, 2H, 1''-H), 2.54 – 2.51 (m, 2H, 2''-H collapses with DMSO).

¹³C-NMR

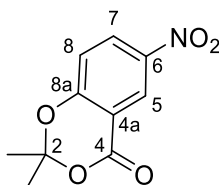
(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3''), 171.4 (1-COOH), 165.7 (4'-CONH), 164.4 (C-2), 163.7 (5-CONH), 141.8 (C-4'), 134.9 (C-4), 130.6 (C-6), 129.2 (C-1'), 127.8 (C-3' and C-5'), 125.4 (C-5), 119.4 (C-2' and C-6'), 117.3 (C-3), 113.0 (C-1), 35.6 (C-1''), 33.9 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3302, 1694, 1679, 1650, 1519, 1440, 1331, 1223, 1200, 841, 758, 658.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₈H₁₅N₂O₇⁻: 371.0885; found: 371.0886.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1f).

2,2-Dimethyl-6-nitro-4*H*-benzo[*d*][1,3]dioxin-4-one (101)



$C_{10}H_9NO_5$

$M_w = 223.18$ g/mol

Prepared according to **General Procedure C** from 5-nitrosalicylic acid (**100**, 1.00 g, 5.46 mmol). The crude product was purified by FCC (EtOAc/hexanes 10:90) and acetone **101** (1.13 g, 5.08 mmol, 93 %) was obtained as a pale yellow solid.

R_f: 0.16 (EtOAc/hexanes 10:90).

m.p.: 94 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.88 (d, $J = 2.8$ Hz, 1H, 5-H), 8.43 (dd, $J = 9.0, 2.8$ Hz, 1H, 7-H), 7.13 (d, $J = 9.1$ Hz, 1H, 8-H), 1.79 (s, 6H, C(CH₃)₂).

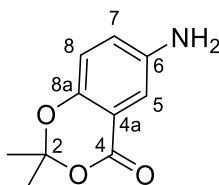
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 160.4 (C-8a), 159.1 (C-4), 143.0 (C-6), 131.4 (C-7), 126.2 (C-5), 118.6 (C-8), 113.6 (C-4a), 107.9 (C-2), 26.1 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2990, 1742, 1592, 1531, 1477, 1336, 1280, 1194, 1050, 924, 746.

HR-MS (EI): $m/z = [M]^{+}$ calcd for C₁₀H₉NO₅⁺: 223.0475; found: 223.0473.

Purity (HPLC): 210 nm: 88 %; 254 nm: 92 % (method 3a).

6-Amino-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (102)^[127]C₁₀H₁₁NO₃M_w = 193.20 g/mol

To a solution of nitro compound **101** (1.00 g, 4.48 mmol) in EtOH (7.5 mL) was added 10 % Pd/C (477 mg, 0.448 mmol) under N₂ atmosphere. Hydrogenation was performed under 1 bar H₂ pressure at room temperature for 12 h. Subsequently, the reaction mixture was filtered through a pad of celite, washed with EtOH (2 x 10 mL) and THF (1 x 10 mL) and concentrated *in vacuo* to give amine **102** (749 mg, 3.88 mmol, 87 %) as a yellow solid.

R_f: 0.45 (EtOAc/hexanes 50:50).

m.p.: 161 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.31 (d, *J* = 2.8 Hz, 1H, 5-H), 6.98 (dd, *J* = 8.7, 2.8 Hz, 1H, 7-H), 6.80 (d, *J* = 8.6 Hz, 1H, 8-H), 4.07 (s, 3H, NH₂), 1.70 (s, 6H, C(CH₃)₂).

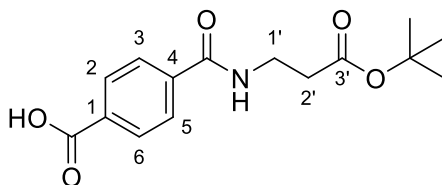
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 161.6 (C-4), 149.5 (C-8a), 140.4 (C-6), 124.7 (C-7), 118.2 (C-8), 115.1 (C-5), 114.2 (C-4a), 106.5 (C-2), 25.8 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3469, 3371, 1711, 1494, 1324, 1275, 1198, 1048, 836.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₀H₁₁NO₃⁺: 193.0733; found: 193.0732.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

4-((3-(*tert*-Butoxy)-3-oxopropyl)carbamoyl)benzoic acid (103)C₁₅H₁₉NO₅M_w = 293.32 g/mol

To a solution of aldehyde **46** (200 mg, 0.721 mmol) in *tert*-butylalcohol (0.93 mL), 2-methyl-2-butene (0.50 mL) was added. To this, a solution containing NaClO₂ (80 %, 106 mg, 0.938 mmol) and NaH₂PO₄ monohydrate (129 mg, 0.938 mmol) in water (0.74 mL) was added dropwise. The reaction mixture was stirred at room temperature for 4 h and then concentrated *in vacuo*, resuspended in water (10 mL), and extracted with hexanes (2 x 10 mL). The aqueous phase was acidified to pH 1 – 2 with 2 N aq. HCl, saturated with sodium chloride, and extracted with ether (3 x 15 mL). The combined organic extracts were dried using a phase separation paper and concentrated *in vacuo* to give benzoic acid **103** (182 mg, 0.622 mmol, 86 %) as a white solid.

R_f: 0.29 (EtOAc/hexanes 50:50).

m.p.: 164 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 13.18 (s, 1H, COOH), 8.69 (t, *J* = 5.6 Hz, 1H, CONH), 8.07 – 7.96 (m, 2H, 2-H and 6-H), 7.94 – 7.87 (m, 2H, 3-H and 5-H), 3.46 (td, *J* = 7.0, 5.4 Hz, 2H, 1'-H), 2.50 – 2.46 (m, 2H, 2'-H, collapses with DMSO), 1.39 (s, 9H, C(CH₃)₃).

¹³C-NMR

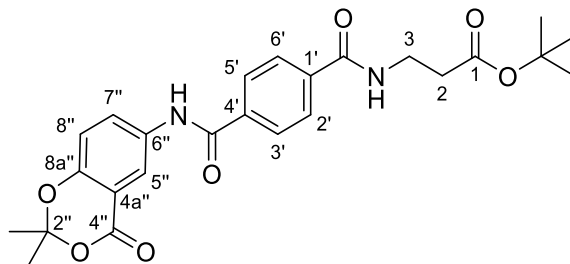
(101 MHz, (CD₃)₂SO): δ (ppm) = 170.6 (C-3'), 166.8 (COOH), 165.5 (CONH), 138.2 (C-4), 133.0 (C-1), 129.3 (C-2 and C-6), 127.4 (C-3 and C-5), 79.9 (C(CH₃)₃), 35.7 (C-1'), 34.9 (C-2'), 27.7 (C(CH₃)₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3324, 2980, 1725, 1688, 1640, 1537, 1432, 1276, 1152, 1074, 875, 690.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₅H₁₈NO₅: 292.1190; found: 292.1190.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

tert-Butyl 3-(4-((2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)carbamoyl)benzamido)propanoate (104)



$C_{25}H_{28}N_2O_7$

$M_w = 468.51$ g/mol

Prepared according to **General Procedure G** from benzoic acid **103** (100 mg, 0.341 mmol) and amine **102** (79.0 mg, 0.409 mmol). The crude product was purified by FCC (EtOAc/hexanes+AcOH 50:50+1) to give amide **104** (125 mg, 0.267 mmol, 78 %) as a white solid.

R_f: 0.29 (EtOAc/hexanes+AcOH 50:50+1).

m.p.: 213 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 10.54 (s, 1H, 4'-CONH), 8.70 (t, $J = 5.6$ Hz, 1H, 1'-CONH), 8.39 (d, $J = 2.6$ Hz, 1H, 5''-H), 8.08 – 8.03 (m, 3H, 3'-H and 5'-H and 7''-H), 7.99 – 7.94 (m, 2H, 2'-H and 6'-H), 7.17 (d, $J = 8.9$ Hz, 1H, 8''-H), 3.49 (td, $J = 7.0, 5.5$ Hz, 2H, 3-H), 2.55 – 2.52 (m, 1H, 2-H), 1.71 (s, 6H, C(CH₃)₂), 1.40 (s, 9H, C(CH₃)₃).

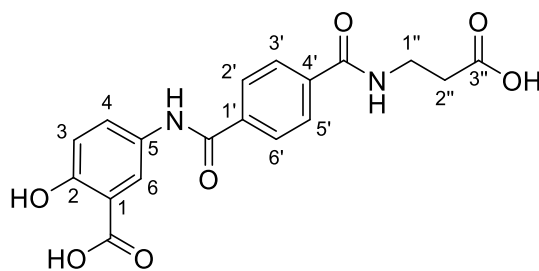
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 170.6 (C-1), 165.5 (1'-CONH), 164.9 (4'-CONH), 160.2 (C-4''), 151.5 (C-8a''), 137.1 (C-1'), 136.7 (C-4'), 134.2 (C-6''), 129.2 (C-7''), 127.7 (C-3' and C-5'), 127.2 (C-2' and C-6'), 120.0 (C-5''), 117.6 (C-8''), 112.9 (C-4a''), 106.5 (C-2''), 79.9 (C(CH₃)₃), 35.7 (C-3), 34.9 (C-2), 27.7 (C(CH₃)₃), 25.2 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3370, 2981, 1751, 1722, 1644, 1538, 1494, 1305, 1283, 1257, 1199, 1137, 832.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₂₅H₂₇N₂O₇⁻: 467.1824; found: 467.1825.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

5-(4-((2-Carboxyethyl)carbamoyl)benzamido)-2-hydroxybenzoic acid (CG_133)C₁₈H₁₆N₂O₇M_w = 372.33 g/mol

Amide **104** (80.0 mg, 0.171 mmol) was dissolved in THF (1.2 mL) followed by the addition of a solution of KOH (47.9 mg, 0.854 mmol) in water (1.2 mL). The reaction mixture was heated to reflux for 2.5 h, cooled to room temperature and the solvents removed *in vacuo*. To the residue was added water (3.0 mL) and 2 N aq. HCl until no further product precipitated. The solid was collected by filtration, washed with 1 N aq. HCl (5.0 mL) and water (5.0 mL), and dried to give product **CG_133** (30.1 mg, 0.0807 mmol, 47 %) as a white solid.

R_f: 0.14 (EtOAc/DCM+AcOH 50:50+1).

m.p.: 286 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 14.02 (s, 1H, 1-COOH or 2''-COOH or 2-OH), 12.28 (s, 1H, 1-COOH or 2''-COOH or 2-OH), 11.08 (s, 1H, 1-COOH or 2''-COOH or 2-OH), 10.33 (s, 1H, 1'-CONH), 8.69 (t, *J* = 5.5 Hz, 1H, 4'-CONH), 8.28 (d, *J* = 2.7 Hz, 1H, 6-H), 8.05 – 8.01 (m, 2H, 2'-H and 6'-H), 7.97 – 7.94 (m, 2H, 3'-H and 5'-H), 7.89 (dd, *J* = 9.0, 2.7 Hz, 1H, 4-H), 6.98 (d, *J* = 8.9 Hz, 1H, 3-H), 3.49 (td, *J* = 7.1, 5.4 Hz, 2H, 1''-H), 2.54 (t, *J* = 7.1 Hz, 2H, 2''-H).

¹³C-NMR

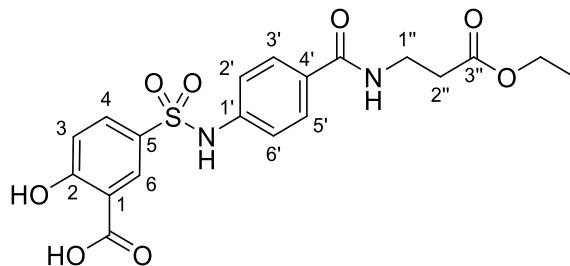
(126 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3''), 171.7 (1-COOH), 165.5 (4'-CONH), 164.5 (1'-CONH), 157.5 (C-2), 136.9 (C-1' or C-4'), 136.8 (C-1' or C-4'), 130.7 (C-5), 128.7 (C-4), 127.6 (C-2' and C-6'), 127.2 (C-3' and C-5'), 121.9 (C-6), 117.1 (C-3), 112.4 (C-1), 35.7 (C-1''), 33.7 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3290, 1677, 1633, 1547, 1451, 1327, 1280, 1196, 860, 684.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₈H₁₅N₂O₇⁻: 371.0885; found: 371.0886.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

5-(*N*-(4-((3-Ethoxy-3-oxopropyl)carbamoyl)phenyl)sulfamoyl)-2-hydroxybenzoic acid
(106)



$C_{19}H_{20}N_2O_8S$

$M_w = 436.44$ g/mol

Prepared according to **General Procedure H** from amine **95** (236 mg, 1.00 mmol). The crude product was purified by FCC (EtOAc/hexanes 70:30 → MeOH/EtOAc 5:95) to give sulfonamide **106** (237 mg, 0.544 mmol, 54 %) as an off-white solid.

R_f: 0.29 (EtOAc/DCM+AcOH 50:50+1).

m.p.: 118 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 8.33 (d, $J = 2.5$ Hz, 1H, 6-H), 7.73 (dd, $J = 8.8, 2.5$ Hz, 1H, 4-H), 7.67 – 7.63 (m, 2H, 3'-H and 5'-H), 7.19 – 7.16 (m, 2H, 2'-H and 6'-H), 6.88 (d, $J = 8.8$ Hz, 1H, 3-H), 4.12 (q, $J = 7.2$ Hz, 2H, CH₂CH₃), 3.58 (t, $J = 6.9$ Hz, 2H, 1''-H), 2.60 (t, $J = 6.8$ Hz, 2H, 2''-H), 1.22 (t, $J = 7.1$ Hz, 3H, CH₃).

¹³C-NMR

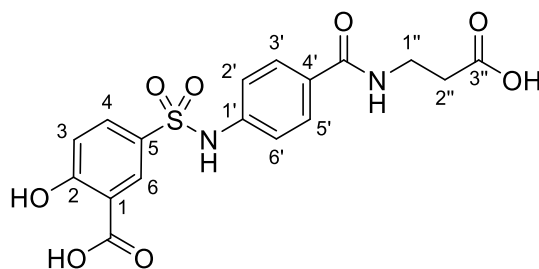
(126 MHz, CD₃OD): δ (ppm) = 173.6 (1-COOH), 173.5 (C-3''), 169.7 (CONH), 166.9 (C-2), 142.6 (C-1'), 133.3 (C-4), 131.8 (C-6), 130.8 (C-4'), 129.9 (C-5), 129.5 (C-3' and C-5'), 120.3 (C-2' and C-6'), 118.5 (C-3), 118.2 (C-1), 61.7 (CH₂CH₃), 37.0 (C-1''), 34.9 (C-2''), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2926, 1726, 1625, 1610, 1568, 1335, 1312, 1198, 1157, 1107, 924, 842, 767, 656.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₉H₁₉N₂O₈S⁻: 435.0868; found: 435.0865.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1f).

5-(N-(4-((2-Carboxyethyl)carbamoyl)phenyl)sulfamoyl)-2-hydroxybenzoic acid
(CG_137)



$C_{17}H_{16}N_2O_8S$

$M_w = 408.38$ g/mol

Prepared according to **General Procedure D** from sulfonamide **106** (100 mg, 0.229 mmol) and heating the reaction mixture to reflux. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo*. The pure product **CG_137** (70.4 mg, 0.172 mmol, 75 %) was obtained as a pale beige solid.

R_f: 0.16 (EtOAc/hexanes+AcOH 80:20+1), streaks.

m.p.: 228 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.18 (s, 1H, 1-COOH or 2''-COOH), 10.53 (s, 1H, OH), 8.37 (t, $J = 5.5$ Hz, 1H, CONH), 8.19 (d, $J = 2.5$ Hz, 1H, 6-H), 7.80 (dd, $J = 8.8, 2.5$ Hz, 1H, 4-H), 7.72 – 7.67 (m, 2H, 3'-H and 5'-H), 7.16 – 7.11 (m, 2H, 2'-H and 6'-H), 7.04 (d, $J = 8.8$ Hz, 1H, 3-H), 3.39 (td, $J = 7.2, 5.5$ Hz, 2H, 1''-H), 2.46 (t, $J = 7.1$ Hz, 2H, 2''-H).

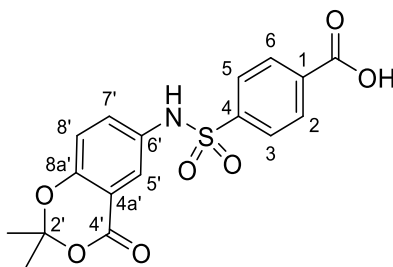
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3''), 170.2 (1-COOH), 165.5 (CONH), 164.5 (C-2), 140.3 (C-1'), 133.0 (C-4), 129.8 (C-6), 129.6 (C-4'), 129.2 (C-5), 128.4 (C-3' and C-5'), 118.5 (C-2' and C-6'), 118.3 (C-3), 114.3 (C-1), 35.5 (C-1''), 33.8 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3401, 3146, 2924, 1693, 1634, 1609, 1504, 1334, 1303, 1208, 1159, 1109, 932, 725.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{17}H_{15}N_2O_8S$: 407.0555; found: 407.0553.

Purity (HPLC): 210 nm: >95 %; 254 nm: 95 % (method 3a).

4-(*N*-(2,2-Dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxin-6-yl)sulfamoyl)benzoic acid (**108**)C₁₇H₁₅NO₇SM_w = 377.37 g/mol

To a solution of amine **102** (300 mg, 1.55 mmol) in DCM (15 mL) was added pyridine (188 μL, 2.33 mmol) and 4-(chlorosulfonyl)benzoic acid (**107**, 343 mg, 1.55 mmol) at 0 °C under N₂ atmosphere. The mixture was stirred for 2 h at 0 °C and then allowed to warm to room temperature. The solvent was removed *in vacuo* and the crude product purified by recrystallisation from hot MeOH to give sulfonamide **108** (125 mg, 0.267 mmol, 78 %) as a white solid.

R_f: 0.67 (EtOAc/DCM+AcOH 50:50+1).

m.p.: 260 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.14 – 8.09 (m, 2H, 2-H and 6-H), 7.84 – 7.80 (m, 2H, 3-H and 5-H), 7.57 (d, *J* = 2.7 Hz, 1H, 5'-H), 7.37 (dd, *J* = 8.8, 2.7 Hz, 1H, 7'-H), 6.95 (d, *J* = 8.8 Hz, 1H, 8'-H), 1.67 (s, 6H, C(CH₃)₂).

¹³C-NMR

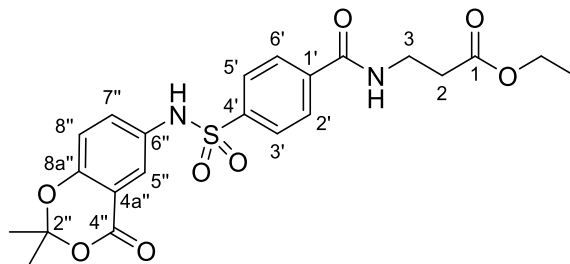
(101 MHz, CD₃OD): δ (ppm) = 168.0 (COOH), 162.0 (C-4'), 154.9 (C-8a'), 144.5 (C-4), 136.1 (C-1), 133.6 (C-6'), 132.3 (C-7'), 131.4 (C-2 and C-6), 128.3 (C-3 and C-5), 123.5 (C-5'), 119.5 (C-8'), 115.0 (C-4a'), 108.1 (C-2'), 25.7 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3270, 1740, 1681, 1493, 1392, 1287, 1162, 1127, 899, 864, 726.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₇H₁₄NO₇S: 376.0496; found: 376.0498.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

Ethyl 3-(4-(*N*-(2,2-dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxin-6-yl)sulfamoyl)benzamido)propanoate (109)



$C_{22}H_{24}N_2O_8S$

$M_w = 476.50$ g/mol

Prepared according to **General Procedure E** from benzoic acid **108** (200 mg, 0.530 mmol) and β -alanine ethyl ester hydrochloride (**76**; 81.4 mg, 0.530 mmol). The crude product was purified by FCC (EtOAc/hexanes 70:30) to give product **109** (136 mg, 0.284 mmol, 54 %) as a pale yellow crystalline solid.

R_f: 0.62 (EtOAc/hexanes 70:30).

m.p.: 57 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 10.47 (s, 1H, SO₂NH), 8.73 (t, $J = 5.4$ Hz, 1H, CONH), 7.95 – 7.90 (m, 2H, 2'-H and 6'-H), 7.81 – 7.76 (m, 2H, 3'-H and 5'-H), 7.52 (d, $J = 2.7$ Hz, 1H, 5''-H), 7.36 (dd, $J = 8.8, 2.7$ Hz, 1H, 7''-H), 7.04 (d, $J = 8.9$ Hz, 1H, 8''-H), 4.05 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 3.47 (t, $J = 6.9$ Hz, 2H, 3-H), 2.56 (t, $J = 6.9$ Hz, 2H, 2-H), 1.63 (s, 6H, C(CH₃)₂), 1.15 (t, $J = 7.1$ Hz, 3H, CH₂CH₃).

¹³C-NMR

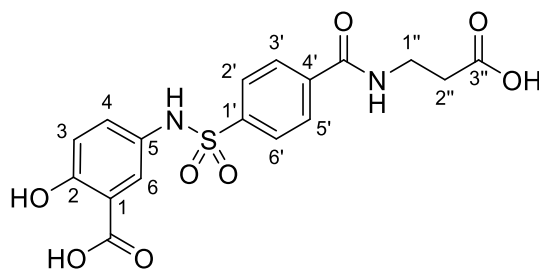
(101 MHz, (CD₃)₂SO): δ (ppm) = 171.2 (C-1), 164.9 (CONH), 159.8 (C-4''), 152.3 (C-8a''), 141.2 (C-4'), 138.2 (C-1'), 132.4 (C-6''), 130.1 (C-7''), 128.1 (C-2' and C-6'), 126.7 (C-3' and C-5'), 120.8 (C-5''), 118.5 (C-8''), 113.3 (C-4a''), 106.6 (C-2''), 60.0 (CH₂CH₃), 35.5 (C-3), 33.5 (C-2), 25.2 (C(CH₃)₂), 14.0 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2988, 1722, 1651, 1490, 1287, 1200, 1166, 1092, 985, 835, 733.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₂₂H₂₃N₂O₈S⁻: 475.1181; found: 475.1179.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

**5-((4-((2-Carboxyethyl)carbamoyl)phenyl)sulfonamido)-2-hydroxybenzoic acid
(CG_140)**



$C_{17}H_{16}N_2O_8S$

$M_w = 408.38$ g/mol

Prepared according to **General Procedure D** from compound **109** (85.0 mg, 0.178 mmol) and heating the reaction mixture to reflux. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give pure sulfonamide **CG_140** (70.4 mg, 0.172 mmol, 97 %) as a white solid.

R_f: 0.14 (EtOAc/DCM+AcOH 50:50+1).

m.p.: 223 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.24 (s, 1H, 1-COOH or 2''-COOH), 10.08 (s, 1H, SO₂NH), 8.71 (t, $J = 5.5$ Hz, 1H, CONH), 7.95 – 7.89 (m, 2H, 3'-H and 5'-H), 7.75 – 7.70 (m, 2H, 2'-H and 6'-H), 7.46 (d, $J = 2.8$ Hz, 1H, 6-H), 7.16 (dd, $J = 8.9, 2.7$ Hz, 1H, 4-H), 6.83 (d, $J = 8.9$ Hz, 1H, 3-H), 3.44 (td, $J = 7.2, 5.4$ Hz, 2H, 1''-H), 2.52 – 2.51 (m, 1H, 2''-H, collapses with DMSO).

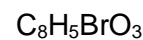
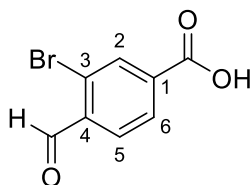
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 172.8 (C-3''), 171.1 (1-COOH), 165.0 (CONH), 158.7 (C-2), 141.3 (C-1'), 138.1 (C-4'), 130.0 (C-4), 128.2 (C-5), 128.0 (C-3' and C-5'), 126.8 (C-2' and C-6'), 123.8 (C-6), 117.8 (C-3), 113.6 (C-1), 35.7 (C-1''), 33.5 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3296, 3248, 1698, 1678, 1633, 1445, 1336, 1227, 1166, 1152, 836, 741, 667.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₇H₁₅N₂O₈S⁻: 407.0555; found: 407.0554.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 3a).

3-Bromo-4-formylbenzoic acid (116)^[139]

M_w = 229.03 g/mol

A solution of 3-bromo-4-methylbenzoic acid (**115**, 1.00 g, 4.65 mmol), NBS (1.90 g, 10.7 mmol) and BPO (56.3 mg, 0.233 mmol) in CCl₄ (10 mL) was heated to reflux for 36 h. The reaction mixture was cooled to room temperature and filtered. The residue was washed with CCl₄ (20 mL) and the filtrate concentrated under reduced pressure to give the crude dibromo product.

AgNO₃ (1.62 g, 9.53 mmol) in hot water (2.5 mL) was added dropwise to a solution of crude dibromo product (4.65 mmol) in EtOH (12 mL) at 50 °C over 10 min. The mixture was stirred at the same temperature for further 45 min. After cooling to room temperature, the mixture was poured into 1 N aq. HCl (15 mL) and filtered. The residue was washed with EtOH (25 mL) and the filtrate concentrated *in vacuo*. To the crude product EtOAc (20 mL) and water (15 mL) were added, and the mixture extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (15 mL), dried using a phase separation paper and evaporated in *vacuo* to give the pure product **116** (495 mg, 2.16 mmol, 47 %) as a white solid.

R_f: 0.36 (EtOAc/hexanes+AcOH 20:80+1).

m.p.: 239 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 13.70 (s, 1H, COOH), 10.25 (d, *J* = 0.8 Hz, 1H, CHO), 8.20 (d, *J* = 1.5 Hz, 1H, 2-H), 8.07 – 8.04 (m, 1H, 6-H), 7.94 (d, *J* = 8.0 Hz, 1H, 5-H).

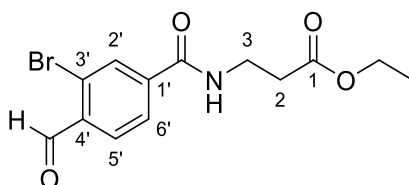
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 191.4 (CHO), 165.3 (COOH), 136.9 (C-1), 136.0 (C-4), 134.3 (C-2), 130.4 (C-5), 128.9 (C-6), 125.3 (C-3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2879, 2658, 2544, 1688, 1554, 1478, 1420, 1378, 1308, 1285, 1197, 1042, 919, 854, 784, 763.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₈H₄BrO₃⁻: 226.9349; found: 226.9349.

Purity (HPLC): 254 nm: >95 % (method 2a).

Ethyl 3-(3-bromo-4-formylbenzamido)propanoate (117)C₁₃H₁₄BrNO₄M_w = 328.16 g/mol

Prepared according to **General Procedure F** from compound **116** (200 mg, 0.873 mmol) and β -alanine ethyl ester hydrochloride (**76**, 134 mg, 0.873 mmol). The crude product was purified by FCC (EtOAc/hexanes+AcOH 40:60+1) to give product **117** (230 mg, 0.701 mmol, 80 %) as a colourless oily solid.

R_f: 0.52 (EtOAc/hexanes+AcOH 40:60+1).

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 10.38 (d, J = 0.8 Hz, 1H, CHO), 8.07 (d, J = 1.6 Hz, 1H, 2'-H), 7.95 (d, J = 8.0 Hz, 1H, 5'-H), 7.75 (ddd, J = 8.0, 1.7, 0.8 Hz, 1H, 6'-H), 6.99 (t, J = 5.3 Hz, 1H, CONH), 4.19 (q, J = 7.2 Hz, 3H, CH₂CH₃), 3.73 (q, J = 6.0 Hz, 2H, 3-H), 2.66 (t, J = 5.8 Hz, 2H, 2-H), 1.28 (t, J = 7.1 Hz, 3H, CH₃).

¹³C-NMR

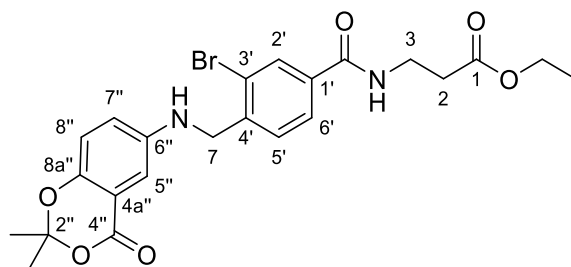
(101 MHz, CDCl₃): δ (ppm) = 191.3 (CHO), 173.1 (C-1), 164.9 (CONH), 140.6 (C-1'), 135.4 (C-4'), 133.0 (C-2'), 130.2 (C-5'), 127.3 (C-3'), 126.2 (C-6'), 61.2 (CH₂CH₃), 35.7 (C-3), 33.8 (C-2), 14.3 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3335, 2981, 1729, 1695, 1640, 1535, 1376, 1306, 1183, 1151, 1025, 758.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₁₃H₁₃BrNO₄⁻: 326.0033; found: 326.0037.

Purity (HPLC): not determinable due to instability of the product; >95 % (NMR).

Ethyl 3-(3-bromo-4-(((2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)amino)methyl)benzamido)propanoate (118)



$$\text{C}_{23}\text{H}_{25}\text{BrN}_2\text{O}_6$$

$$M_w = 505.37 \text{ g/mol}$$

Aldehyde **117** (200 mg, 0.609 mmol) and amine **102** (78.5 mg, 0.406 mmol) were added to MeOH (4.0 mL). AcOH (7.0 μL) was added and the reaction mixture stirred at room temperature for 16 h. Then, more MeOH (5.00 mL) and NaBH_3CN (80.6 mg, 1.22 mmol) were added. After stirring for 1 h, water (10 mL) was added, and the mixture extracted with EtOAc (3 x 10 mL). The solvents were removed *in vacuo* and amine **118** (196 mg, 0.389 mmol, 96 %) obtained as a yellow oily solid.

R_f: 0.34 (EtOAc/hexanes 50:50).

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 8.64 (t, J = 5.5 Hz, 1H, CONH), 8.06 (d, J = 1.7 Hz, 1H, 2'-H), 7.78 (dd, J = 8.0, 1.7 Hz, 1H, 6'-H), 7.45 (d, J = 8.0 Hz, 1H, 5'-H), 6.93 (dd, J = 8.9, 2.8 Hz, 1H, 7''-H), 6.89 (d, J = 8.7 Hz, 1H, 8''-H), 6.86 (d, J = 2.7 Hz, 1H, 5''-H), 6.53 (t, J = 6.0 Hz, 1H, CH₂NH), 4.33 (d, J = 5.9 Hz, 2H, CH₂NH), 4.06 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.47 (td, J = 6.7, 5.4 Hz, 2H, 3-H), 2.56 (t, J = 6.9 Hz, 2H, 2-H), 1.62 (s, 6H, C(CH₃)₂), 1.17 (t, J = 7.1 Hz, 3H, CH₂CH₃).

¹³C-NMR

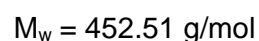
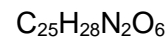
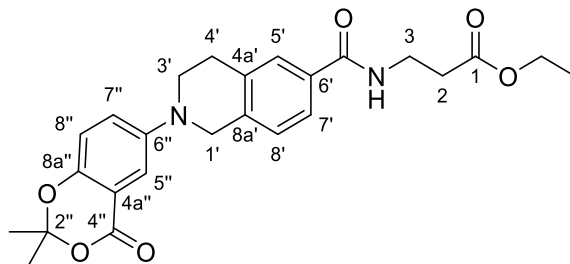
(101 MHz, (CD₃)₂SO): δ (ppm) = 171.2 (C-1), 164.6 (CONH), 160.7 (C-4''), 146.7 (C-8a''), 143.9 (C-6''), 141.2 (C-4'), 134.8 (C-1'), 131.1 (C-2'), 128.6 (C-5'), 126.6 (C-6'), 122.4 (C-3'), 121.8 (C-7''), 117.9 (C-8''), 113.5 (C-4a''), 109.3 (C-5''), 105.9 (C-2''), 59.9 (CH₂CH₃), 47.1 (CH₂NH), 35.6 (C-3), 33.6 (C-2), 25.2 (C(CH₃)₂), 14.1 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3369, 2989, 2938, 2322, 2178, 1715, 1648, 1536, 1495, 1378, 1295, 1278, 1196, 1126, 1051, 1035, 980, 934, 826.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₂₃H₂₄BrN₂O₆⁻: 503.0823; found: 503.0827.

Purity (HPLC): 210 nm: 94 %; 254 nm: 94 % (method 2a).

Ethyl 3-(2-(2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)propanoate (120)



N-Aryl 2-bromobenzylamine **118** (190 mg, 0.376 mmol), Pd(PPh₃)₄ (43.4 mg, 0.0376 mmol; 10 mol%) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 149 mg, 0.752 mmol) were dissolved in degassed THF (2.3 mL) under N₂ atmosphere and stirred for 10 min at room temperature. Cs₂CO₃ (612 mg, 1.88 mmol) was dissolved in degassed water (3.7 mL) under N₂ atmosphere and added to the reaction mixture. Stirring was continued at 75 °C for 19 h. The reaction mixture was allowed to cool to room temperature. Sat. aq. NH₄Cl solution (10 mL) was added. The organic material was extracted with EtOAc (3 x 15 mL) and the combined organic phases washed with brine (20 mL) and dried using a phase separation paper. The solvent was removed *in vacuo* to give the crude enol ether **119** (R_f = 0.55 (EtOAc/hexanes 60:40)).

The crude product (**119**, 0.376 mmol) was suspended in dry DCM (1.8 mL) under N₂ atmosphere. TFA (367 μL, 4.89 mmol) and triethylsilane (152 μL, 0.940 mmol) were added under N₂ atmosphere at room temperature in rapid succession. After 2 h, 2 M aq. NaOH (5.0 mL) was added and the mixture was extracted with DCM (3 x 15 mL). The combined organic extracts were dried using a phase separation paper. The solvent was removed *in vacuo* and the crude product was purified by FCC (EtOAc/DCM 15:85) to give product **120** (108 mg, 0.238 mmol, 63 %) as a fluorescent yellow oily solid.

R_f: 0.22 (EtOAc/DCM 15:85).

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 7.60 (d, *J* = 1.8 Hz, 1H, 5'-H), 7.56 (dd, *J* = 7.9, 1.9 Hz, 1H, 7'-H), 7.48 (d, *J* = 3.0 Hz, 1H, 5''-H), 7.24 (dd, *J* = 9.0, 3.1 Hz, 1H, 7''-H), 7.20 (d, *J* = 8.0 Hz, 1H, 8'-H), 6.91 (d, *J* = 9.0 Hz, 1H, 8''-H), 6.84 (t, *J* = 6.1 Hz, 1H, CONH), 4.39 (s, 2H, 1'-H), 4.17 (q, *J* = 7.2 Hz, 2H, CH₂CH₃), 3.73 (q, *J* = 6.0 Hz, 2H, 3-H), 3.52 (t, *J* = 5.9 Hz, 2H, 3'-H), 3.03 (t, *J* = 5.8 Hz, 2H, 4'-H), 2.64 (dd, *J* = 6.4, 5.4 Hz, 2H, 2-H), 1.72 (s, 6H, C(CH₃)₂), 1.27 (t, *J* = 7.2 Hz, 3H, CH₂CH₃).

¹³C-NMR

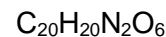
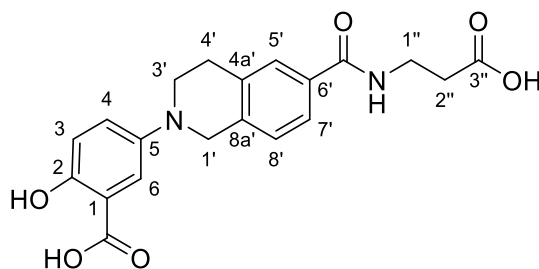
(126 MHz, CDCl₃): δ (ppm) = 173.0 (C-1), 167.1 (CONH), 161.7 (C-4^{''}), 149.2 (C-8a^{''}), 146.1 (C-6^{''}), 137.6 (C-8a[']), 134.9 (C-4a[']), 132.7 (C-6[']), 127.5 (C-5[']), 126.8 (C-8[']), 124.9 (C-7^{''}), 124.5 (C-7[']), 117.9 (C-8^{''}), 114.6 (C-5^{''}), 113.9 (C-4a^{''}), 106.3 (C-2^{''}), 60.9 (CH₂CH₃), 51.4 (C-1[']), 47.2 (C-3[']), 35.3 (C-3), 34.0 (C-2), 29.0 (C-4[']), 25.8 (C(CH₃)₂), 14.2 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3353, 2986, 2940, 1726, 1641, 1495, 1294, 1276, 1185, 1141, 1049, 985, 923, 775.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₂₅H₂₉N₂O₆⁺: 453.2020; found: 453.2017.

Purity (HPLC): not determinable due to instability of the product.

5-(6-((2-Carboxyethyl)carbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-hydroxybenzoic acid (CG_224)



$$M_w = 384.39 \text{ g/mol}$$

N-Aryl 2-bromobenzylamine **118** (150 mg, 0.208 mmol), Pd(PPh₃)₄ (24.0 mg, 0.0208 mmol; 10 mol%) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 88.0 μL, 0.416 mmol) were dissolved in degassed THF (1.5 mL) under N₂ atmosphere and stirred for 10 min at room temperature. Cs₂CO₃ (338 mg, 1.04 mmol) was dissolved in degassed water (1.0 mL) under N₂ atmosphere and added to the reaction mixture. Stirring was continued at 75 °C for 19 h. The reaction mixture was allowed to cool to room temperature. Sat. aq. NH₄Cl solution (10 mL) was added. The organic material was extracted with EtOAc (3 x 15 mL) and the combined organic phases washed with brine (20 mL) and dried using a phase separation paper. The solvent was removed *in vacuo* to give the crude enol ether **119** (R_f = 0.55 (EtOAc/hexanes 60:40)).

The crude product (**119**, 0.208 mmol) was suspended in dry DCM (1.5 mL) under N₂ atmosphere. TFA (203 μL, 4.89 mmol) and triethylsilane (84.0 μL, 0.520 mmol) were added under N₂ atmosphere at room temperature in rapid succession. After 2 h, 2 M aq. NaOH (5.0 mL) was added and the mixture was extracted with DCM (3 x 15 mL). The combined organic extracts were dried using a phase separation paper. The solvent was removed *in vacuo* to give the crude cyclised tetrahydroisoquinoline **120**.

Without further purification, crude **120** (0.208 mmol) was dissolved in THF (1.4 mL) followed by the addition of a solution of KOH (58.3 mg, 1.04 mmol) in water (1.4 mL). The reaction mixture was stirred at room temperature for 1.5 h. Then water (10 mL) was added, and the mixture acidified to pH 1 using 2 N aq. HCl. The organic material was extracted with EtOAc (3 x 15 mL). The combined organic layers were extracted with 0.5 N aq. NaOH (3 x 15 mL). The aqueous phase was then again acidified to pH 1 using 2 N aq. HCl and extracted with EtOAc (3 x 15 mL). The combined organic phases were dried using a phase separation paper and the solvents were removed *in vacuo*. The crude product was purified by FCC (MeOH/DCM+AcOH 5:95+1) and dried under high vacuum to give product **CG_224** (47.9 mg, 0.125 mmol, 60 % over three steps) as a yellow to brown oily solid.

R_f: 0.14 (MeOH/DCM+AcOH 5:95+1).

¹H-NMR

(500 MHz, (CD₃)₂SO): contains residual AcOH.

δ (ppm) = 12.22 (s, 2H, 1-COOH and 2''-COOH), 8.46 (t, J = 5.4 Hz, 1H, CONH), 7.64 (s, 1H, 5'-H or 7'-H), 7.64 – 7.61 (m, 1H, 5'-H or 7'-H), 7.40 (s, 1H, 6-H), 7.28 (d, J = 7.9 Hz, 1H, 8'-H), 7.10 (dd, J = 9.2, 2.7 Hz, 1H, 4-H), 6.69 (d, J = 8.7 Hz, 1H, 3-H), 4.23 (s, 2H, 1'-H), 3.45 (q, J = 6.7 Hz, 2H, 1''-H), 3.36 (t, J = 5.8 Hz, 3H, 3'-H), 2.95 (t, J = 5.8 Hz, 2H, 4'-H), 2.53 (d, J = 1.6 Hz, 2H, 2''-H, collapses with DMSO).

¹³C-NMR

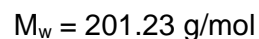
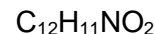
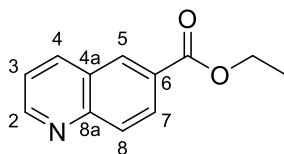
(126 MHz, (CD₃)₂SO): contains residual AcOH.

δ (ppm) = 172.9 (C-3''), 171.8 (1-COOH), 166.2 (CONH), 156.2 (C-2), 141.8 (C-5), 138.1 (C-8a'), 134.3 (C-4a'), 132.3 (C-6'), 127.4 (C-5'), 126.5 (C-8'), 124.5 (C-7'), 122.8 (C-4), 117.6 (C-6), 116.4 (C-3), 52.3 (C-1'), 48.2 (C-3'), 35.6 (C-1''), 33.8 (C-2''), 28.6 (C-4''), C-1 not clearly visible.

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3333, 2922, 2586, 1709, 1632, 1547, 1488, 1434, 1372, 1229, 1009, 828, 750.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₂₀H₁₉N₂O₆⁻: 383.1249; found: 383.1249.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl quinoline-6-carboxylate (122)^[140]

To a solution of 6-quinolinecarboxylic acid (**121**, 3.00 g, 17.3 mmol) in EtOH (120 mL), H₂SO₄ (6.0 mL) was added. The solution was heated to reflux for 16 h. After cooling to room temperature, water (250 mL) was added, and the solution was basified using solid K₂CO₃. The solution was then extracted with DCM (3 x 100 mL). The combined organic layers were dried using a phase separation paper and the solvent was removed *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 20:80) to give ethyl ester **122** (2.79 g, 13.9 mmol, 80 %) as a pale yellow to white solid.

R_f: 0.21 (EtOAc/hexanes 20:80).

m.p.: 47 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 9.01 (dd, *J* = 4.3, 1.7 Hz, 1H, 2-H), 8.59 (d, *J* = 1.9 Hz, 1H, 5-H), 8.31 (dd, *J* = 8.8, 1.9 Hz, 1H, 7-H), 8.29 – 8.25 (m, 1H, 4-H), 8.17 – 8.12 (m, 1H, 8-H), 7.47 (dd, *J* = 8.3, 4.2 Hz, 1H, 3-H), 4.46 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 1.45 (t, *J* = 7.1 Hz, 3H, CH₃).

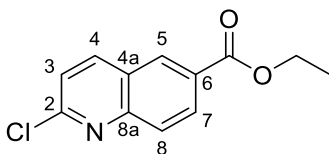
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 166.3 (COOEt), 152.6 (C-2), 150.2 (C-8a), 137.5 (C-4), 131.0 (C-5), 129.9 (C-8), 129.1 (C-7), 128.6 (C-6), 127.6 (C-4a), 122.0 (C-3), 61.5 (CH₂CH₃), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3248, 2991, 1720, 1625, 1502, 1480, 1462, 1362, 1273, 1243, 1187, 1095, 1022, 908, 846, 797, 784, 743.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₂H₁₂NO₂⁺: 202.0863; found: 202.0861.

Purity (HPLC): 254 nm: >95 % (method 4a).

Ethyl 2-chloroquinoline-6-carboxylate (124)^[141]C₁₂H₁₀ClNO₂M_w = 235.67 g/mol

To a mixture of ethyl quinoline-6-carboxylate (**122**, 735 mg, 3.65 mmol) in dry DCM (10.5 mL), *m*-chloroperbenzoic acid (819 mg, 4.75 mmol) was added at 0 °C and the reaction mixture was stirred at room temperature for 16 h (formation of the product confirmed *via* LC-MS). The mixture was then washed with 10 % aq. Na₂SO₃ (15 mL), sat. aq. NaHCO₃ (15 mL) and brine (15 mL). The organic layer was dried using a phase separation paper and concentrated *in vacuo* to give crude *N*-oxide intermediate **123**.

To the residue, dry DCM (8.5 mL) and phosphoryl chloride (4.47 mL, 47.5 mmol) were added, and the mixture was stirred at 50 °C for 16 h. The reaction mixture was concentrated *in vacuo*, the residue was diluted with DCM (20 mL) and washed with sat. aq. NaHCO₃ (2 x 15 mL) and brine (15 mL). The organic phase was concentrated *in vacuo* and the crude product was purified by FCC (EtOAc/toluene 1:99) to give product **124** (179 mg, 0.760 mmol, 21 %) as a white solid.

R_f: 0.32 (EtOAc/toluene 1:99).

m.p.: 97 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.58 (d, *J* = 1.9 Hz, 1H, 5-H), 8.34 (dd, *J* = 8.8, 1.9 Hz, 1H, 7-H), 8.22 – 8.20 (m, 1H, 4-H), 8.06 (dd, *J* = 8.8, 0.7 Hz, 1H, 8-H), 7.46 (d, *J* = 8.6 Hz, 1H, 3-H), 4.45 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 1.45 (t, *J* = 7.1 Hz, 3H, CH₃).

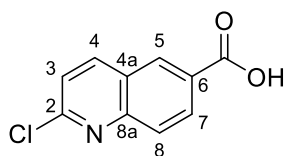
¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 165.9 (6-COOEt), 153.1 (C-2), 149.8 (C-8a), 140.1 (C-4), 130.6 (C-5), 130.4 (C-7), 129.1 (C-6), 129.0 (C-8), 126.2 (C-4a), 123.4 (C-3), 61.7 (CH₂CH₃), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3064, 2903, 1715, 1621, 1453, 1393, 1261, 1197, 1142, 1085, 1023, 917, 814, 781, 748, 664.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₂H₁₁ClNO₂⁺: 236.0473; found: 236.0472.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

2-Chloroquinoline-6-carboxylic acid (125)^[141]C₁₀H₆ClNO₂M_w = 207.61 g/mol

A suspension of compound **124** (150 mg, 0.636 mmol) in HCl (32 %, 3.0 mL) was heated to 95 °C for 75 min, cooled to room temperature and concentrated to dryness. The residue was purified FCC (EtOAc/hexanes+AcOH 30:70+1 → 40:60+1) to give product **125** (82.0 mg, 0.395 mmol, 62 %) as a white solid.

R_f: 0.38 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 244 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 13.37 (s, 1H, COOH), 8.73 (d, *J* = 1.9 Hz, 1H, 5-H), 8.65 (dd, *J* = 8.7, 0.7 Hz, 1H, 4-H), 8.26 (dd, *J* = 8.8, 2.0 Hz, 1H, 7-H), 8.04 – 8.01 (m, 1H, 8-H), 7.70 (d, *J* = 8.6 Hz, 1H, 3-H).

¹³C-NMR

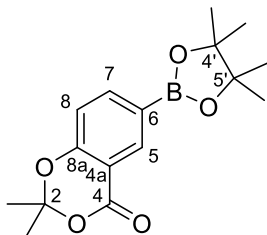
(126 MHz, (CD₃)₂SO): δ (ppm) = 166.7 (COOH), 152.0 (C-2), 148.9 (C-8a), 141.3 (C-4), 130.8 (C-5), 130.2 (C-7), 129.3 (C-6), 128.2 (C-8), 126.2 (C-4a), 123.3 (C-3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2922, 2528, 1677, 1621, 1580, 1450, 1311, 1280, 1196, 1137, 1088, 809, 852, 813, 785, 771, 750.

HR-MS (EI): *m/z* = [M]⁺⁺ calcd for C₁₀H₆ClNO₂⁺⁺: 207.0082; found: 207.0085.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

2,2-Dimethyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4*H*-benzo[*d*][1,3]dioxin-4-one (126)



$C_{16}H_{21}BO_5$

$M_w = 304.15$ g/mol

A solution of iodo compound **93** (152 mg, 0.500 mmol), KOAc (147 mg, 0.500 mmol), and B_2pin_2 (140 mg, 0.551 mmol) in 1,4-dioxane (5.0 mL) was degassed for 10 min and put under N_2 atmosphere. $PdCl_2(dppf) \cdot DCM$ (12.2 mg, 0.0150 mmol) was added, and the mixture stirred at 80 °C for 16 h. Another 0.2 eq of B_2pin_2 were added and stirring continued at 80 °C for further 16 h. The reaction mixture was cooled to room temperature, water (15 mL) was added, and the organic materials extracted with EtOAc (3 x 20 mL). The combined organic layers were dried using a phase separation paper and concentrated *in vacuo*. The crude product was put through a short silica gel column (EtOAc/hexanes 10:90) and concentrated *in vacuo*. The resulting material was then diluted with EtOAc (10 mL) and IBX (406 mg, 1.45 mmol) was added under N_2 atmosphere. The reaction mixture was heated to 80 °C for 2 h while stirring rapidly. The heterogeneous mixture was cooled to room temperature, filtered through a plug of silica and concentrated under reduced pressure. The crude product was purified by FCC (EtOAc/hexanes 5:95) to give pinacol ester **126** (33.9 mg, 0.111 mmol, 22 %) as a white solid.

R_f: 0.17 (EtOAc/hexanes 10:90).

m.p.: 96 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.45 (d, $J = 1.7$ Hz, 1H, 5-H), 7.96 (dd, $J = 8.2, 1.6$ Hz, 1H, 7-H), 6.94 (d, $J = 8.2$ Hz, 1H, 8-H), 1.73 (s, 6H, 2-(CH₃)₂), 1.33 (s, 12H, 4'-(CH₃)₂ and 5'-(CH₃)₂).

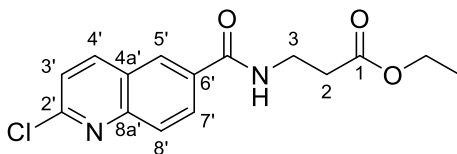
¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 161.1 (C-4), 158.4 (C-8a), 142.7 (C-7), 137.1 (C-5), 123.4 (C-6), 116.7 (C-8), 113.2 (C-4a), 106.5 (C-2), 84.3 (C-4' and C-5'), 26.0 (2-(CH₃)₂), 25.0 (4'-(CH₃)₂ and 5'-(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2987, 2362, 1738, 1613, 1379, 1353, 1323, 1276, 1202, 1141, 1128, 1076, 1047, 982, 930, 851, 841, 783, 680, 656.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for $C_{16}H_{22}BO_5^+$: 305.1555; found: 305.1556.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(2-chloroquinoline-6-carboxamido)propanoate (128)C₁₅H₁₅ClN₂O₃M_w = 306.75 g/mol

Prepared according to **General Procedure F** from quinoline **125** (70.0 mg, 0.337 mmol) and β -alanine ethyl ester hydrochloride (**76**, 51.8 mg, 0.337 mmol). The crude product was purified by FCC (EtOAc/hexanes 50:50) to give amide **128** (89.2 mg, 0.291 mmol, 86 %) as a white solid.

R_f: 0.30 (EtOAc/hexanes 50:50).

m.p.: 130 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.30 (t, J = 1.4 Hz, 1H, 5'-H), 8.19 (d, J = 8.6 Hz, 1H, 4'-H), 8.07 (s, 1H, 7'-H), 8.06 (s, 1H, 8'-H), 7.46 (d, J = 8.6 Hz, 1H, 3'-H), 7.07 (t, J = 5.1 Hz, 1H, CONH), 4.19 (q, J = 7.2 Hz, 2H, CH₂CH₃), 3.79 (q, J = 5.9 Hz, 2H, 3-H), 2.69 (t, J = 5.8 Hz, 2H, 2-H), 1.29 (t, J = 7.1 Hz, 3H, CH₃).

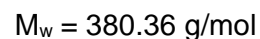
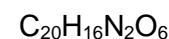
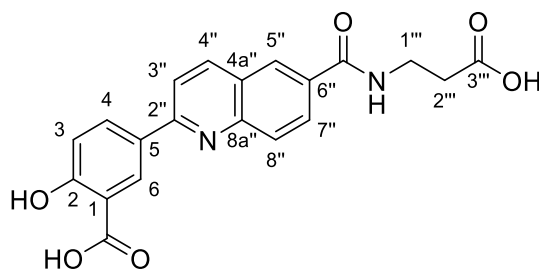
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 173.2 (C-1), 166.3 (CONH), 152.6 (C-2'), 149.2 (C-8a'), 139.8 (C-4'), 132.9 (C-6'), 129.3 (C-8'), 128.4 (C-7'), 127.5 (C-5'), 126.4 (C-4a'), 123.5 (C-3'), 61.1 (CH₂CH₃), 35.7 (C-3), 34.0 (C-2), 14.3 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3358, 2983, 1719, 1638, 1620, 1538, 1504, 1441, 1318, 1279, 1216, 1179, 1147, 1094, 1027, 908, 820, 780, 748, 664.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₁₅H₁₆ClN₂O₃⁺: 307.0844; found: 307.0847.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

5-(6-((2-Carboxyethyl)carbamoyl)quinolin-2-yl)-2-hydroxybenzoic acid (CG_268)

Under N_2 atmosphere, iodo compound **93** (76.0 mg, 0.2500 mmol) and $\text{Pd}(\text{PPh}_3)_4$ (8.67 mg, 7.50 μmol) were dissolved in dry 1,4-dioxane (1.5 mL) and purged with N_2 for 10 min. The reaction mixture was heated to 80 $^\circ\text{C}$ and dry triethylamine (348 μL , 2.50 mmol) and HBPIn (72.6 μL , 0.500 mmol) were added. After 3 h at 80 $^\circ\text{C}$, the reaction mixture was allowed to cool to room temperature. Dry MeOH (1.5 mL), compound **128** (76.7 mg, 0.250 mmol) and Cs_2CO_3 (204 mg, 0.625 mmol) were added sequentially and the resulting mixture heated to 100 $^\circ\text{C}$ for 16 h. The reaction mixture was allowed to cool to room temperature and water (10 mL) was added. The organic material was extracted with EtOAc (3 x 15 mL). The aqueous phase was then additionally acidified with 1 N aq. HCl and again extracted with EtOAc (3 x 15 mL). The combined organic phases were dried using a phase separation paper and the solvent evaporated *in vacuo*. To the residue was added THF (1.7 mL) followed by the addition of a solution of KOH (70.1 mg, 1.25 mmol) in water (1.7 mL). The reaction mixture was stirred at 60 $^\circ\text{C}$ for 2 h. Water (5 mL) was added, and the mixture acidified to pH 1 with 2 N aq. HCl. The resulting precipitates were collected by filtration and dried *in vacuo*. Product **CG_268** (28.6 mg, 0.0752 mmol, 30 % over three steps) was obtained as a brown solid.

R_f: 0.15 (MeOH/DCM+AcOH 5:95+1).

m.p.: 170 $^\circ\text{C}$.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 11.62 (s, 1H, 1-COOH or 2'''-COOH), 8.81 – 8.77 (m, 2H, 6-H and CONH), 8.56 (d, J = 8.8 Hz, 1H, 4''-H), 8.51 (d, J = 1.9 Hz, 1H, 5''-H), 8.47 (dd, J = 8.8, 2.4 Hz, 1H, 4-H), 8.22 (d, J = 8.9 Hz, 1H, 3''-H), 8.19 (dd, J = 9.0, 1.9 Hz, 1H, 7''-H), 8.13 (d, J = 8.8 Hz, 1H, 8''-H), 7.17 (d, J = 8.7 Hz, 1H, 3-H), 3.54 (q, J = 6.7 Hz, 2H, 1'''-H), 2.58 (t, J = 7.1 Hz, 2H, 2'''-H).

¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3'''), 171.7 (1-COOH), 165.7 (CONH), 162.6 (C-2), 156.1 (C-2''), 148.1 (C-8a''), 138.7 (C-4''), 134.4 (C-4), 131.9 (C-6''), 228

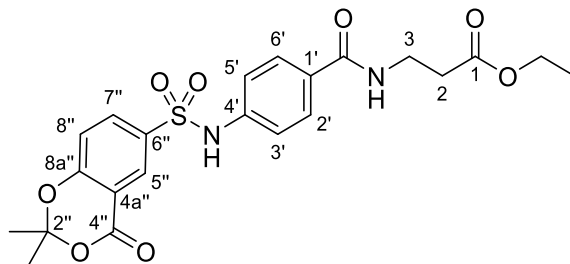
129.6 (C-6), 129.1 (C-5), 128.5 (C-8''), 128.3 (C-7''), 127.7 (C-5''),
126.0 (C-4a''), 118.9 (C-3''), 117.8 (C-3), 113.6 (C-1), 35.8 (C-1'''), 33.8
(C-2''').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2919, 2505, 1713, 1634, 1594, 1538, 1493, 1432, 1393,
1353, 1297, 1207, 1153, 903, 826.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₂₀H₁₅N₂O₆⁻: 379.0936; found: 379.0937.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-((2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxine)-6-sulfonamido)benzamido)propanoate (129)



$$\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8\text{S}$$

$$M_w = 476.50 \text{ g/mol}$$

Prepared according to **General Procedure C** from sulfonamide **106** (75.0 mg, 0.172 mmol). The crude product was purified by FCC (EtOAc/hexanes+AcOH 50:50+1) to give product **129** (43.3 mg, 0.0909 mmol, 53 %) as a yellow solid.

R_f: 0.37 (EtOAc/hexanes+AcOH 70:30+1).

m.p.: 164 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.49 (d, J = 2.4 Hz, 1H, 5''-H), 7.93 (dd, J = 8.7, 2.4 Hz, 1H, 7''-H), 7.69 – 7.64 (m, 2H, 2'-H and 6'-H), 7.48 (s, 1H, SO₂NH), 7.20 – 7.15 (m, 2H, 3'-H and 5'-H), 7.01 (d, J = 8.7 Hz, 1H, 8''-H), 6.85 (t, J = 5.9 Hz, 1H, CONH), 4.17 (q, J = 7.2 Hz, 2H, CH₂CH₃), 3.70 (q, J = 6.0 Hz, 2H, 3-H), 2.63 (t, J = 5.8 Hz, 2H, 2-H), 1.74 (s, 6H, C(CH₃)₂), 1.27 (t, J = 7.1 Hz, 3H, CH₂CH₃).

¹³C-NMR

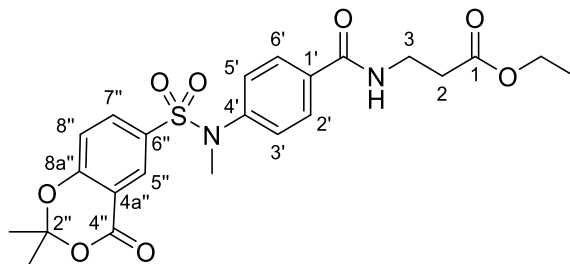
(101 MHz, CDCl₃): δ (ppm) = 173.2 (C-1), 166.6 (CONH), 159.6 (C-4''), 159.4 (C-8a''), 139.5 (C-4'), 135.1 (C-7''), 133.7 (C-6''), 131.1 (C-1'), 129.8 (C-5''), 128.7 (C-2' and C-6'), 120.1 (C-3' and C-5'), 118.6 (C-8''), 113.8 (C-4a''), 107.7 (C-2''), 61.1 (CH₂CH₃), 35.5 (C-3), 34.0 (C-2), 26.1 (C(CH₃)₂), 14.3 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3330, 3256, 2923, 2853, 1732, 1635, 1610, 1542, 1391, 1335, 1288, 1185, 1164, 1123, 1075, 935, 828, 694.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₂₂H₂₃N₂O₈S⁻: 475.1181; found: 475.1181.

Purity (HPLC): 254 nm: >95 % (method 4a).

Ethyl 3-(4-((*N*,2,2-trimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxine)-6-sulfonamido)benzamido)propanoate (130**)**



$$\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_8\text{S}$$

$$M_w = 490.53 \text{ g/mol}$$

Methyl iodide (13.0 μL , 0.210 mmol) was added to a mixture of compound **129** (50.0 mg, 0.105 mmol) and K_2CO_3 (43.6 mg, 0.262 mmol) in acetone (7.3 mL). The mixture was stirred at room temperature for 16 h, filtered and the solvent was removed *in vacuo* to give *N*-methylated sulfonamide **130** (51.4 mg, 0.105 mmol, quant.) as a yellowish solid.

R_f: 0.39 (toluene/EtOAc+AcOH 65:35+1).

m.p.: 55 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.03 (dd, $J = 2.3, 0.5$ Hz, 1H, 5''-H), 7.80 – 7.75 (m, 2H, 2'-H and 6'-H), 7.71 (dd, $J = 8.7, 2.4$ Hz, 1H, 7''-H), 7.30 – 7.26 (m, 2H, 3'-H and 5'-H), 7.18 (dd, $J = 8.7, 0.5$ Hz, 1H, 8''-H), 4.15 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 3.63 (t, $J = 6.8$ Hz, 2H, 3-H), 3.24 (s, 3H, NCH₃), 2.65 (t, $J = 6.8$ Hz, 2H, 2-H), 1.76 (s, 6H, C(CH₃)₂), 1.24 (t, $J = 7.2$ Hz, 3H, CH₂CH₃).

¹³C-NMR

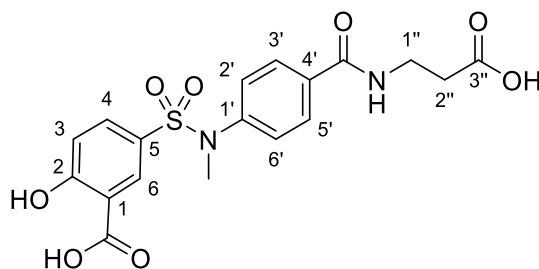
(101 MHz, CD₃OD): δ (ppm) = 173.5 (C-1), 169.2 (CONH), 160.9 (C-4''), 160.7 (C-8a''), 145.6 (C-4'), 136.7 (C-7''), 134.3 (C-1'), 132.2 (C-6''), 130.6 (C-5''), 129.1 (C-2' and C-6'), 127.2 (C-3' and C-5'), 119.8 (C-8''), 114.7 (C-4a''), 108.9 (C-2''), 61.7 (CH₂CH₃), 38.4 (NCH₃), 37.1 (C-3), 34.9 (C-2), 25.9 (C(CH₃)₂), 14.5 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3410, 2985, 1728, 1643, 1607, 1477, 1353, 1291, 1255, 1173, 1158, 1131, 1058, 1024, 930, 876, 852, 771, 720.

HR-MS (ESI): $m/z = [\text{M}+\text{H}]^+$ calcd for C₂₃H₂₇N₂O₈S⁺: 491.1483; found: 491.1480.

Purity (HPLC): 254 nm: >95 % (method 4a).

5-(N-(4-((2-Carboxyethyl)carbamoyl)phenyl)-N-methylsulfamoyl)-2-hydroxybenzoic acid (CG_168)



$C_{18}H_{18}N_2O_8S$

$M_w = 422.41$ g/mol

Prepared according to **General Procedure D** at room temperature from compound **130** (46.0 mg, 0.0938 mmol). The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give the pure product **CG_168** (39.2 mg, 0.0928 mmol, quant.) as a yellow solid.

R_f: 0.30 (EtOAc/hexanes+AcOH 40:60+1), streaks.

m.p.: 219 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.03 (d, $J = 2.4$ Hz, 1H, 6-H), 7.81 – 7.76 (m, 2H, 3'-H and 5'-H), 7.50 (dd, $J = 8.8, 2.4$ Hz, 1H, 4-H), 7.28 – 7.22 (m, 2H, 2'-H and 6'-H), 6.99 (d, $J = 8.8$ Hz, 1H, 3-H), 3.62 (t, $J = 7.0$ Hz, 2H, 1''-H), 3.20 (s, 3H, CH₃), 2.64 (t, $J = 7.0$ Hz, 2H, 2''-H).

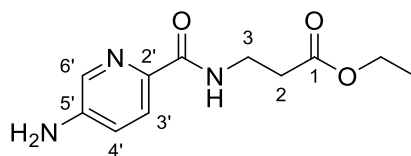
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 175.4 (C-3''), 172.6 (1-COOH), 169.3 (CONH), 166.9 (C-2), 145.9 (C-1'), 134.9 (C-4), 134.0 (C-4'), 132.2 (C-6), 129.0 (C-3' and C-5'), 127.4 (C-5), 127.1 (C-2' and C-6'), 119.0 (C-3), 115.5 (C-1), 38.3 (CH₃), 37.0 (C-1''), 34.6 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2923, 1731, 1652, 1608, 1540, 1500, 1476, 1429, 1351, 1293, 1256, 1174, 1062, 931, 875, 771, 700.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₈H₁₇N₂O₈S⁻: 421.0711; found: 421.0710.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1f).

Ethyl 3-(5-aminopyridinamido)propanoate (134)C₁₁H₁₅N₃O₃M_w = 237.26 g/mol

Prepared according to **General Procedure F** from 5-aminopyridine-2-carboxylic acid (**131**, 500 mg, 3.62 mmol) and β -alanine ethyl ester hydrochloride (**76**, 556 mg, 3.62 mmol). The crude product was purified by FCC (EtOAc/hexanes 70:30) to give amine **134** (763 mg, 3.22 mmol, 89 %) as a pale yellow oil.

R_f: 0.50 (EtOAc/hexanes 80:20)

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 7.96 (dd, J = 2.7, 0.7 Hz, 1H, 6'-H), 7.77 (dd, J = 8.5, 0.7 Hz, 1H, 3'-H), 7.02 (dd, J = 8.5, 2.7 Hz, 1H, 4'-H), 4.15 (q, J = 7.2 Hz, 2H, CH₂CH₃), 3.64 (t, J = 6.6 Hz, 2H, 3-H), 2.62 (t, J = 6.6 Hz, 2H, 2-H), 1.24 (t, J = 7.2 Hz, 3H, CH₃).

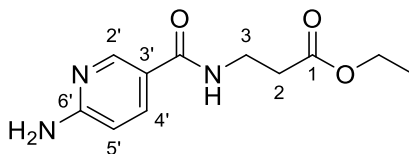
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 173.7 (C-1), 167.6 (CONH), 149.1 (C-5'), 138.9 (C-2'), 136.3 (C-6'), 124.2 (C-3'), 120.7 (C-4'), 61.7 (CH₂CH₃), 36.08 (C-3), 35.2 (C-2), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3340, 3229, 2982, 1720, 1644, 1585, 1519, 1478, 1323, 1252, 1188, 1161, 1025, 1014, 849, 734.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₁₁H₁₆N₃O₃⁺: 238.1186; found: 238.1186.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1d).

Ethyl 3-(6-aminonicotinamido)propanoate (135)C₁₁H₁₅N₃O₃M_w = 237.26 g/mol

Prepared according to **General Procedure F** from 6-aminopyridine-3-carboxylic acid (**132**, 500 mg, 3.62 mmol) and β-alanine ethyl ester hydrochloride (**76**, 556 mg, 3.62 mmol). The crude product was purified by FCC (MeOH/DCM 7:93) to give the amine **135** (142 mg, 0.598 mmol, 17 %) as a white solid.

R_f: 0.48 (MeOH/DCM 7:93)

m.p.: 147 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 8.39 (dd, *J* = 2.4, 0.8 Hz, 1H, 2'-H), 7.84 (dd, *J* = 8.8, 2.5 Hz, 1H, 4'-H), 6.56 (dd, *J* = 8.8, 0.8 Hz, 1H, 5'-H), 4.14 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 3.60 (t, *J* = 6.8 Hz, 2H, 3-H), 2.62 (t, *J* = 6.8 Hz, 2H, 2-H), 1.24 (t, *J* = 7.1 Hz, 3H, CH₃).

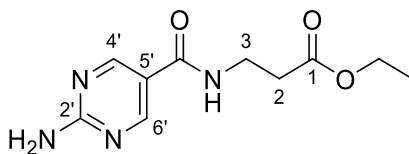
¹³C-NMR

(126 MHz, CD₃OD): δ (ppm) = 173.5 (C-1), 168.6 (CONH), 162.9 (C-6'), 148.9 (C-2'), 138.0 (C-4'), 119.8 (C-3'), 109.1 (C-5'), 61.7 (CH₂CH₃), 36.8 (C-3), 35.1 (C-2), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3390, 3253, 3157, 1724, 1622, 1600, 1546, 1503, 1333, 1317, 1186, 1164, 1083, 1023, 836, 778.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₁H₁₆N₃O₃⁺: 238.1186; found: 238.1186.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1f).

Ethyl 3-(2-aminopyrimidine-5-carboxamido)propanoate (136) $C_{10}H_{14}N_4O_3$ $M_w = 238.25$ g/mol

Prepared according to **General Procedure F** from 2-aminopyrimidine-5-carboxylic acid (**133**, 504 mg, 3.62 mmol) and β -alanine ethyl ester hydrochloride (**76**, 556 mg, 3.62 mmol). The crude product was purified by FCC (MeOH/DCM 7:93) to give amine **136** (376 mg, 1.58 mmol, 44 %) as a white solid.

R_f: 0.29 (MeOH/DCM 7:93).

m.p.: 169 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.66 (s, 2H, 4'-H and 6'-H), 4.14 (q, $J = 7.2$ Hz, 2H, CH₂CH₃), 3.60 (t, $J = 6.8$ Hz, 2H, 3-H), 2.63 (t, $J = 6.8$ Hz, 2H, 2-H), 1.24 (t, $J = 7.1$ Hz, 3H, CH₃).

¹³C-NMR

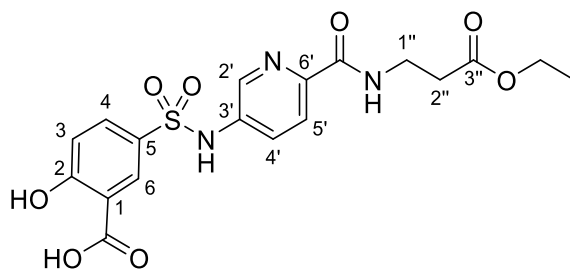
(101 MHz, CD₃OD): δ (ppm) = 173.4 (C-1), 166.9 (CONH), 165.7 (C-2'), 159.3 (C-4' and C-6'), 118.2 (C-5'), 61.7 (CH₂CH₃), 36.8 (C-3), 35.0 (C-2), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3307, 3122, 1725, 1689, 1626, 1608, 1550, 1325, 1303, 1190, 1162, 1085, 1023, 954, 858, 801.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for C₁₁H₁₅N₄O₃⁺: 239.1139; found: 239.1138.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1f).

5-(N-(6-((3-Ethoxy-3-oxopropyl)carbamoyl)pyridin-3-yl)sulfamoyl)-2-hydroxybenzoic acid (137)



$C_{18}H_{19}N_3O_8S$

$M_w = 437.42$ g/mol

Prepared according to **General Procedure H** from compound **134** (100 mg, 0.421 mmol). The crude product was purified by FCC (MeOH/DCM+AcOH 3:97+1 → 10:90+1) to give product **137** (58.2 mg, 0.133 mmol, 32 %) as a white solid.

R_f: 0.33 (MeOH/DCM+AcOH 10:90+1).

m.p.: 209 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 8.37 (d, $J = 2.5$ Hz, 1H, 6-H), 8.32 (d, $J = 2.6$ Hz, 1H, 2'-H), 7.91 (d, $J = 8.5$ Hz, 1H, 5'-H), 7.72 (d, $J = 8.6$ Hz, 1H, 4-H), 7.66 (dd, $J = 8.6, 2.6$ Hz, 1H, 4'-H), 6.87 (d, $J = 8.6$ Hz, 1H, 3-H), 4.12 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 3.63 (t, $J = 6.7$ Hz, 2H, 1''-H), 2.61 (t, $J = 6.7$ Hz, 2H, 2''-H), 1.21 (t, $J = 7.1$ Hz, 3H, CH₃).

¹³C-NMR

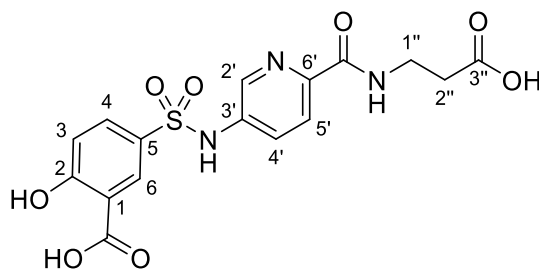
(126 MHz, CD₃OD): δ (ppm) = 174.6 (1-COOH), 173.5 (C-3''), 167.2 (C-2), 166.4 (CONH), 145.7 (C-6'), 141.1 (C-2'), 139.4 (C-3'), 132.7 (C-4), 131.9 (C-6), 129.3 (C-5), 128.1 (C-4'), 123.6 (C-5'), 120.0 (C-1), 118.5 (C-3), 61.8 (CH₂CH₃), 36.3 (C-1''), 34.9 (C-2''), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2925, 1716, 1636, 1582, 1442, 1375, 1324, 1261, 1163, 1110, 1072, 1021, 917, 834, 661.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₈H₁₈N₃O₈S⁻: 436.0820; found: 436.0819.

Purity (HPLC): 210 nm: >95 %; 254 nm: 95 % (method 1f).

**5-(N-(6-((2-Carboxyethyl)carbamoyl)pyridin-3-yl)sulfamoyl)-2-hydroxybenzoic acid
(CG_163)**



$C_{16}H_{15}N_3O_8S$

$M_w = 409.37$ g/mol

Prepared according to **General Procedure D** at room temperature from compound **137** (50.0 mg, 0.114 mmol). The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give the pure product **CG_163** (18.6 mg, 0.0454 mmol, 40 %) as a white solid.

R_f: 0.328 (MeOH/DCM+AcOH 10:90+1).

m.p.: 228 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.34 – 8.32 (m, 2H, 6-H and 2'-H), 7.96 (dd, $J = 8.5, 0.7$ Hz, 1H, 5'-H), 7.85 (dd, $J = 8.8, 2.5$ Hz, 1H, 4-H), 7.69 (dd, $J = 8.6, 2.6$ Hz, 1H, 4'-H), 7.01 (d, $J = 8.8$ Hz, 1H, 3-H), 3.62 (t, $J = 6.7$ Hz, 2H, 1''-H), 2.60 (t, $J = 6.7$ Hz, 2H, 2''-H).

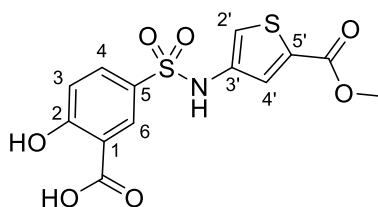
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 175.4 (C-3''), 172.4 (1-COOH), 166.8 (C-2), 166.2 (CONH), 146.4 (C-6'), 141.2 (C-2'), 138.8 (C-3'), 134.5 (C-4), 131.7 (C-6), 130.7 (C-5), 128.5 (C-4'), 123.7 (C-5'), 119.4 (C-3), 115.1 (C-1), 36.3 (C-1''), 34.6 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2923, 1694, 1637, 1587, 1538, 1455, 1327, 1215, 1161, 1110, 1075, 920, 873, 845, 706.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{16}H_{14}N_3O_8S^-$: 408.0507; found: 408.0505.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1f).

2-Hydroxy-5-((5-(methoxycarbonyl)thiophene)-3-sulfonamido)benzoic acid (141)C₁₃H₁₁NO₇S₂M_w = 357.35 g/mol

Prepared according to **General Procedure H** from methyl 4-aminothiophene-2-carboxylate (**140**, 490 mg, 3.12 mmol) and 5-chlorosulfonyl-2-hydroxybenzoic acid (**105**, 738 mg, 3.12 mmol). The crude product was purified by FCC (MeOH/DCM+AcOH 5:95+1) to give sulfonamide **141** (847 mg, 2.37 mmol, 76 %) as an off-white solid.

R_f: 0.33 (MeOH/DCM+AcOH 5:95+1).

m.p.: 206 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.26 (d, *J* = 2.4 Hz, 1H, 6-H), 7.79 (dd, *J* = 8.8, 2.5 Hz, 1H, 4-H), 7.49 (d, *J* = 1.7 Hz, 1H, 4'-H), 7.18 (d, *J* = 1.7 Hz, 1H, 2'-H), 7.00 (d, *J* = 8.8 Hz, 1H, 3-H), 3.83 (s, 3H, CH₃).

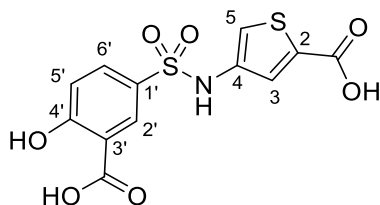
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 172.6 (1-COOH), 166.6 (C-2), 163.5 (5'-COOMe), 137.4 (C-3'), 134.5 (C-4), 134.0 (C-5'), 131.6 (C-6), 130.9 (C-5), 129.2 (C-4'), 120.4 (C-2'), 119.1 (C-3), 114.8 (C-1), 52.8 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3229, 1702, 1660, 1608, 1445, 1364, 1293, 1156, 1073, 991, 761, 663.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₃H₁₀NO₇S₂⁻: 355.9904; found: 355.9905.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1e).

4-((3-Carboxy-4-hydroxyphenyl)sulfonamido)thiophene-2-carboxylic acid (142)C₁₂H₉NO₇S₂M_w = 343.32 g/mol

Prepared according to **General Procedure D** from compound **141** (500 mg, 1.40 mmol) and heating the reaction mixture to reflux. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give the pure product **142** (433 mg, 1.26 mmol, 90 %) as an off-white solid.

R_f: 0.40 (EtOAc/DCM+AcOH 30:70+1).

m.p.: 259 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 8.27 (d, *J* = 2.4 Hz, 1H, 2'-H), 7.80 (dd, *J* = 8.9, 2.4 Hz, 1H, 6'-H), 7.47 (d, *J* = 1.7 Hz, 1H, 3-H), 7.16 (d, *J* = 1.7 Hz, 1H, 5-H), 7.02 (d, *J* = 8.8 Hz, 1H, 5'-H).

¹³C-NMR

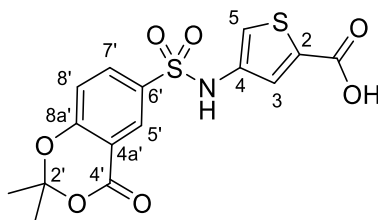
(126 MHz, CD₃OD): δ (ppm) = 172.4 (3'-COOH), 166.5 (C-4'), 164.6 (2-COOH), 137.3 (C-4), 135.2 (C-2), 134.7 (C-6'), 131.6 (C-2'), 131.0 (C-1'), 129.2 (C-3), 120.3 (C-5), 119.2 (C-5'), 114.4 (C-3').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3241, 1675, 1437, 1356, 1259, 1159, 969, 797, 733, 715, 659.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₂H₈NO₇S₂⁻: 341.9748; found: 341.9747.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1f).

4-((2,2-Dimethyl-4-oxo-4*H*-benzo[d][1,3]dioxine)-6-sulfonamido)thiophene-2-carboxylic acid (143)



$C_{15}H_{13}NO_7S_2$

$M_w = 383.39$ g/mol

Prepared according to **General Procedure C** from compound **142** (250 mg, 0.728 mmol). The crude product was purified by FCC (EtOAc/DCM+AcOH 30:70+1) to give acetonide **143** (87.3 mg, 0.228 mmol, 31 %) as a dark yellow oily solid.

R_f: 0.55 (EtOAc/DCM+AcOH 30:70+1).

m.p.: 70 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.29 (d, $J = 2.3$ Hz, 1H, 5'-H), 7.96 (dd, $J = 8.7, 2.4$ Hz, 1H, 7'-H), 7.44 (d, $J = 1.7$ Hz, 1H, 3-H), 7.22 (d, $J = 1.7$ Hz, 1H, 5-H), 7.17 (d, $J = 8.8$ Hz, 1H, 8'-H), 1.73 (s, 7H, C(CH₃)₂).

¹³C-NMR

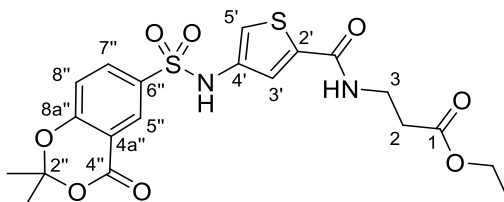
(101 MHz, CD₃OD): δ (ppm) = 164.5 (2-COOH), 161.0 (C-4'), 160.4 (C-8a'), 136.9 (C-4), 136.1 (C-7'), 135.4 (C-6'), 135.3 (C-2), 130.0 (C-5'), 129.2 (C-3), 120.8 (C-5), 119.7 (C-8'), 114.7 (C-4a'), 108.9 (C-2'), 25.8 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3109, 2923, 1683, 1609, 1476, 1435, 1293, 1162, 1132, 1077, 980, 782, 681.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₅H₁₂NO₇S₂⁻: 382.0061; found: 382.0064.

Purity (HPLC): 210 nm: 94 %; 254 nm: 89 % (method 1e).

Ethyl 3-(4-((2,2-dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxine)-6-sulfonamido)thiophene-2-carboxamido)propanoate (144)



$$\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_8\text{S}_2$$

$$M_w = 482.52 \text{ g/mol}$$

Prepared according to **General Procedure E** from compound **143** (51.0 mg, 0.133 mmol) and β -alanine ethyl ester hydrochloride (**76**, 40.8 mg, 0.266 mmol). The crude product was purified by FCC (EtOAc/hexanes+AcOH 50:50+1) to give product **144** (28.0 mg, 0.0580 mmol, 44 %) as a beige solid.

R_f: 0.45 (EtOAc/hexanes+AcOH 50:50+1).

m.p.: 88 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.29 (d, J = 2.3 Hz, 1H, 5''-H), 7.96 (dd, J = 8.7, 2.4 Hz, 1H, 7''-H), 7.48 (d, J = 1.6 Hz, 1H, 3'-H), 7.17 (d, J = 8.7 Hz, 1H, 8''-H), 7.07 (d, J = 1.6 Hz, 1H, 5'-H), 4.13 (q, J = 7.2 Hz, 2H, CH₂CH₃), 3.57 (t, J = 6.9 Hz, 2H, 3-H), 2.60 (t, J = 6.9 Hz, 2H, 2-H), 1.73 (s, 6H, C(CH₃)₂), 1.23 (t, J = 7.1 Hz, 3H, CH₂CH₃).

¹³C-NMR

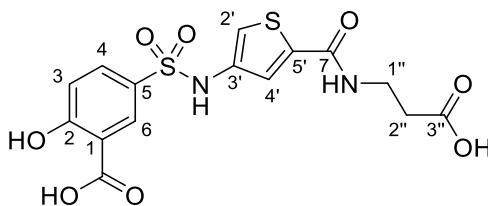
(101 MHz, CD₃OD): δ (ppm) = 173.4 (C-1), 163.7 (CONH), 161.0 (C-4'), 160.4 (C-8a''), 139.8 (C-2'), 136.7 (C-4'), 136.1 (C-7''), 135.4 (C-6''), 130.0 (C-5''), 125.0 (C-3'), 119.7 (C-8''), 118.9 (C-5'), 114.7 (C-4a''), 108.8 (C-2''), 61.7 (CH₂CH₃), 36.9 (C-3), 34.9 (C-2), 25.8 (C(CH₃)₂), 14.5 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3243, 1742, 1726, 1611, 1559, 1531, 1477, 1367, 1293, 1163, 1120, 1077, 980, 846, 723.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₂₀H₂₁N₂O₈S₂: 481.0745; found: 481.0744.

Purity (HPLC): 210 nm: 89 %; 254 nm: >95 % (method 1e).

5-(N-(5-((2-Carboxyethyl)carbamoyl)thiophen-3-yl)sulfamoyl)-2-hydroxybenzoic acid
(CG_169)



$C_{15}H_{14}N_2O_8S_2$

$M_w = 414.40 \text{ g/mol}$

Prepared according to **General Procedure D** from compound **144** (60.0 mg, 0.124 mmol) at room temperature. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give the pure product **CG_169** (44.9 mg, 0.108 mmol, 87 %) as a beige solid.

R_f: 0.20 (MeOH/DCM 10:90).

m.p.: 246 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 8.25 (d, $J = 2.5 \text{ Hz}$, 1H, 6-H), 7.80 (dd, $J = 8.8, 2.4 \text{ Hz}$, 1H, 4-H), 7.47 (d, $J = 1.6 \text{ Hz}$, 1H, 4'-H), 7.04 (d, $J = 1.6 \text{ Hz}$, 1H, 2'-H), 7.02 (d, $J = 8.9 \text{ Hz}$, 1H, 3-H), 3.56 (t, $J = 7.0 \text{ Hz}$, 2H, 1''-H), 2.60 (t, $J = 7.0 \text{ Hz}$, 2H, 2''-H).

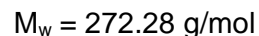
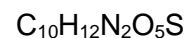
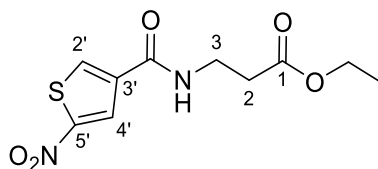
¹³C-NMR

(126 MHz, CD₃OD): δ (ppm) = 175.3 (C-3''), 172.3 (1-COOH), 166.5 (C-2), 163.8 (CONH), 139.6 (C-5'), 137.1 (C-3'), 134.8 (C-4), 131.6 (C-6), 131.1 (C-5), 125.2 (C-4'), 119.2 (C-3), 118.8 (C-2'), 114.1 (C-1), 36.9 (C-1''), 34.6 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3374, 3239, 2924, 2502, 2417, 1723, 1678, 1602, 1342, 1215, 1166, 1076, 805, 670.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{15}H_{13}N_2O_8S_2^-$: 413.0119; found: 413.0118.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(5-nitrothiophene-3-carboxamido)propanoate (147)

Prepared according to **General Procedure E** from 5-nitrothiophene-3-carboxylic acid (**145**, 500 mg, 2.89 mmol) and β -alanine ethyl ester hydrochloride (**76**, 444 mg, 2.89 mmol). The crude product was purified by FCC (EtOAc/hexanes 40:60) to give **147** (725 mg, 2.66 mmol, 92 %) as a white solid.

R_f: 0.25 (EtOAc/hexanes 40:60).

m.p.: 102 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.34 (d, J = 1.9 Hz, 1H, 4'-H), 8.32 (d, J = 1.9 Hz, 1H, 2'-H), 4.15 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.61 (t, J = 6.8 Hz, 2H, 3-H), 2.64 (t, J = 6.8 Hz, 2H, 2-H), 1.24 (t, J = 7.1 Hz, 3H, CH₃).

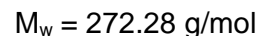
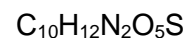
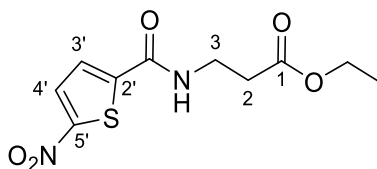
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 173.3 (C-1), 163.5 (CONH), 153.5 (C-5'), 137.4 (C-3'), 135.9 (C-2'), 128.1 (C-4'), 61.7 (CH₂CH₃), 36.9 (C-3), 34.8 (C-2), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3373, 3283, 1716, 1624, 1556, 1512, 1335, 1294, 1186, 1014, 830, 815, 729.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₁₀H₁₁N₂O₅S⁻: 271.0394; found: 271.0393.

Purity (HPLC): 210 nm: >95 %; 254 nm: 95 % (method 1e).

Ethyl 3-(5-nitrothiophene-2-carboxamido)propanoate (148)

Prepared according to **General Procedure E** from 5-nitrothiophene-2-carboxylic acid (**146**, 500 mg, 2.89 mmol) and β -alanine ethyl ester hydrochloride (**76**, 444 mg, 2.89 mmol). The crude product was purified by FCC (EtOAc/hexanes 40:60) to give **148** (729 mg, 2.68 mmol, 93 %) as a yellow solid.

R_f: 0.28 (EtOAc/hexanes 40:60).

m.p.: 86 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 7.96 (d, J = 4.3 Hz, 1H, 4'-H), 7.62 (d, J = 4.4 Hz, 1H, 3'-H), 4.15 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.63 (t, J = 6.8 Hz, 2H, 3-H), 2.65 (t, J = 6.8 Hz, 2H, 2-H), 1.24 (t, J = 7.2 Hz, 3H, CH₃).

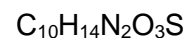
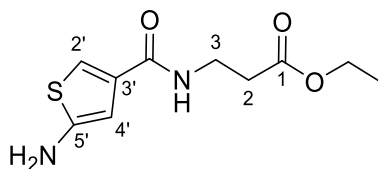
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 173.2 (C-1), 162.4 (CONH), 155.4 (C-5'), 146.4 (C-2'), 129.8 (C-4'), 128.1 (C-3'), 61.8 (CH₂CH₃), 37.2 (C-3), 34.7 (C-2), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3334, 1714, 1633, 1566, 1513, 1337, 1321, 1292, 1214, 1192, 1029, 817, 754, 655.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₁₀H₁₁N₂O₅S⁻: 271.0394; found: 271.0393.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 1e).

Ethyl 3-(5-aminothiophene-3-carboxamido)propanoate (149)

$$M_w = 242.29 \text{ g/mol}$$

To a mixture of nitro compound **147** (100 mg, 0.358 mmol) and AcOH (1.0 mL), iron powder (78.9 mg, 1.41 mmol) was added, and the resulting reaction mixture was stirred at room temperature for 4 h. The reaction mixture was filtered through a pad of celite and the solvent removed *in vacuo*. To the residue was added EtOAc (40 mL) and sat. aq. NaHCO₃ (40 mL). Phases were separated and the aq. phase extracted with EtOAc (3 x 40 mL). The combined organic extracts were washed with brine (20 mL), dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 55:45 → 60:40) to give the amine **149** (234 mg, 0.966 mmol, 53 %) as a dark brown oil.

R_f: 0.16 (EtOAc/hexanes 40:60).

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 7.07 (d, *J* = 1.7 Hz, 1H, 2'-H), 6.41 (d, *J* = 1.7 Hz, 1H, 4'-H), 4.14 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 3.55 (t, *J* = 6.9 Hz, 2H, 3-H), 2.60 (t, *J* = 6.9 Hz, 2H, 2-H), 1.24 (t, *J* = 7.1 Hz, 3H, CH₃).

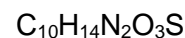
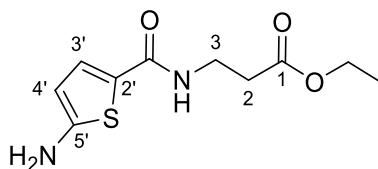
¹³C-NMR

(101 MHz, CD₃OD): δ (ppm) = 173.5 (C-1), 166.2 (CONH), 154.6 (C-5'), 136.8 (C-3'), 115.8 (C-2'), 106.4 (C-4'), 61.7 (CH₂CH₃), 36.6 (C-3), 35.0 (C-2), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3314, 2981, 1718, 1627, 1522, 1473, 1374, 1294, 1249, 1183, 1026, 668.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₀H₁₅N₂O₃S⁺: 243.0798; found: 243.0799.

Purity (HPLC): 210 nm: 87 %; 254 nm: 87 % (method 2a).

Ethyl 3-(5-aminothiophene-2-carboxamido)propanoate (150)

$$M_w = 242.29 \text{ g/mol}$$

To a mixture of nitro compound **148** (500 mg, 1.84 mmol) and AcOH (4.80 mL), iron powder (410 mg, 7.35 mmol) was added, and the resulting reaction mixture was stirred at room temperature for 2 h. The reaction mixture was filtered through a pad of celite and the solvent removed *in vacuo*. To the residue was added EtOAc (40 mL) and sat. aq. NaHCO₃ (40 mL). Phases were separated and the aq. phase extracted with EtOAc (3 x 40 mL). The combined organic extracts were washed with brine (20 mL), dried using a phase separation paper and concentrated *in vacuo* to give amine **150** (272 mg, 1.12 mmol, 61 %) in sufficient purity as a pale brown oil.

R_f: 0.35 (EtOAc/hexanes 70:30).

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 7.25 (d, J = 4.0 Hz, 1H, 3'-H), 5.97 (d, J = 4.1 Hz, 1H, 4'-H), 4.14 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.54 (t, J = 6.9 Hz, 2H, 3-H), 2.59 (t, J = 6.9 Hz, 2H, 2-H), 1.24 (t, J = 7.1 Hz, 4H, CH₃).

¹³C-NMR

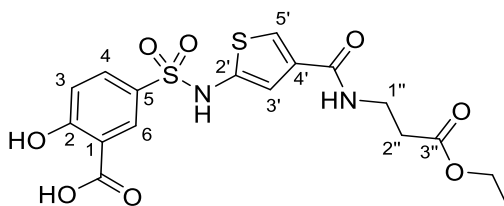
(101 MHz, CD₃OD): δ (ppm) = 173.6 (C-1), 165.5 (CONH), 161.6 (C-5'), 131.2 (C-3'), 121.3 (C-2'), 106.8 (C-4'), 61.7 (CH₂CH₃), 36.7 (C-3), 35.3 (C-2), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3315, 3210, 1715, 1607, 1516, 1460, 1299, 1184, 1144, 1025, 735.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₁₀H₁₅N₂O₃S⁺: 243.0798; found: 243.0798.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

5-(*N*-(4-((3-Ethoxy-3-oxopropyl)carbamoyl)thiophen-2-yl)sulfamoyl)-2-hydroxybenzoic acid (151)



$C_{17}H_{18}N_2O_8S_2$

$M_w = 442.46$ g/mol

Prepared according to **General Procedure H** from amine **149** (100 mg, 0.413 mmol). The crude product was purified by FCC (MeOH/DCM+AcOH 3:97+1 → 7:93+1) to give sulfonamide **151** (91.1 mg, 0.206 mmol, 50 %) as a brown solid.

R_f: 0.44 (MeOH/DCM+AcOH 10:90+1).

m.p.: 90 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.27 (s, 1H, 6-H), 7.73 (d, $J = 8.6$ Hz, 1H, 4-H), 7.62 (s, 1H, 5'-H), 6.98 (t, $J = 4.6$ Hz, 2H, 3-H and 3'-H), 4.12 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 3.54 (t, $J = 6.8$ Hz, 2H, 1''-H), 2.59 (t, $J = 6.7$ Hz, 2H, 2''-H), 1.22 (t, $J = 7.1$ Hz, 3H, CH₃).

¹³C-NMR

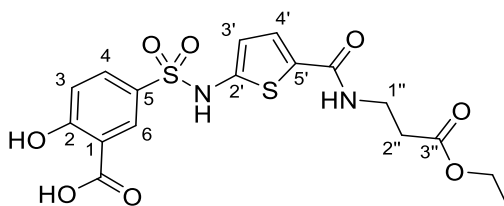
(126 MHz, CD₃OD): δ (ppm) = 173.4 (C-3''), 173.2 (1-COOH), 166.9 (C-2), 165.2 (CONH), 141.1 (C-2'), 136.3 (C-4'), 134.3 (C-4), 132.0 (C-6), 129.7 (C-5), 125.5 (C-5'), 120.3 (C-3'), 118.9 (C-3), 116.5 (C-1), 61.7 (CH₂CH₃), 36.7 (C-1''), 34.9 (C-2''), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3102, 1704, 1631, 1476, 1362, 1327, 1294, 1162, 1109, 1073, 900, 836, 657.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₇H₁₇N₂O₈S₂⁻: 441.0432; found: 441.0434.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

5-(*N*-(5-((3-Ethoxy-3-oxopropyl)carbamoyl)thiophen-2-yl)sulfamoyl)-2-hydroxybenzoic acid (152)



$C_{17}H_{18}N_2O_8S_2$

$M_w = 442.46$ g/mol

Prepared according to **General Procedure H** from compound **150** (100 mg, 0.413 mmol). The crude product was purified by FCC (MeOH/DCM+AcOH 3:97+1 → 7:93+1) to give sulfonamide **152** (73.3 mg, 0.166 mmol, 40 %) as a brown solid.

R_f: 0.20 (MeOH/DCM 5:95).

m.p.: 93 °C.

¹H-NMR

(400 MHz, CD₃OD): δ (ppm) = 8.34 – 8.29 (m, 1H, 6-H), 7.77 (dd, $J = 8.8, 1.8$ Hz, 1H, 4-H), 7.34 (d, $J = 4.0$ Hz, 1H, 4'-H), 6.99 (d, $J = 8.7$ Hz, 1H, 3-H), 6.59 (d, $J = 4.0$ Hz, 1H, 3'-H), 4.12 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 3.54 (t, $J = 6.8$ Hz, 2H, 1''-H), 2.58 (t, $J = 6.8$ Hz, 2H, 2''-H), 1.21 (t, $J = 7.1$ Hz, 3H, CH₃).

¹³C-NMR

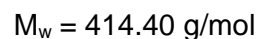
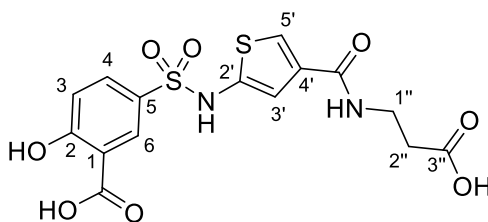
(126 MHz, CD₃OD): δ (ppm) = 173.4 (C-3''), 173.0 (1-COOH), 167.0 (C-2), 164.2 (CONH), 145.7 (C-2'), 134.2 (C-4), 133.4 (C-5'), 132.0 (C-6), 129.8 (C-5), 128.7 (C-4'), 119.5 (C-3'), 118.9 (C-3), 116.4 (C-1), 61.7 (CH₂CH₃), 36.8 (C-1''), 35.0 (C-2''), 14.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2982, 1711, 1609, 1528, 1457, 1318, 1202, 1162, 1110, 1074, 1018, 892, 832, 805.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₇H₁₇N₂O₈S₂⁻: 441.0432; found: 441.0434.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

5-(N-(4-((2-Carboxyethyl)carbamoyl)thiophen-2-yl)sulfamoyl)-2-hydroxybenzoic acid
(CG_176)



Prepared according to **General Procedure D** from compound **151** (70.0 mg, 0.158 mmol) at room temperature. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give the pure product **CG_176** (32.4 mg, 0.0782 mmol, 49 %) as a white solid.

R_f: 0.25 (MeOH/DCM+AcOH 10:90+1).

m.p.: 254 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 8.26 (d, *J* = 2.4 Hz, 1H, 6-H), 7.81 (dd, *J* = 8.8, 2.4 Hz, 1H, 4-H), 7.64 (d, *J* = 1.7 Hz, 1H, 5'-H), 7.04 (d, *J* = 8.9 Hz, 1H, 3-H), 6.99 (d, *J* = 1.7 Hz, 1H, 3'-H), 3.54 (t, *J* = 7.0 Hz, 2H, 1''-H), 2.58 (t, *J* = 6.9 Hz, 2H, 2''-H).

¹³C-NMR

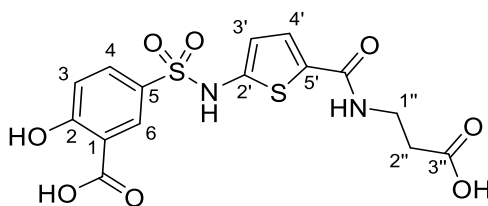
(126 MHz, CD₃OD): δ (ppm) = 175.3 (C-3''), 172.3 (1-COOH), 166.7 (C-2), 165.1 (CONH), 140.9 (C-2'), 136.3 (C-4'), 135.1 (C-4), 131.9 (C-6), 130.5 (C-5), 125.6 (C-5'), 120.5 (C-3'), 119.3 (C-3), 114.1 (C-1), 36.7 (C-1''), 34.6 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3384, 3234, 2877, 1723, 1678, 1602, 1565, 1535, 1330, 1201, 1167, 1109, 1076, 896, 738, 660.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₅H₁₃N₂O₈S₂⁻: 413.0119; found: 413.0118.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

5-(N-(5-((2-Carboxyethyl)carbamoyl)thiophen-2-yl)sulfamoyl)-2-hydroxybenzoic acid
(CG_177)



$$\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_8\text{S}_2$$

$$M_w = 414.40 \text{ g/mol}$$

Prepared according to **General Procedure D** from compound **152** (60.0 mg, 0.136 mmol) at room temperature. The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo* to give the pure product **CG_177** (47.3 mg, 0.114 mmol, 84 %) as a yellow solid.

R_f: 0.24 (MeOH/DCM+AcOH 10:90+1), streaks.

m.p.: 86 °C.

¹H-NMR

(500 MHz, CD₃OD): δ (ppm) = 8.30 (d, *J* = 2.4 Hz, 1H, 6-H), 7.82 (dd, *J* = 8.9, 2.4 Hz, 1H, 4-H), 7.36 (d, *J* = 4.1 Hz, 1H, 4'-H), 7.03 (d, *J* = 8.8 Hz, 1H, 3-H), 6.60 (d, *J* = 4.0 Hz, 1H, 3'-H), 3.54 (t, *J* = 7.0 Hz, 2H, 1''-H), 2.58 (t, *J* = 7.0 Hz, 2H, 2''-H).

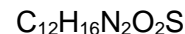
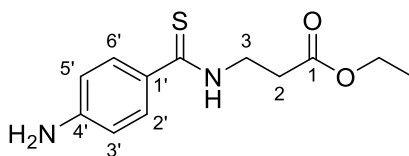
¹³C-NMR

(126 MHz, CD₃OD): δ (ppm) = 175.3 (C-3''), 172.4 (1-COOH), 166.8 (C-2), 164.2 (CONH), 145.6 (C-2'), 134.8 (C-4), 133.6 (C-5'), 131.9 (C-6), 130.3 (C-5), 128.7 (C-4'), 119.7 (C-3'), 119.2 (C-3), 114.7 (C-1), 36.9 (C-1''), 34.7 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3076, 2931, 1682, 1558, 1525, 1455, 1317, 1201, 1157, 1106, 1073, 894, 798, 654.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₅H₁₃N₂O₈S₂⁻: 413.0119; found: 413.0118.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-aminophenylthioamido)propanoate (153)M_w = 252.33 g/mol

A mixture of amine **95** (640 mg, 2.71 mmol), LAWESSON's reagent (657 mg, 1.63 mmol) and THF (8.3 mL) was heated to reflux for 30 min under N₂ atmosphere. The resulting solution was concentrated *in vacuo* and the crude product purified by FCC (EtOAc/hexanes 60:40) to give the thioamide **153** (262 mg, 1.04 mmol, 38 %) as a yellow oil.

R_f: 0.25 (EtOAc/hexanes 50:50).

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.50 (s, 1H, CSNH), 7.65 (d, *J* = 8.0 Hz, 2H, 2'-H and 6'-H), 6.80 (d, *J* = 8.0 Hz, 2H, 3'-H and 5'-H), 4.16 (q, *J* = 7.2 Hz, 2H, CH₂CH₃), 4.17 – 4.06 (m, 2H, 3-H), 2.78 (t, *J* = 6.1 Hz, 2H, 2-H), 1.26 (t, *J* = 7.2 Hz, 3H, CH₃).

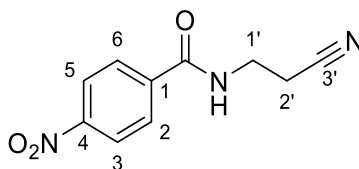
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 197.7 (CSNH), 173.2 (C-1), 128.8 (C-2' and C-6'), 115.2 (C-3' and C-5'), 61.2 (CH₂CH₃), 41.7 (C-3), 32.6 (C-2), 14.3 (CH₃), C-1' and C-4' not visible.

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3331, 3216, 2980, 2933, 1714, 1622, 1601, 1532, 1504, 1442, 1372, 1295, 1249, 1178, 1039, 1025, 831.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₂H₁₇N₂O₂S⁺: 253.1005; found: 253.1005.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

***N*-(2-Cyanoethyl)-4-nitrobenzamide (158)**^[154]C₁₀H₉N₃O₃M_w = 219.20 g/mol

At 0 °C, 4-nitrobenzoyl chloride (**1**, 1.30 g, 6.99 mmol) and 10 % aq. NaOH (3.22 mL, 8.04 mmol) were gradually added alternately into 3-aminopropionitrile (**157**, 522 μL, 7.13 mmol). After stirring for 30 min, the precipitates were collected by filtration, washed with water (15 mL) and dried *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes+AcOH 50:50+1) and subsequent recrystallisation from hot acetone. Amide **158** (471 mg, 2.15 mmol, 31 %) was obtained as white crystals.

R_f: 0.20 (EtOAc/hexanes+AcOH 50:50+1).

m.p.: 156 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 9.18 (t, *J* = 5.7 Hz, 1H, CONH), 8.36 – 8.33 (m, 2H, 3-H and 5-H), 8.10 – 8.06 (m, 2H, 2-H and 6-H), 3.53 (td, *J* = 6.4, 5.5 Hz, 2H, 1'-H), 2.80 (t, *J* = 6.5 Hz, 2H, 2'-H).

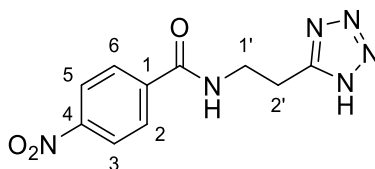
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 164.9 (CONH), 149.2 (C-4), 139.5 (C-1), 128.7 (C-2 and C-6), 123.7 (C-3 and C-5), 119.2 (C-3'), 35.6 (C-1'), 17.4 (C-2').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3306, 3078, 2252, 1650, 1598, 1548, 1521, 1422, 1346, 1320, 1297, 1081, 876, 846, 689, 676.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₀H₈N₃O₃⁻: 218.0571; found: 218.0571.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

N-(2-(1H-Tetrazol-5-yl)ethyl)-4-nitrobenzamide (159)C₁₀H₁₀N₆O₃M_w = 262.23 g/mol

Nitrile **158** (300 mg, 1.37 mmol), NaN₃ (93.4 mg, 1.44 mmol) and NH₄Cl (80.5 mg, 1.51 mmol) were suspended in dry DMF (1.2 mL) and the reaction mixture heated to 120 °C. After 16 h, the mixture was cooled to room temperature and the solid residue triturated with 2 M aq. HCl (5.0 mL), filtered, washed with water (25 mL) and dried *in vacuo*. Tetrazole **159** (274 mg, 1.05 mmol, 77 %) was obtained as an off-white solid.

R_f: 0.29 (MeOH/DCM+AcOH 5:95+0.5).

m.p.: 225 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 16.15 (s, 1H, tetrazole-H), 8.97 (t, *J* = 5.7 Hz, 1H, CONH), 8.33 – 8.30 (m, 2H, 3-H and 5-H), 8.04 – 8.01 (m, 2H, 2-H and 6-H), 3.65 (td, *J* = 6.9, 5.6 Hz, 2H, 1'-H), 3.18 (t, *J* = 6.9 Hz, 2H, 2'-H).

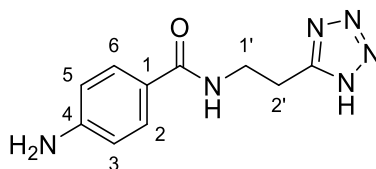
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 164.8 (CONH), 154.1 (tetrazole-C), 149.1 (C-4), 139.9 (C-1), 128.7 (C-2 and C-6), 123.5 (C-3 and C-5), 37.8 (C-1'), 23.2 (C-2').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3302, 3026, 2889, 2755, 1643, 1596, 1554, 1512, 1353, 1345, 1324, 1311, 1265, 1108, 1056, 872, 846, 724, 716.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₀H₉N₆O₃⁻: 261.0742; found: 261.0740.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

***N*-(2-(1*H*-Tetrazol-5-yl)ethyl)-4-aminobenzamide (160)** $C_{10}H_{12}N_6O$ $M_w = 232.25 \text{ g/mol}$

Prepared according to **General Procedure A** from nitro compound **159** (250 mg, 0.953 mmol). Aryl amine **160** (1.76 g, 8.44 mmol, 75 %) was obtained as a beige solid.

R_f: 0.15 (MeOH/DCM+AcOH 5:95+0.5).

m.p.: 252 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 16.05 (s, 1H, tetrazole-H), 8.18 (t, $J = 5.7$ Hz, 1H, CONH), 7.54 – 7.51 (m, 2H, 2-H and 6-H), 6.54 – 6.51 (m, 2H, 3-H and 5-H), 5.61 (s, 2H, NH₂), 3.57 (td, $J = 7.1, 5.6$ Hz, 2H, 1'-H), 3.11 (t, $J = 7.1$ Hz, 2H, 2'-H).

¹³C-NMR

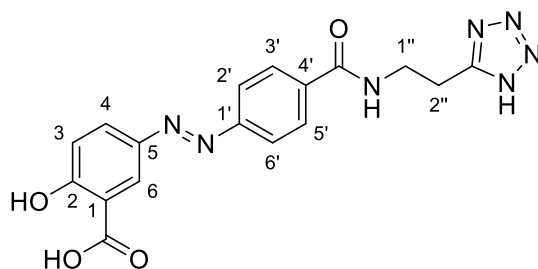
(126 MHz, (CD₃)₂SO): δ (ppm) = 166.4 (CONH), 154.3 (tetrazole-C), 151.7 (C-4), 128.7 (C-2 and C-6), 120.9 (C-1), 112.5 (C-3 and C-5), 37.4 (C-1'), 23.7 (C-2').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3487, 3386, 2606, 1619, 1595, 1548, 1508, 1425, 1309, 1293, 1272, 1190, 1032, 843, 768.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₀H₁₁N₆O: 231.1000; found: 231.0999.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

**(E)-5-((4-((2-(1H-Tetrazol-5-yl)ethyl)carbamoyl)phenyl)diazenyl)-2-hydroxybenzoic acid
(CG_238)**



$C_{17}H_{15}N_7O_4$

$M_w = 381.35$ g/mol

Prepared according to **General Procedure B** from aryl amine **160** (150 mg, 0.646 mmol) and salicylic acid (91.9 mg, 0.665 mmol). The crude product was purified by FCC (MeOH/DCM+AcOH 5:95+1) to give tetrazole **CG_238** (58.0 mg, 0.152 mmol, 24 %) as an orange solid.

R_f: 0.24 (MeOH/DCM+AcOH 7:93+1).

m.p.: 264 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 16.16 (s, 1H, tetrazole-H), 11.96 (s, 1H, 1-COOH), 8.79 (t, $J = 5.6$ Hz, 1H, CONH), 8.33 (d, $J = 2.6$ Hz, 1H, 6-H), 7.97 (d, $J = 8.2$ Hz, 2H, 3'-H and 5'-H), 7.93 (dd, $J = 8.8, 2.5$ Hz, 1H, 4-H), 7.87 (d, $J = 8.3$ Hz, 2H, 2'-H and 6'-H), 6.91 (d, $J = 8.8$ Hz, 1H, 3-H), 3.66 (q, $J = 6.5$ Hz, 2H, 1''-H), 3.18 (t, $J = 7.0$ Hz, 2H, 2''-H).

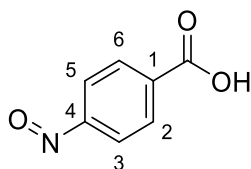
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 170.8 (1-COOH), 167.8 (C-2), 165.8 (CONH), 154.1 (tetrazole-C), 153.7 (C-1'), 143.1 (C-5), 135.2 (C-4'), 128.4 (C-3' and C-5'), 127.6 (C-4), 126.4 (C-6), 121.8 (C-2' and C-6'), 118.2 (C-3), 117.3 (C-1), 37.7 (C-1''), 23.4 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3318, 2924, 1628, 1540, 1485, 1386, 1296, 1248, 1178, 1158, 1071, 856, 835, 799, 770, 699.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for $C_{17}H_{16}N_7O_4^+$: 382.1258; found: 382.1253.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

4-Nitrosobenzoic acid (161)^[156] $C_7H_5NO_3$ $M_w = 151.12 \text{ g/mol}$

4-Aminobenzoic acid (**14**, 500 mg, 3.65 mmol) was dissolved in DCM (5.0 mL). A solution of Oxone[®] (1.79 g, 7.29 mmol) in water (23 mL) was added and the solution stirred under N_2 atmosphere for 1 h. The precipitate was collected by filtration, washed with water (20 mL), and dried *in vacuo* to give nitroso derivative **161** (411 mg, 2.72 mmol, 75 %) as a yellow solid.

R_f: 0.43 (EtOAc/hexanes+AcOH 30:70+1).

m.p.: 220 °C (decomposition).

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 13.49 (s, 1H, COOH), 8.28 – 8.24 (m, 2H, 2-H and 6-H), 8.05 – 8.01 (m, 2H, 3-H and 5-H).

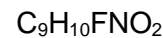
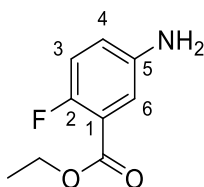
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 166.2 (COOH), 165.0 (C-4), 136.6 (C-1), 131.0 (C-2 and C-6), 120.6 (C-3 and C-5).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3060, 2830, 2666, 2552, 1686, 1600, 1427, 1294, 1262, 1112, 928, 871, 845, 792, 762, 690.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_7H_4NO_3^-$: 150.0197; found: 150.0196.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 5-amino-2-fluorobenzoate (163)^[157]

M_w = 183.18 g/mol

A solution of 5-amino-2-fluorobenzoic acid (**162**, 500 mg, 3.22 mmol) in EtOH (10 mL) was cooled to 0 °C and SOCl₂ (470 μL, 6.45 mmol) was added dropwise over 10 min. The resulting solution was then allowed to warm to room temperature over 1 h and then heated to reflux for 3 h. After this time, the mixture was concentrated *in vacuo* and water (25 mL) was added. The obtained purple acidic mixture was washed with an EtOAc/hexanes mixture (1:1; 2 x 15 mL). The organic phase was further extracted with 0.5 N aq. HCl (1 x 15 mL). The combined aqueous acidic layers were washed with an EtOAc/hexanes mixture (1:1; 15 mL), cooled to 0 °C, basified using solid NaHCO₃ and then extracted with EtOAc (3 x 15 mL). The combined organic phase was washed with brine (20 mL), dried using a phase separation paper and concentrated under reduced pressure to give product **163** (541 mg, 2.95 mmol, 92 %) as a purple oil, which solidifies below room temperature.

R_f: 0.54 (EtOAc/hexanes 50:50).

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 7.19 (dd, *J* = 5.8, 3.1 Hz, 1H, 6-H), 6.92 (dd, *J* = 10.3, 8.7 Hz, 1H, 3-H), 6.78 (ddd, *J* = 8.7, 3.9, 3.1 Hz, 1H, 4-H), 4.37 (q, *J* = 7.1 Hz, 2H, CH₂), 3.64 (s, 2H, NH₂), 1.38 (t, *J* = 7.1 Hz, 3H, CH₃).

¹³C-NMR

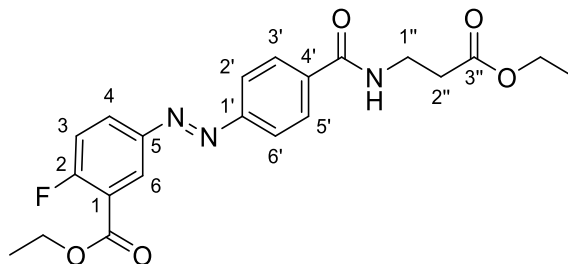
(126 MHz, CDCl₃): δ (ppm) = 164.8 (d, COOEt), 155.5 (d, C-2), 142.4 (d, C-5), 120.7 (d, C-4), 119.2 (d, C-1), 117.7 (d, C-3), 117.3 (C-6), 61.4 (CH₂), 14.4 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3441, 3358, 3211, 2981, 1698, 1633, 1589, 1500, 1446, 1367, 1308, 1264, 1243, 1213, 1138, 1088, 1023, 817, 769.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₉H₁₀FNO₂⁺: 183.0690; found: 183.0688.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl (E)-5-((4-((3-ethoxy-3-oxopropyl)carbamoyl)phenyl)diazenyl)-2-fluorobenzoate
(165)



$$\text{C}_{21}\text{H}_{22}\text{FN}_3\text{O}_5$$

$$M_w = 415.42 \text{ g/mol}$$

Nitroso derivative **161** (60.4 mg, 0.400 mmol) and amine **163** (61.1 mg, 0.333 mmol) were suspended in AcOH/DMSO (1:1; 2.5 mL). The reaction mixture was stirred at room temperature for 48 h. Water (10 mL) was added, the precipitate collected by filtration, washed with water (15 mL), and dried *in vacuo* to give crude azobenzene **164**.

Amide coupling was then performed according to **General Procedure F** from crude azo-derivative **164** (0.333 mmol) and β -alanine ethyl ester hydrochloride (**76**, 51.2 mg, 0.333 mmol). The crude product was purified by FCC (EtOAc/hexanes 40:60) to give azobenzene **165** (71.2 mg, 0.171 mmol, 52 % over two steps) as an orange solid.

R_f: 0.34 (EtOAc/hexanes 40:60).

m.p.: 113 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.54 (dd, J = 6.8, 2.6 Hz, 1H, 6-H), 8.11 (ddd, J = 8.8, 4.4, 2.6 Hz, 1H, 4-H), 7.98 – 7.95 (m, 2H, 2'-H and 6'-H), 7.94 – 7.91 (m, 2H, 3'-H and 5'-H), 7.29 (dd, J = 10.0, 8.8 Hz, 1H, 3-H), 6.97 (t, J = 5.8 Hz, 1H, CONH), 4.45 (q, J = 7.1 Hz, 2H, 1-COOCH₂CH₃), 4.19 (q, J = 7.1 Hz, 2H, 2''-COOCH₂CH₃), 3.76 (q, J = 6.0 Hz, 2H, 1''-H), 2.68 (t, J = 6.0 Hz, 2H, 2''-H), 1.44 (t, J = 7.1 Hz, 3H, 1-COOCH₂CH₃), 1.29 (t, J = 7.2 Hz, 3H, 2''-COOCH₂CH₃).

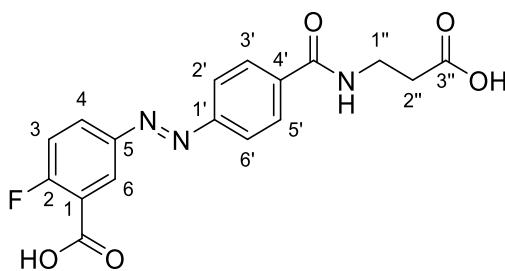
¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 173.2 (C-3''), 166.6 (CONH), 163.8 (d, 1-COOCH₂CH₃), 163.6 (d, C-2), 154.0 (C-1'), 148.6 (d, C-5), 136.7 (C-4'), 128.2 (C-3' and C-5'), 128.0 (d, C-4), 127.9 (d, C-6), 123.2 (C-2' and C-6'), 120.0 (d, C-1), 118.1 (d, C-3), 61.9 (1-COOCH₂CH₃), 61.1 (2''-COOCH₂CH₃), 35.6 (C-1''), 34.0 (C-2''), 14.4 (1-COOCH₂CH₃), 14.3 (2''-COOCH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3298, 2983, 1728, 1713, 1636, 1617, 1605, 1537, 1481, 1422, 1287, 1240, 1188, 1154, 1074, 1018, 858, 844, 781.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₂₁H₂₃FN₃O₅⁺: 416.1616; found: 416.1615.

Purity (HPLC): 210 nm: 88 %; 254 nm: 88 % (method 2a).

(E)-5-((4-((2-carboxyethyl)carbamoyl)phenyl)diazenyl)-2-fluorobenzoic acid (CG_267)C₁₇H₁₄FN₃O₅M_w = 359.31 g/mol

Prepared according to **General Procedure D** from azobenzene **165** (60.0 mg, 0.144 mmol) at room temperature. The resulting precipitates were collected by filtration and dried *in vacuo* to give product **CG_267** (39.6 mg, 0.110 mmol, 76 %) as an orange solid.

R_f: 0.15 (MeOH/DCM+AcOH 2:98+1).

m.p.: 248 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 13.52 (s, 1H, 1-COOH or 2''-COOH), 12.36 (s, 1H, 1-COOH or 2''-COOH), 8.73 (t, *J* = 5.5 Hz, 1H, CONH), 8.38 (dd, *J* = 6.9, 2.7 Hz, 1H, 6-H), 8.21 (ddd, *J* = 8.8, 4.4, 2.7 Hz, 1H, 4-H), 8.07 – 8.03 (m, 2H, 3'-H and 5'-H), 8.01 – 7.97 (m, 2H, 2'-H and 6'-H), 7.59 (dd, *J* = 10.3, 8.8 Hz, 1H, 3-H), 3.49 (td, *J* = 7.1, 5.4 Hz, 2H, 1''-H), 2.55 (t, *J* = 7.1 Hz, 2H, 2''-H).

¹³C-NMR

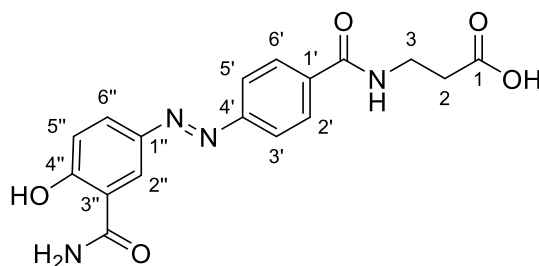
(126 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3''), 165.4 (CONH), 164.3 (d, 1-COOH), 162.8 (d, C-2), 153.0 (C-1'), 148.0 (d, C-5), 136.9 (C-4'), 129.1 (d, C-4), 128.5 (C-3' and C-5'), 125.8 (d, C-6), 122.6 (C-2' and C-6'), 120.4 (d, C-1), 118.6 (d, C-3), 35.7 (C-1''), 33.7 (C-2'').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3361, 2921, 2666, 1697, 1683, 1639, 1619, 1536, 1449, 1284, 1237, 1134, 1080, 920, 855, 834, 772, 696.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₇H₁₃FN₃O₅⁻: 358.0845; found: 358.0846.

Purity (HPLC): 210 nm: 90 %; 254 nm: 90 % (method 2a).

(E)-3-(4-((3-Carbamoyl-4-hydroxyphenyl)diazenyl)benzamido)propanoic acid (CG_254)



$C_{17}H_{16}N_4O_5$

$M_w = 356.33 \text{ g/mol}$

Prepared according to **General Procedure B** from amine **3** (100 mg, 0.480 mmol) and salicylamide (67.8 mg, 0.495 mmol). Azobenzene **CG_254** (136 mg, 0.382 mmol, 80 %) was obtained as an orange solid.

R_f: 0.28 (MeOH/DCM+AcOH 5:95+1).

m.p.: 278 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 13.75 (s, 1H, OH), 12.28 (s, 1H, COOH), 8.76 (s, 1H, CONH₂), 8.69 (t, $J = 5.5 \text{ Hz}$, 1H, CONH), 8.60 (d, $J = 2.4 \text{ Hz}$, 1H, 2''-H), 8.14 – 8.10 (m, 1H, CONH₂), 8.05 – 8.01 (m, 2H, 2'-H and 6'-H), 7.99 (dd, $J = 8.9, 2.4 \text{ Hz}$, 1H, 6''-H), 7.91 – 7.87 (m, 2H, 3'-H and 5'-H), 7.07 (d, $J = 9.0 \text{ Hz}$, 1H, 5''-H), 3.49 (td, $J = 7.1, 5.4 \text{ Hz}$, 2H, 3-H), 2.54 (t, $J = 7.1 \text{ Hz}$, 2H, 2-H).

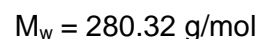
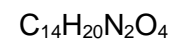
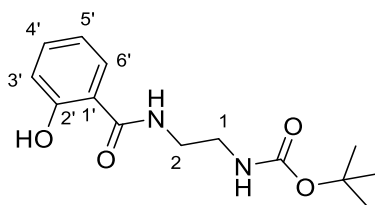
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (COOH), 171.4 (CONH₂), 165.5 (CONH), 164.8 (C-4''), 153.4 (C-4'), 144.3 (C-1''), 135.9 (C-1'), 128.5 (C-2' and C-6'), 126.9 (C-2''), 125.9 (C-6''), 122.0 (C-3' and C-5'), 118.9 (C-5''), 114.9 (C-3''), 35.7 (C-3), 33.7 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3439, 3346, 3815, 1691, 1657, 1630, 1527, 1488, 1436, 1375, 1311, 1252, 1218, 1115, 954, 892, 855, 769.

HR-MS (EI): $m/z = [M]^{++}$ calcd for $C_{17}H_{16}N_4O_5^{++}$: 356.1115; found: 356.1113.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

***tert*-Butyl (2-(2-hydroxybenzamido)ethyl)carbamate (167)^[158]**

To a solution of salicylic acid (150 mg, 1.09 mmol) and *tert*-butyl *N*-(2-aminoethyl)carbamate (**166**, 174 mg, 1.09 mmol) in dry THF (1.2 mL), a solution of 1,3-dicyclocarbodiimide (246 mg, 1.19 mmol) in DCM (0.40 mL) was added dropwise at 0 °C. The reaction was stirred for 18 h and then filtered. The filtrate was concentrated *in vacuo*. The residue was dissolved in EtOAc (20 mL), washed with 5 % aq. NaHCO₃ (10 mL), dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 30:70) to give **167** (220 mg, 0.786 mmol, 72 %) as a colourless oil.

R_f: 0.27 (EtOAc/hexanes 30:70).

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 7.73 (s, 1H, 1-NHCO), 7.47 (dd, *J* = 8.0, 1.5 Hz, 1H, 6'-H), 7.37 (ddd, *J* = 8.6, 7.2, 1.5 Hz, 1H, 4'-H), 6.96 (dd, *J* = 8.3, 1.2 Hz, 1H, 3'-H), 6.84 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H, 5'-H), 5.00 (s, 1H, 2-NHCO), 3.57 – 3.50 (m, 2H, 2-H), 3.43 (dd, *J* = 6.7, 3.9 Hz, 2H, 1-H), 1.44 (s, 9H, C(CH₃)₃).

¹³C-NMR

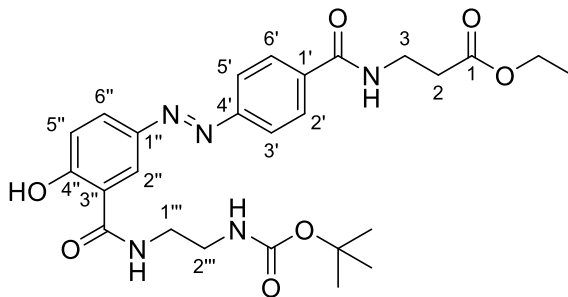
(126 MHz, CDCl₃): δ (ppm) = 170.7 (2-NHCO), 161.7 (C-2'), 158.2 (1-NHCO), 134.2 (C-4'), 126.1 (C-6'), 118.8 (C-5'), 118.5 (C-3'), 114.3 (C-1'), 80.7 (C(CH₃)₃), 42.6 (C-2), 39.7 (C-1), 28.4 (C(CH₃)₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3345, 2979, 2936, 1688, 1636, 1533, 1489, 1365, 1250, 1161, 1147, 856, 751.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₄H₁₉N₂O₄⁻: 279.1350; found: 279.1350.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl (E)-3-(4-((3-((2-((tert-butoxycarbonyl)amino)ethyl)carbamoyl)-4-hydroxyphenyl)diazenyl)benzamido)propanoate (168)



$C_{26}H_{33}N_5O_7$

$M_w = 527.58$ g/mol

Prepared according to **General Procedure B** from amine **95** (100 mg, 0.423 mmol) and compound **167** (122 mg, 0.436 mmol). Azobenzene **168** (119 mg, 0.226 mmol, 53 %) was obtained as a yellow solid.

R_f: 0.38 (EtOAc/hexanes 60:40).

m.p.: 180 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 13.36 (s, 1H, OH), 9.12 (t, $J = 5.8$ Hz, 1H, 1'''-NHCO), 8.71 (t, $J = 5.5$ Hz, 1H, 3-NHCO), 8.56 (d, $J = 2.4$ Hz, 1H, 2''-H), 8.05 – 8.01 (m, 2H, 2'-H and 6'-H), 7.99 (dd, $J = 8.9, 2.4$ Hz, 1H, 6''-H), 7.90 (dd, $J = 8.5, 1.7$ Hz, 2H, 3'-H and 5'-H), 7.10 (d, $J = 8.8$ Hz, 1H, 5''-H), 6.97 (t, $J = 5.9$ Hz, 1H, 2'''-NHCO), 4.08 (q, $J = 7.1$ Hz, 2H, CH₂CH₃), 3.52 (td, $J = 6.9, 5.4$ Hz, 2H, 3-H), 3.37 (q, $J = 6.0$ Hz, 2H, 1'''-H), 3.17 (q, $J = 6.2$ Hz, 2H, 2'''-H), 2.60 (t, $J = 7.0$ Hz, 2H, 2-H), 1.36 (s, 9H, C(CH₃)₃), 1.18 (t, $J = 7.1$ Hz, 3H, CH₂CH₃).

¹³C-NMR

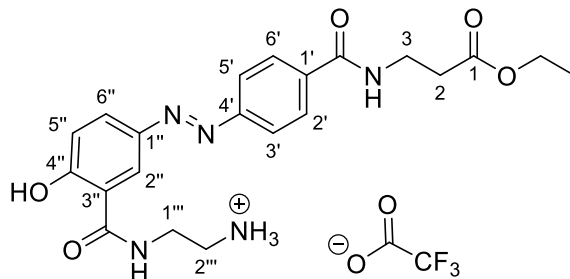
(126 MHz, (CD₃)₂SO): δ (ppm) = 171.3 (C-1), 168.6 (1'''-NHCO), 165.6 (3-NHCO), 163.5 (C-4''), 155.8 (2'''-NHCO), 153.4 (C-4'), 144.5 (C-1''), 135.9 (C-1'), 128.5 (C-2' and C-6'), 126.1 (C-6''), 125.8 (C-2''), 122.0 (C-3' and C-5'), 118.6 (C-5''), 115.6 (C-3''), 77.7 (C(CH₃)₃), 60.0 (CH₂CH₃), 39.5 (C-1''', collapses with DMSO), 39.2 (C-2''', collapses with DMSO), 35.6 (C-3), 33.7 (C-2), 28.2 (C(CH₃)₃), 14.1 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3345, 2980, 2937, 1731, 1688, 1634, 1592, 1536, 1487, 1367, 1275, 1249, 1169, 1026, 989, 856, 833.

HR-MS (EI): $m/z = [M]^{+}$ calcd for C₂₆H₃₃N₅O₇⁺: 527.2374; found:527.2399.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl (*E*)-3-(4-((3-((2-aminoethyl)carbamoyl)-4-hydroxyphenyl)diazenyl)benzamido)propanoate • TFA (169)



$$\text{C}_{21}\text{H}_{25}\text{N}_5\text{O}_5 \cdot \text{CF}_3\text{CO}_2\text{H}$$

$$M_w = 541.48 \text{ g/mol}$$

Azobenzene **168** (70.0 mg, 0.133 mmol) was dissolved in DCM (1.2 mL) and TFA (0.80 mL) was added dropwise. The reaction mixture was stirred for 2 h at room temperature. After this time, the product was concentrated *in vacuo* to give TFA-salt **169** (69.6 mg, 0.129 mmol, quant.) as an orange solid.

R_f: 0.17 (MeOH/DCM+AcOH 10:90+1).

m.p.: 190 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 13.05 (s, 1H, OH), 9.17 (t, *J* = 5.7 Hz, 1H, 1'''-NHCO), 8.73 (t, *J* = 5.5 Hz, 1H, 3-NHCO), 8.55 (d, *J* = 2.4 Hz, 1H, 2''-H), 8.07 – 7.98 (m, 3H, 2'-H and 6'-H and 6''-H), 7.92 – 7.88 (m, 2H, 3'-H and 5'-H), 7.86 (s, 3H, 2'''-NH₃⁺), 7.15 (d, *J* = 8.9 Hz, 1H, 5''-H), 4.08 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 3.60 (q, *J* = 6.0 Hz, 2H, 1'''-H), 3.52 (td, *J* = 6.9, 5.4 Hz, 2H, 3-H), 3.06 (s, 2H, 2'''-H), 2.60 (t, *J* = 7.0 Hz, 2H, 2-H), 1.18 (t, *J* = 7.1 Hz, 3H, CH₃).

¹³C-NMR

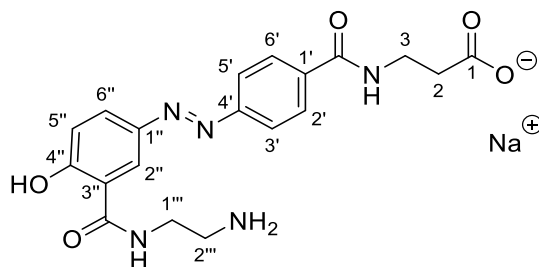
(126 MHz, (CD₃)₂SO): δ (ppm) = 171.3 (C-1), 168.7 (1'''-NHCO), 165.5 (3-NHCO), 163.0 (C-4''), 153.4 (C-4'), 144.5 (C-1''), 136.0 (C-1'), 128.5 (C-2' and C-6'), 126.5 (C-6''), 126.0 (C-2''), 122.0 (C-5'), 118.6 (C-5''), 116.1 (C-3''), 60.0 (CH₂CH₃), 38.5 (C-2'''), 37.0 (C-1'''), 35.6 (C-3), 33.7 (C-2), 14.1 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3282, 3081, 2921, 1723, 1690, 1631, 1550, 1480, 1302, 1258, 1200, 1161, 1128, 811, 974, 720.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₂₁H₂₆N₅O₅⁺: 428.1928; found: 428.1924.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

(E)-3-(4-((3-((2-Aminoethyl)carbamoyl)-4-hydroxyphenyl)diazenyl)benzamido)propanoic acid sodium salt (CG_190)



$C_{19}H_{20}N_5O_5 \cdot Na$

$M_w = 421.39 \text{ g/mol}$

Prepared according to **General Procedure D** from compound **169** (40.0 mg, 0.0739 mmol) at room temperature. After 1.5 h, the pH of the reaction mixture was adjusted to 5 (calculated isoelectric point of the product) with 1 N aq. NaOH/1 N aq. HCl and concentrated *in vacuo*. The crude product was dissolved in MeOH/water (1:9) and an SPE purification was performed (two column volumes of MeOH/water (1:9) to wash out any remaining salts, followed by two column volumes MeOH to elute the product). Product **CG_190** (17.4 mg, 0.0413 mmol, 56 %) was obtained as an orange solid.

R_f: not determinable.

m.p.: 234 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 11.67 (s, 1H, 1'''-NHCO), 8.57 (t, $J = 5.4$ Hz, 1H, 3-NHCO), 8.37 (d, $J = 2.8$ Hz, 1H, 2''-H), 7.97 – 7.89 (m, 2H, 2'-H and 6'-H), 7.74 – 7.70 (m, 2H, 3'-H and 5'-H), 7.68 (dd, $J = 9.1, 2.9$ Hz, 1H, 6''-H), 6.44 (d, $J = 9.2$ Hz, 1H, 5''-H), 3.56 – 3.50 (m, 1H, 1'''-H), 3.48 (q, $J = 7.0$ Hz, 1H, 3-H), 2.98 (s, 2H, 2'''-H), 2.56 – 2.52 (m, 2H, 2-H), NH₂ not clearly visible.

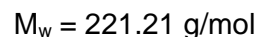
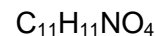
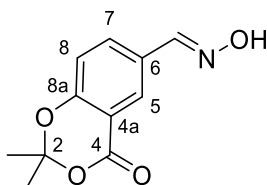
¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 176.7 (C-4''), 173.0 (C-1), 169.0 (1'''-NHCO), 165.8 (3-NHCO), 154.7 (C-4'), 138.6 (C-1''), 133.1 (C-1'), 131.1 (C-2''), 128.2 (C-2' and C-6'), 124.7 (C-6''), 123.4 (C-5''), 120.8 (C-3' and C-5'), 117.9 (C-3''), 40.0 (C-2'''), 36.8 (C-1'''), 35.7 (C-3), 34.0 (C-2).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3632, 3295, 2359, 1628, 1529, 1491, 1409, 1360, 1300, 1258, 1181, 894, 857, 840, 767, 662.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₁₉H₂₀N₅O₅⁻: 398.1470; found: 398.1468.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

(E)-2,2-Dimethyl-4-oxo-4H-benzo[d][1,3]dioxine-6-carbaldehyde oxime (170)^[142]

Aldehyde **50** (200 mg, 0.970 mmol), hydroxylamine hydrochloride (135 mg, 1.94 mmol) and pyridine (204 μL, 2.52 mmol) were dissolved in EtOH (5.0 mL) and the reaction mixture was heated to 80 °C for 16 h. The reaction mixture was cooled to room temperature and the solvent evaporated *in vacuo*. Water (10 mL) and EtOAc (10 mL) were added, layers were separated, and the aqueous phase further extracted with EtOAc (2 x 15 mL). The combined organic layers were dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 30:70) to give aldoxime **170** (177 mg, 0.798 mmol, 82 %) as a white solid.

R_f: 0.44 (EtOAc/hexanes 30:70).

m.p.: 160 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.12 (s, 1H, CH=N-OH), 8.10 (d, *J* = 2.2 Hz, 1H, 5-H), 7.88 (dd, *J* = 8.6, 2.2 Hz, 1H, 7-H), 7.00 (d, *J* = 8.6 Hz, 1H, 8-H), 1.75 (s, 6H, C(CH₃)₂).

¹³C-NMR

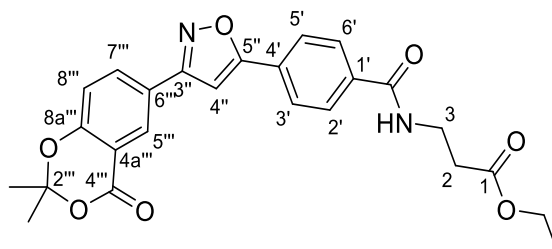
(101 MHz, CDCl₃): δ (ppm) = 160.7 (C-4), 157.3 (C-8a), 148.7 (CH=N-OH), 134.2 (C-7), 129.1 (C-5), 127.0 (C-6), 118.2 (C-8), 113.8 (C-4a), 107.0 (C-2), 26.0 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3289, 2946, 1740, 1616, 1498, 1380, 1298, 1263, 1202, 1136, 1115, 1048, 967, 946, 848, 751, 667.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₁H₁₁NO₄⁺: 221.0683; found: 221.0689.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-(3-(2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)isoxazol-5-yl)benzamido)propanoate (171)



$$\text{C}_{25}\text{H}_{24}\text{N}_2\text{O}_7$$

$$M_w = 464.47 \text{ g/mol}$$

Aldoxime **170** (67.6 mg, 0.306 mmol) and alkyne **91** (50.0 mg, 0.204 mmol) were suspended in MeOH/water (5:1; 2.0 mL, 0.1 M with respect to alkyne). PIFA (131 mg, 0.306 mmol) was added as 0.5 eq per 2 h. After addition of 1.5 eq of PIFA in total, the reaction mixture was stirred at room temperature for 16 h. Then EtOAc (15 mL) was added, the mixture filtered through a pad of silica and solvents evaporated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 50:50) to give isoxazole **171** (41.7 mg, 0.0898 mmol, 44 %) as a white solid.

R_f: 0.24 (EtOAc/hexanes 50:50).

m.p.: 200 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.37 (d, J = 2.3 Hz, 1H, 5'''-H), 8.19 (dd, J = 8.6, 2.2 Hz, 1H, 7'''-H), 7.90 (s, 4H, 2'-, 3'-, 5'- and 6'-H), 7.10 (d, J = 8.6 Hz, 1H, 8'''-H), 6.97 (t, J = 6.1 Hz, 1H, CONH), 6.94 (s, 1H, 4''-H), 4.19 (q, J = 7.2 Hz, 2H, CH₂CH₃), 3.76 (q, J = 6.0 Hz, 2H, 3-H), 2.67 (t, J = 5.9 Hz, 2H, 2-H), 1.79 (s, 6H, C(CH₃)₂), 1.29 (t, J = 7.2 Hz, 3H, CH₂CH₃).

¹³C-NMR

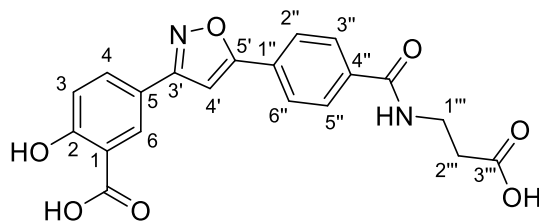
(126 MHz, CDCl₃): δ (ppm) = 173.2 (C-1), 169.9 (C-5''), 166.4 (CONH), 161.7 (C-3''), 160.7 (C-4''), 157.5 (C-8a'''), 136.0 (C-1'), 134.7 (C-7'''), 129.9 (C-4'), 128.3 (C-5'''), 127.9 (C-2' and C-6'), 126.1 (C-3' and C-5'), 124.0 (C-6'''), 118.4 (C-8'''), 113.9 (C-4a'''), 107.1 (C-2'''), 98.5 (C-4''), 61.1 (CH₂CH₃), 35.6 (C-3), 34.0 (C-2), 26.0 (C(CH₃)₂), 14.3 (CH₂CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3408, 2981, 2905, 1735, 1724, 1648, 1624, 1539, 1500, 1378, 1292, 1251, 1181, 1142, 1056, 927, 850, 764.

HR-MS (ESI): m/z = [M+H]⁺ calcd for C₂₅H₂₅N₂O₇⁺: 465.1656; found: 465.1654.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

**5-(5-(4-((2-Carboxyethyl)carbamoyl)phenyl)isoxazol-3-yl)-2-hydroxybenzoic acid
(CG_209)**



$C_{20}H_{16}N_2O_7$

$M_w = 396.36 \text{ g/mol}$

Prepared according to **General Procedure D** at room temperature from isoxazole **171** (35.0 mg, 0.0754 mmol). The organic material was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried using a phase separation paper and concentrated *in vacuo*. The pure product **CG_209** (23.0 mg, 0.0580 mmol, 77 %) was obtained as a white solid.

R_f: 0.27 (EtOAc/hexanes+AcOH 80:20+1).

m.p.: 272 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.24 (s, 1H, 1-COOH or 2'''-COOH), 8.69 (t, $J = 5.5 \text{ Hz}$, 1H, CONH), 8.33 (d, $J = 2.3 \text{ Hz}$, 1H, 6-H), 8.05 (dd, $J = 8.6, 2.3 \text{ Hz}$, 1H, 4-H), 8.01 (s, 4H, 2''-, 3''-, 5''- and 6''-H), 7.76 (s, 1H, 4'-H), 7.13 (d, $J = 8.7 \text{ Hz}$, 1H, 3-H), 3.49 (q, $J = 6.7 \text{ Hz}$, 2H, 1'''-H), 2.54 (t, $J = 7.2 \text{ Hz}$, 2H, 2'''-H).

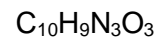
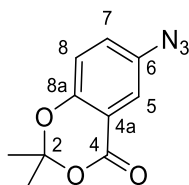
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3'''), 171.3 (1-COOH), 168.9 (C-5'), 165.4 (CONH), 162.6 (C-2), 161.8 (C-3'), 135.7 (C-4''), 133.3 (C-4), 129.0 (C-1''), 128.6 (C-6), 128.1 (C-3'' and C-5''), 125.5 (C-2'' and C-6''), 119.4 (C-5), 118.2 (C-3), 114.0 (C-1), 99.5 (C-4'), 35.7 (C-1'''), 33.7 (C-2''').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3332, 2922, 2853, 1697, 1668, 1638, 1587, 1538, 1423, 1399, 1286, 1237, 1201, 855, 794, 769.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{20}H_{15}N_2O_7^-$: 395.0885; found: 395.0885.

Purity (HPLC): 210 nm: 93 %; 254 nm: 93 % (method 2a).

6-Azido-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (172)M_w = 219.20 g/mol

Conc. HCl (0.62 mL) and a solution of NaNO₂ (85.7 mg, 1.24 mmol) in water (0.16 mL) was slowly added to amine **102** (200 mg, 1.04 mmol) in water (0.60 mL) at 0 °C. After 10 min, a solution of NaN₃ (1.24 mg, 1.24 mmol) in water (0.16 mL) was added dropwise and the reaction mixture stirred at 0 °C for 40 min. The mixture was then extracted with diethyl ether (3 x 25 mL). The combined organic extracts were dried using a phase separation paper and concentrated *in vacuo* to give product **172** (199 mg, 0.906 mmol, 88 %) as a yellow solid.

R_f: 0.42 (EtOAc/hexanes 10:90).

m.p.: 85 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 7.64 (d, *J* = 2.8 Hz, 1H, 5-H), 7.18 (dd, *J* = 8.7, 2.8 Hz, 1H, 7-H), 6.97 (d, *J* = 8.8 Hz, 1H, 8-H), 1.73 (s, 6H, C(CH₃)₂).

¹³C-NMR

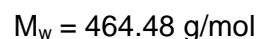
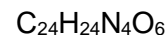
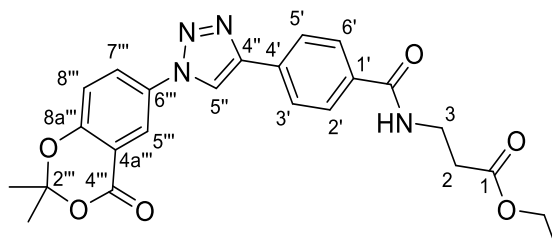
(101 MHz, CDCl₃): δ (ppm) = 160.4 (C-4), 153.1 (C-8a), 135.1 (C-6), 127.3 (C-7), 119.0 (C-5), 118.9 (C-8), 114.5 (C-4a), 106.8 (C-2), 25.8 (C(CH₃)₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2132, 1727, 1484, 1439, 1380, 1328, 1294, 1242, 1198, 1138, 1050, 979, 934, 836, 780, 749.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₀H₉N₃O₃⁺: 219.0638; found: 219.0638.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-(1-(2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)-1H-1,2,3-triazol-4-yl)benzamido)propanoate (173)



An alkyne **91** stock solution (0.33 M in *tert*-BuOH, 600 μL , 0.200 mmol) and an azide **172** stock solution (0.13 M in *tert*-BuOH/DMSO 21:1, 628 μL , 0.0800 mmol) were added in a 15 mL round bottom flask. Then, a CuSO_4 stock solution (26.7 mM in water, 600 μL , 16.0 μmol) and a sodium ascorbate stock solution (0.13 M in water, 600 μL , 0.0800 mmol) were added. The reaction mixture was stirred at room temperature for 4 days. EtOAc (20 mL) was added and the organic phase was washed with 1 N aq. HCl (10 mL), sat. aq. NaHCO_3 (10 mL) and brine (10 mL). The organic phase was dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 60:40) to give triazole **173** (21.0 mg, 0.0452 mmol, 57 %) as a white solid.

R_f: 0.29 (EtOAc/hexanes 60:40).

m.p.: 215 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.29 (s, 1H, 5''-H), 8.26 (d, $J = 2.6$ Hz, 1H, 5'''-H), 8.16 (dd, $J = 8.9, 2.7$ Hz, 1H, 7'''-H), 7.98 (d, $J = 8.2$ Hz, 2H, 3'-H and 5'-H), 7.88 (d, $J = 8.1$ Hz, 2H, 2'-H and 6'-H), 7.20 (d, $J = 8.9$ Hz, 1H, 8'''-H), 6.93 (t, $J = 6.1$ Hz, 1H, CONH), 4.19 (q, $J = 7.1$ Hz, 2H, CH_2CH_3), 3.76 (q, $J = 5.9$ Hz, 2H, 3-H), 2.67 (t, $J = 5.8$ Hz, 2H, 2-H), 1.80 (s, 6H, $\text{C}(\text{CH}_3)_2$), 1.29 (t, $J = 7.2$ Hz, 3H, CH_2CH_3).

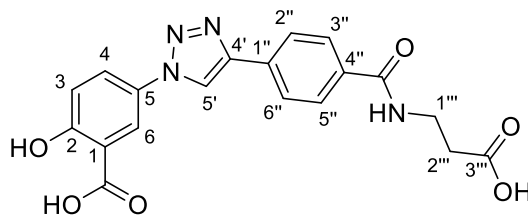
¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 173.2 (C-1), 166.8 (CONH), 160.1 (C4'''), 156.2 (C-8a'''), 147.9 (C-4''), 134.4 (C-1'), 133.1 (C-4'), 132.1 (C-6'''), 129.1 (C-7'''), 127.9 (C-2' and C-6'), 126.1 (C-3' and C-5'), 120.9 (C-5'''), 119.4 (C-8'''), 118.4 (C-5''), 114.3 (C-4a'''), 107.5 (C-2'''), 61.0 (CH_2CH_3), 35.5 (C-3), 34.1 (C-2), 26.0 ($\text{C}(\text{CH}_3)_2$), 14.4 (CH_2CH_3).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3414, 1739, 1725, 1647, 1533, 1506, 1492, 1302, 1184, 1149, 1057, 1030, 930, 826, 768.

HR-MS (ESI): $m/z = [M+H]^+$ calcd for $C_{24}H_{25}N_4O_6^+$: 465.1769; found: 465.1765.
Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

5-(4-(4-((2-Carboxyethyl)carbamoyl)phenyl)-1H-1,2,3-triazol-1-yl)-2-hydroxybenzoic acid (CG_220)



$C_{19}H_{16}N_4O_6$

$M_w = 396.36 \text{ g/mol}$

Prepared according to **General Procedure D** from triazole **173** (30.0 mg, 0.0646 mmol) at room temperature. The solvents were evaporated *in vacuo*. To the crude product was added MeOH (10 mL) and the suspension filtered. The filtrate was concentrated *in vacuo* to give product **CG_220** (25.6 mg, 0.0646 mmol, quant.) as a white solid.

R_f: 0.10 (EtOAc/hexanes+AcOH 80:20+1).

m.p.: 295 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 12.22 (s, 2H, 1-COOH and 2'''-COOH), 9.41 (s, 1H, 5'-H), 8.62 (t, $J = 5.5 \text{ Hz}$, 1H, CONH), 8.30 (d, $J = 2.8 \text{ Hz}$, 1H, 6-H), 8.08 (dd, $J = 9.0, 2.8 \text{ Hz}$, 1H, 4-H), 8.03 (d, $J = 8.5 \text{ Hz}$, 2H, 2''-H and 6''-H), 7.96 (d, $J = 8.5 \text{ Hz}$, 2H, 3''-H and 5''-H), 7.22 (d, $J = 8.9 \text{ Hz}$, 1H, 3-H), 3.48 (td, $J = 7.1, 5.3 \text{ Hz}$, 2H, 1'''-H), 2.54 (t, $J = 7.1 \text{ Hz}$, 2H, 2'''-H).

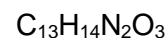
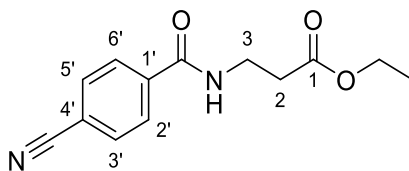
¹³C-NMR

(101 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3'''), 170.8 (1-COOH), 165.7 (CONH), 160.9 (C-2), 146.5 (C-4'), 133.8 (C-4''), 132.9 (C-1''), 128.5 (C-5), 127.9 (C-3'' and C-5''), 127.3 (C-4), 125.0 (C-2'' and C-6''), 121.6 (C-6), 120.5 (C-5'), 118.7 (C-3), 114.1 (C-1), 35.6 (C-1'''), 33.8 (C-2''').

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3310, 3076, 2921, 2852, 1677, 1633, 1621, 1536, 1447, 1294, 1219, 1199, 1186, 1040, 850, 829, 767, 694.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{19}H_{15}N_4O_6^-$: 395.0997; found: 395.0994.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-cyanobenzamido)propanoate (**175**)

$$M_w = 246.27 \text{ g/mol}$$

4-Cyanobenzoic acid (**174**, 368 mg, 2.50 mmol) was treated with solid CDI (422 mg, 2.60 mmol) in dry DMF (1.5 mL) at 0 °C. After the initial vigorous evolution of gas, the reaction mixture was allowed to warm to room temperature and stirred until all gas evolution ceased. Solid β -alanine ethyl ester hydrochloride (**76**, 388 mg, 2.52 mmol) was added, and the mixture was stirred until a clear solution resulted. Water (10 mL) was added, and the mixture extracted with EtOAc (3 x 15 mL). The combined organic phase was washed with 0.5 N aq. NaOH (15 mL) and brine (15 mL), dried using a phase separation paper and concentrated *in vacuo* to give product **175** (414 mg, 1.68 mmol, 67 %) as a white solid.

R_f: 0.25 (EtOAc/hexanes+AcOH 40:60+1).

m.p.: 82 °C.

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 7.88 – 7.85 (m, 2H, 2'-H and 6'-H), 7.74 – 7.72 (m, 2H, 3'-H and 5'-H), 7.00 (t, J = 5.3, 4.7 Hz, 1H, CONH), 4.18 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.73 (q, J = 6.0 Hz, 2H, 3-H), 2.65 (t, J = 5.9 Hz, 2H, 2-H), 1.28 (t, J = 7.1 Hz, 3H, CH₃).

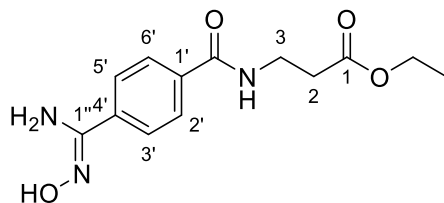
¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 173.2 (C-1), 165.6 (CONH), 138.4 (C-1'), 132.6 (C-3' and C-5'), 127.8 (C-2' and C-6'), 118.2 (CN), 115.3 (C-4'), 61.1 (CH₂CH₃), 35.6 (C-3), 33.8 (C-2), 14.3 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3344, 3298, 2980, 2230, 1727, 1637, 1543, 1324, 1278, 1192, 1150, 1090, 1026, 857.

HR-MS (EI): m/z = [M]⁺⁺ calcd for C₁₃H₁₄N₂O₃⁺⁺: 246.0999; found: 246.0998.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl (Z)-3-(4-(N'-hydroxycarbamimidoyl)benzamido)propanoate (176)C₁₃H₁₇N₃O₄M_w = 279.30 g/mol

To a stirred solution of nitrile **175** (300 mg, 1.22 mmol) in MeOH (3.0 mL), NaHCO₃ (113 mg, 1.34 mmol) and hydroxylamine hydrochloride (84.7 mg, 1.22 mmol) were added. The resulting reaction mixture was heated to reflux for 4 h. Then, the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give product **176** (339 mg, 1.21 mmol, quant.) as a white solid.

R_f: 0.30 (EtOAc/hexanes 90:10).

m.p.: 159 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): δ (ppm) = 9.79 (s, 1H, OH), 8.62 (t, *J* = 5.6 Hz, 1H, CONH), 7.84 – 7.80 (m, 2H, 2'-H and 6'-H), 7.76 – 7.73 (m, 2H, 3'-H and 5'-H), 5.89 (s, 2H, NH₂), 4.06 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 3.49 (td, *J* = 6.9, 5.4 Hz, 2H, 3-H), 2.58 (t, *J* = 7.0 Hz, 2H, 2-H), 1.17 (t, *J* = 7.1 Hz, 3H, CH₃).

¹³C-NMR

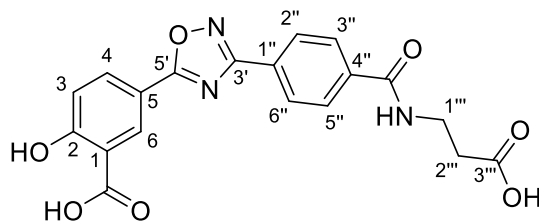
(101 MHz, (CD₃)₂SO): δ (ppm) = 171.3 (C-1), 165.8 (CONH), 150.2 (C-1''), 135.8 (C-4'), 134.4 (C-1'), 127.0 (C-2' and C-6'), 125.1 (C-3' and C-5'), 59.9 (CH₂CH₃), 35.5 (C-3), 33.8 (C-2), 14.1 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3442, 3299, 2983, 1653, 1623, 1539, 1472, 1372, 1323, 1300, 1267, 1205, 1021, 929, 856, 821, 776, 687.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₃H₁₆N₃O₄⁻: 278.1146; found: 278.1147.

Purity (HPLC): 210 nm: 92 %; 254 nm: 92 % (method 2a).

5-(3-(4-((2-Carboxyethyl)carbamoyl)phenyl)-1,2,4-oxadiazol-5-yl)-2-hydroxybenzoic acid (CG_264)



$C_{19}H_{15}N_3O_7$

$M_w = 397.34$ g/mol

Under N_2 atmosphere, carboxylic acid **98** (100 mg, 0.450 mmol) was dissolved in dry DMSO (0.50 mL). Solid CDI (79.6 mg, 0.491 mmol) was added, and the reaction mixture stirred at room temperature for 30 min. After this time, amidoxime **176** (114 mg, 0.409 mmol) was added. The reaction mixture was stirred at room temperature for another 18 h. Then, solid NaOH (19.6 mg, 0.491 mmol) was added rapidly. The mixture was stirred at room temperature for 2 h and afterwards diluted with cold water. The resulting precipitate was collected by filtration, washed with cold water (15 mL), and dried *in vacuo*. The crude product was purified by FCC (MeOH/DCM+AcOH 4:96+1 \rightarrow 7:93+1) to give 1,2,4-oxadiazole **CG_264** (46.6 mg, 0.117 mmol, 29 %) as a pink solid.

R_f: 0.31 (MeOH/DCM+AcOH 5:95+1).

m.p.: 248 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.25 (s, 1H, 1-COOH or 2'''-COOH), 8.74 (t, $J = 5.4$ Hz, 1H, CONH), 8.56 (d, $J = 2.3$ Hz, 1H, 6-H), 8.20 (dd, $J = 8.7, 2.3$ Hz, 1H, 4-H), 8.17 (dd, $J = 8.3, 1.7$ Hz, 2H, 2''-H and 6''-H), 8.04 – 8.01 (m, 2H, 3''-H and 5''-H), 7.12 (d, $J = 8.7$ Hz, 1H, 3-H), 3.49 (td, $J = 7.1, 5.4$ Hz, 2H, 1'''-H), 2.55 (t, $J = 7.1$ Hz, 2H, 2'''-H).

¹³C-NMR

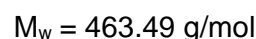
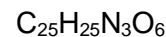
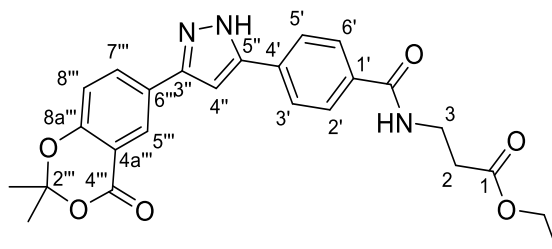
(126 MHz, (CD₃)₂SO): δ (ppm) = 175.2 (C-5'), 172.9 (C-3'''), 170.5 (1-COOH), 167.6 (C-3'), 166.0 (C-2), 165.5 (CONH), 136.9 (C-4''), 133.8 (C-4), 130.8 (C-6), 128.6 (C-3'' and C-5''), 127.1 (C-2'' and C-6''), 118.7 (C-3), 115.8 (C-5), 113.2 (C-1), 35.7 (C-1'''), 33.7 (C-2'''), C-1'' not visible.

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3307, 2925, 1688, 1632, 1548, 1471, 1411, 1354, 1302, 1247, 1206, 909, 863, 756, 720.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for $C_{19}H_{14}N_3O_7^-$: 396.0837; found: 396.0835.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

Ethyl 3-(4-(3-(2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)-1H-pyrazol-5-yl)benzamido)propanoate (177)



Method A:

To compound **179** (53.4 mg, 0.143 mmol; 0.5 eq) in MeCN (1.2 mL), 5 N aq. NaOH (28.5 μL) was added and the mixture was stirred for 20 min. Then the mixture was added to the dipolarophile **91** (70.0 mg, 0.285 mmol; 1.0 eq), and the reaction mixture was stirred at 50 °C for 6 h. Then again, a mixture of **179** (0.5 eq) in MeCN and 5 N aq. NaOH (stirred for 20 min) was added and the mixture heated to 50 °C for further 16 h. After this time, again a mixture of **179** (0.5 eq) in MeCN and 5 N aq. NaOH (stirred for 20 min) was added and the mixture stirred further at 50 °C for 16 h, now containing 1.5 eq of compound **179** in total. After cooling to room temperature, EtOAc (15 mL) was added and the organic phase was washed with 1 N aq. HCl (10 mL), sat. aq. NaHCO_3 (10 mL) and brine (10 mL). The organic phase was dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 60:40) to give pyrazole **177** (37.0 mg, 0.0798 mmol, 28 %) as a pale yellow solid.

Method B:

To a mixture of PdCl_2 (5.0 mol%, 3.87 mg, 0.0218 mmol), $\text{Cr}(\text{CO})_6$ (96.1 mg, 0.437 mmol), iodo compound **93** (133 mg, 0.437 mmol), and alkyne **91** (150 mg, 0.612 mmol) in DMF (1.5 mL) was added hydrazine monohydrate (31.8 μL , 0.655 mmol). The resulting mixture was heated to 70 °C for 4 h, then cooled to room temperature and quenched with water (10 mL). The mixture was extracted with EtOAc (3 x 10 mL), the organic phase was dried using a phase separation paper and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc/hexanes 65:35) to give pyrazole **177** (15.0 mg, 0.0324 mmol, 7 %) as a pale yellow solid.

R_f: 0.13 (EtOAc/hexanes 60:40).

m.p.: 197 °C.

¹H-NMR

(400 MHz, (CD₃)₂SO): due to tautomerisation NMR measured at 100 °C.

δ (ppm) = 13.26 (s, 1H, pyrazole-H), 8.32 (d, J = 2.2 Hz, 1H, 5'''-H), 8.23 (t, J = 5.1 Hz, 1H, CONH), 8.13 (d, J = 8.6 Hz, 1H, 7'''-H), 7.90 (s, 4H, 2', 3', 5'- and 6'-H), 7.21 (s, 1H, 4''-H), 7.19 (d, J = 8.6 Hz, 1H, 8'''-H), 4.12 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.56 (td, J = 7.0, 5.6 Hz, 2H, 3-H), 2.61 (t, J = 7.0 Hz, 2H, 2-H), 1.75 (s, 6H, C(CH₃)₂), 1.21 (t, J = 7.1 Hz, 3H, CH₂CH₃).

¹³C-NMR

(126 MHz, (CD₃)₂SO): due to tautomerisation NMR measured at 100 °C.

δ (ppm) = 170.7 (C-1), 165.6 (CONH), 159.4 (C-4'''), 154.6 (C-8a'''), 133.2 (C-7'''), 127.2 (C-3' and C-5'), 124.9 (C-5'''), 124.5 (C-2' and C-6'), 117.3 (C-8'''), 113.5 (C-6'''), 113.1 (C-4a'''), 106.1 (C(CH₃)₂), 99.9 (C-4''), 59.3 (CH₂CH₃), 35.2 (C-3), 33.6 (C-2), 25.0 (C(CH₃)₂), 13.5 (CH₂CH₃), at 100 °C C-3'' and C-5'' not visible.

IR (ATR):

$\tilde{\nu}$ (cm⁻¹) = 3315, 3238, 2927, 1742, 1702, 1628, 1542, 1494, 1277, 1259, 1183, 1139, 930, 796, 769.

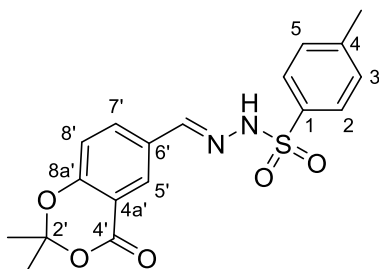
HR-MS (ESI):

m/z = [M+H]⁺ calcd for C₂₅H₂₆N₃O₆⁺: 464.1816; found: 464.1814.

Purity (HPLC):

210 nm: >95 %; 254 nm: >95 % (method 2a).

(E)-N'-((2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-6-yl)methylene)-4-methylbenzenesulfonohydrazide (179)



C₁₈H₁₈N₂O₅S

M_w = 374.41 g/mol

To a stirred solution of *p*-toluenesulfonyl hydrazide (**178**, 93.1 mg, 0.500 mmol) in MeOH (1.2 mL), aldehyde **50** (103 mg, 0.500 mmol) was added portionwise at room temperature. After 3 h, the reaction mixture was cooled to 0 °C and the product was collected by filtration, washed with a small quantity of cold MeOH (5.0 mL) and dried *in vacuo* to give the product **179** (153.3 mg, 0.409 mmol, 82 %) as a white solid.

R_f: 0.37 (EtOAc/hexanes 35:65).

m.p.: 199 °C.

¹H-NMR

(400 MHz, CDCl₃): δ (ppm) = 8.02 (d, *J* = 2.2 Hz, 1H, 5'-H), 7.92 (dd, *J* = 8.7, 2.2 Hz, 1H, 7'-H), 7.89 – 7.85 (m, 2H, 2-H and 6-H), 7.75 (s, 1H, 6'-CH), 7.33 (d, *J* = 8.1 Hz, 2H, 3-H and 5-H), 6.97 (d, *J* = 8.6 Hz, 1H, 8'-H), 2.41 (s, 3H, 4-CH₃), 1.73 (s, 6H, C(CH₃)₂).

¹³C-NMR

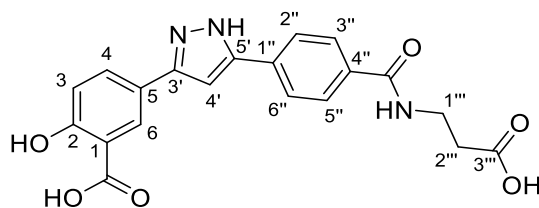
(101 MHz, CDCl₃): δ (ppm) = 160.6 (C-4'), 157.6 (C-8a'), 145.7 (6'-CH), 144.6 (C-4), 135.3 (C-1), 134.3 (C-7'), 129.9 (C-3 and C-5), 129.5 (C-5'), 128.4 (C-6'), 128.1 (C-2 and C-6), 118.2 (C-8'), 113.6 (C-4a'), 107.1 (C-2'), 26.0 (C(CH₃)₂), 21.8 (4-CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3247, 1716, 1623, 1498, 1362, 1286, 1217, 1163, 1067, 1051, 966, 932, 881, 809, 667.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₈H₁₉N₂O₅S⁺: 375.1009; found: 375.1007.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

**5-(5-(4-((2-Carboxyethyl)carbamoyl)phenyl)-1H-pyrazol-3-yl)-2-hydroxybenzoic acid
(CG_232)**



$C_{20}H_{17}N_3O_6$

$M_w = 395.37$ g/mol

Prepared according to **General Procedure D** from compound **173** (30.0 mg, 0.0646 mmol) at room temperature. The solvents were evaporated *in vacuo*. To the crude product water (10 mL) was added, the remaining solid collected by filtration and dried *in vacuo* to give pyrazole **CG_232** (21.3 mg, 0.0539 mmol, quant.) as a beige solid.

R_f: 0.10 (EtOAc/hexanes+AcOH 80:20+1).

m.p.: 288 °C.

¹H-NMR

(500 MHz, (CD₃)₂SO): δ (ppm) = 12.42 (s, 1H, 1-COOH or 2'''-COOH), 11.34 (s, 1H, pyrazol-NH), 8.57 (t, $J = 5.5$ Hz, 1H, CONH), 8.27 (d, $J = 2.3$ Hz, 1H, 6-H), 7.98 (dd, $J = 8.6, 2.3$ Hz, 1H, 4-H), 7.95 – 7.88 (m, 4H, 2''-, 3''-, 5''- and 6''-H), 7.26 (s, 1H, 4'-H), 7.07 (d, $J = 8.6$ Hz, 1H, 3-H), 3.48 (td, $J = 7.1, 5.4$ Hz, 2H, 1'''-H), 2.53 (t, $J = 7.2$ Hz, 2H, 2'''-H).

¹³C-NMR

(126 MHz, (CD₃)₂SO): δ (ppm) = 172.9 (C-3'''), 171.7 (1-COOH), 165.8 (CONH), 160.7 (C-2), 133.3 (C-1'' and C-4''), 132.5 (C-4), 127.8 (C-3'' and C-5''), 126.7 (C-6), 124.8 (C-2'' and C-6''), 122.7 (C-5), 117.8 (C-3), 113.3 (C-1), 99.8 (C-4'), 35.6 (C-1'''), 33.8 (C-2'''), C-3' and C-5' not visible.

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3391, 3141, 2923, 2503, 1722, 1666, 1598, 1568, 1546, 1489, 1301, 1181, 865, 858, 801, 770.

HR-MS (ESI): $m/z = [M-H]^-$ calcd for C₂₀H₁₆N₃O₆⁻: 394.1045; found: 394.1047.

Purity (HPLC): 210 nm: >95 %; 254 nm: >95 % (method 2a).

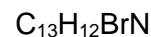
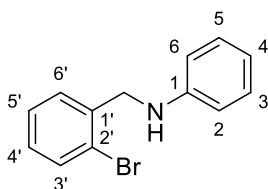
6.4.2. *N*-Aryl-1,2,3,4-tetrahydroisoquinolines

General Procedure 1 – Reductive amination

The appropriate 2-bromobenzaldehyde (1.2 equivalents) and the appropriate aniline (1.0 equivalents) were dissolved in MeOH to a concentration of 0.15 M (with respect to the aniline). A catalytic amount of AcOH was then added, and the reaction mixture stirred at room temperature for 16 h. NaCNBH₃ (3.0 equivalents) was added in portions at room temperature. After stirring for additional 2.5 – 3 h (TLC monitoring), water was added, and the mixture extracted with EtOAc (3 x). The organic phase was dried using a phase separation paper and the solvent was evaporated *in vacuo*. The crude product was then purified by FCC using the indicated eluent.

General Procedure 2 – Suzuki-Miyaura cross-coupling and subsequent cyclisation

The appropriate *N*-(2-bromobenzyl)aniline (1.0 equivalents), Pd(PPh₃)₄ (0.1 equivalents) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 2.0 equivalents) were dissolved in degassed 1,4-dioxane to a concentration of 0.15 M (with respect to the aniline) under N₂ atmosphere and stirred for 10 min at room temperature. Cs₂CO₃ (5.0 equivalents) was dissolved in degassed water to a concentration of 0.5 M (with respect to the aniline) under N₂ atmosphere and added to the reaction mixture. Stirring was then continued at 75 °C for 16 – 19 h. The reaction mixture was allowed to cool to room temperature. Sat. aq. NH₄Cl solution was added. The organic material was extracted with EtOAc (3 x) and the combined organic phases were dried using a phase separation paper. The solvent was removed *in vacuo*. The crude product was then dissolved in dry DCM to a concentration of 0.33 M (with respect to the aniline) under N₂ atmosphere. TFA (13 equivalents) and triethylsilane (2.5 equivalents) were added under N₂ atmosphere at room temperature in rapid succession. After completion of the reaction (2.5 – 4.5 h; TLC monitoring), 2 M aq. NaOH was added and the mixture was extracted with DCM (3 x). The combined organic extracts were dried using a phase separation paper. The solvent was removed *in vacuo* and the crude product was purified by FCC using the indicated eluent.

***N*-(2-Bromobenzyl)aniline (112a[#])**

M_w = 262.15 g/mol

Prepared according to **General Procedure 1** but with 2.0 equivalents of 2-bromobenzaldehyde (**111**, 1.85 g, 10.0 mmol) and aniline (**4**, 0.456 mL, 5.00 mmol). The crude product was purified by FCC (hexanes/EtOAc 95:5) to give product **112a[#]** (1.31 g, 5.00 mmol, quant.) as a white solid.

R_f: 0.25 (hexanes/EtOAc 95:5).

m.p.: 40 – 42 °C.

¹H-NMR

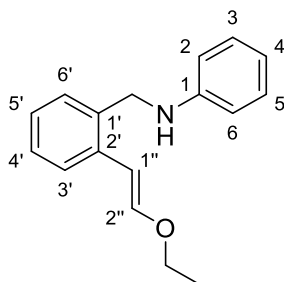
(400 MHz, CDCl₃): δ (ppm) = 7.56 (dd, *J* = 7.9, 1.3 Hz, 1H, 3'-H), 7.47 (dd, *J* = 7.7, 1.7 Hz, 1H, 6'-H), 7.25 (td, 1H, 5'-H), 7.22 – 7.16 (m, 2H, 2-H and 6-H), 7.13 (td, *J* = 7.6, 1.8 Hz, 1H, 4'-H), 6.82 – 6.76 (m, 1H, 4-H), 6.73 – 6.67 (m, 2H, 3-H and 5-H), 4.43 (s, 2H, 1'-CH₂).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 146.4 (C-1), 137.4 (C-1'), 133.0 (C-3'), 129.7 (C-6'), 129.5 (C-2 and C-6), 129.1 (C-4'), 127.7 (C-5'), 123.7 (C-2'), 119.1 (C-4), 114.3 (C-3 and C-5), 49.2 (1'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3381, 3049, 2837, 1601, 1504, 1464, 1433, 1319, 1253, 1178, 1100, 1067, 1043, 1023, 991, 748, 691, 658.

HR-MS (EI): *m/z* = [M+H]⁺ calcd for C₁₃H₁₃BrN⁺: 262.0226; found: 262.0227.

(E)-N-(2-(2-Ethoxyvinyl)benzyl)aniline (113a^{#*})C₁₇H₁₉NOM_w = 253.35 g/mol

Under N₂ atmosphere, compound **112a[#]** (131 mg, 0.500 mmol), Pd(PPh₃)₄ (57.8 mg, 0.0500 mmol; 10 mol%) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 212 μL, 1.00 mmol) were dissolved in degassed 1,4-dioxane (3.0 mL) and stirred for 10 min at room temperature. Under N₂ atmosphere Cs₂CO₃ (815 mg, 2.50 mmol) was dissolved in degassed water (1.0 mL) and added to the reaction mixture. Stirring was then continued at 75 °C for 19 h. The reaction mixture was allowed to cool to room temperature. Sat. aq. NH₄Cl (10.0 mL) was then added. The organic material was extracted with EtOAc (3 x 15.0 mL) and the combined organic phases were dried using a phase separation paper. The solvent was removed *in vacuo* and the crude product was purified by FCC (hexanes/EtOAc 95:5) to give enol ether **113a^{#*}** (63.4 mg, 0.250 mmol, 50 %) as a yellow oil.

R_f: 0.48 (hexanes/EtOAc 95:5).

¹H-NMR

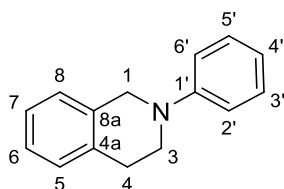
(400 MHz, CDCl₃): δ (ppm) = 7.34 (ddd, *J* = 9.3, 7.6, 1.4 Hz, 2H, 3'-H and 6'-H), 7.25 – 7.21 (m, 1H, 4'-H), 7.22 – 7.18 (m, 2H, 3-H and 5-H), 7.16 (td, *J* = 7.5, 1.4 Hz, 1H, 5'-H), 6.89 (d, *J* = 12.8 Hz, 1H, 2''-H), 6.73 (tt, *J* = 7.3, 1.1 Hz, 1H, 4-H), 6.67 – 6.63 (m, 2H, 2-H and 6-H), 6.05 (d, *J* = 12.8 Hz, 1H, 1''-H), 4.28 (s, 2H, 1'-CH₂), 3.85 (q, *J* = 7.0 Hz, 2H, CH₂CH₃), 1.31 (t, *J* = 7.0 Hz, 3H, CH₃).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 149.4 (C-2''), 148.4 (C-1), 135.6 (C-2'), 135.3 (C-1'), 129.4 (C-3 and C-5), 129.2 (C-6'), 127.9 (C-4'), 126.3 (C-5'), 125.5 (C-3'), 117.6 (C-4), 112.9 (C-2 and C-6), 103.0 (C-1''), 65.8 (CH₂CH₃), 46.9 (1'-CH₂), 14.9 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3430, 3061, 2976, 1631, 1599, 1507, 1327, 1158, 930, 747, 691.

HR-MS (ESI): *m/z* = [M+H]⁺ calcd for C₁₇H₂₀NO⁺: 254.1539; found: 254.1536.

2-Phenyl-1,2,3,4-tetrahydroisoquinoline (114a^{#*})C₁₅H₁₅NM_w = 209.29 g/mol

Prepared according to **General Procedure 2** from *N*-(2-bromo-benzyl)aniline (**112a[#]**, 131 mg, 0.500 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 212 μL, 1.00 mmol). The crude product was purified by FCC (hexanes/EtOAc 95:5) to give product **114a^{#*}** (35.6 mg, 0.169 mmol, 34 %) as a dark yellow-orange oily solid.

R_f: 0.38 (hexanes/EtOAc 95:5).

¹H-NMR

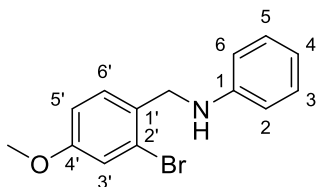
(500 MHz, CDCl₃): δ (ppm) = 7.32 – 7.27 (m, 2H, 3'-H and 5'-H), 7.21 – 7.15 (m, 4H, 5-H, 6-H, 7-H and 8-H), 7.01 – 6.97 (m, 2H, 2'-H and 6'-H), 6.84 (tt, J = 7.3, 1.1 Hz, 1H, 4'-H), 4.42 (s, 2H, 1-H), 3.57 (t, J = 5.8 Hz, 2H, 3-H), 3.00 (t, J = 5.9 Hz, 2H, 4-H).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 150.7 (C-1'), 135.0 (C-4a or C-8a), 134.6 (C-4a or C-8a), 129.3 (C-3' and C-5'), 128.7 (C-5), 126.7 (C-8), 126.5 (C-6 or C-7), 126.2 (C-6 or C-7), 118.8 (C-4'), 115.3 (C-2' and C-6'), 50.9 (C-1), 46.7 (C-3), 29.3 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3023, 2922, 2821, 1661, 1598, 1498, 1460, 1387, 1293, 1212, 1151, 1112, 1034, 989, 935, 741, 690.

HR-MS (ESI): m/z = [M-H]⁻ calcd for C₁₅H₁₄N⁻: 208.1126; found: 208.1120.

N-(2-Bromo-4-methoxybenzyl)aniline (112b[#])C₁₄H₁₄BrNOM_w = 292.18 g/mol

Prepared according to **General Procedure 1** from 2-bromo-4-methoxybenzaldehyde (**111b**, 129 mg, 0.600 mmol) and aniline (**4**, 45.6 μ L, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 95:5) to give product **112b[#]** (123 mg, 0.422 mmol, 85 %) as a pale yellow solid.

R_f: 0.25 (hexanes/EtOAc 95:5).

m.p.: 69 – 73 °C.

¹H-NMR

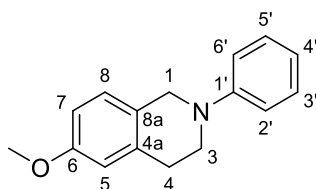
(400 MHz, CDCl₃): δ (ppm) = 7.37 (d, J = 8.5 Hz, 1H, 6'-H), 7.22 – 7.15 (m, 2H, 3-H and 5-H), 7.11 (d, J = 2.6 Hz, 1H, 3'-H), 6.83 – 6.77 (m, 2H, 4-H and 5'-H), 6.72 (d, J = 7.7 Hz, 2H, 2-H and 6-H), 4.37 (s, 2H, 1'-CH₂), 3.78 (s, 3H, 4'-OCH₃).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 159.6 (C-4'), 146.2 (C-1), 130.6 (C-6'), 129.5 (C-3 and C-5), 129.1 (C-1'), 124.1 (C-2'), 119.4 (C-4 and C-5'), 118.3 (C-3'), 114.6 (C-2 or C-6), 113.7 (C-2 or C-6), 55.7 (4'-OCH₃), 48.8 (1'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3439, 3062, 2937, 2832, 1601, 1567, 1507, 1486, 1456, 1435, 1319, 1270, 1229, 1176, 1028, 988, 879, 809, 749, 688.

HR-MS (EI): m/z = [M]⁺ calcd for C₁₄H₁₄BrNO⁺: 291.0253; found: 291.0253.

6-Methoxy-2-phenyl-1,2,3,4-tetrahydroisoquinoline (114b[#])C₁₆H₁₇NOM_w = 239.32 g/mol

Prepared according to **General Procedure 2** from *N*-(2-bromo-4-methoxybenzyl)aniline (**112b[#]**, 73.0 mg, 0.250 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 106 μL, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 92:8) to give product **114b[#]** (28.6 mg, 0.120 mmol, 48 %) as a yellow solid.

R_f: 0.32 (hexanes/EtOAc 92:8).

m.p.: 39 – 44 °C.

¹H-NMR

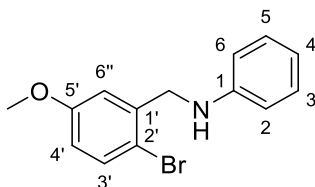
(500 MHz, CDCl₃): δ (ppm) = 7.32 – 7.27 (m, 2H, 3'-H and 5'-H), 7.08 (d, *J* = 8.4 Hz, 1H, 8-H), 7.01 (d, *J* = 8.0 Hz, 2H, 2'-H and 6'-H), 6.85 (t, *J* = 6.9 Hz, 1H, 4'-H), 6.77 (dd, *J* = 8.4, 2.7 Hz, 1H, 7-H), 6.70 (d, *J* = 2.6 Hz, 1H, 5-H), 4.37 (s, 2H, 1-H), 3.80 (s, 3H, 6-OCH₃), 3.56 (t, *J* = 5.9 Hz, 2H, 3-H), 2.97 (t, *J* = 5.9 Hz, 2H, 4-H).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 158.2 (C-6), 150.7 (C-1'), 136.2 (C-4a), 129.3 (C-3' and C-5'), 127.6 (C-8), 126.8 (C-8a), 118.8 (C-4'), 115.3 (C-2' and C-6'), 113.3 (C-5), 112.5 (C-7), 55.4 (6-OCH₃), 50.4 (C-1), 46.6 (C-3), 29.5 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3060, 2933, 2833, 1731, 1597, 1504, 1460, 1388, 1331, 1275, 1227, 1194, 1152, 1032, 913, 849, 805, 751, 690.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₆H₁₆NO⁻: 238.1232; found: 238.1227.

N-(2-Bromo-5-methoxybenzyl)aniline (112c#) $C_{14}H_{14}BrNO$ $M_w = 292.18 \text{ g/mol}$

Prepared according to **General Procedure 1** from 2-bromo-5-methoxybenzaldehyde (**111c**, 129 mg, 0.600 mmol) and aniline (**4**, 45.6 μL , 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 95:5) to give product **112c#** (108 mg, 0.370 mmol, 74 %) as a yellow oil.

R_f: 0.33 (hexanes/EtOAc 95:5).

¹H-NMR

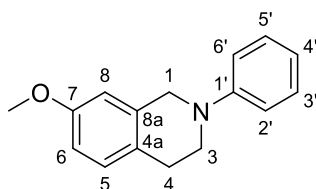
(400 MHz, CDCl₃): δ (ppm) = 7.43 (d, $J = 8.8 \text{ Hz}$, 1H, 3'-H), 7.22 – 7.16 (m, 2H, 3-H and 5-H), 7.05 (d, $J = 2.9 \text{ Hz}$, 1H, 6'-H), 6.78 (t, $J = 7.3, 1.1 \text{ Hz}$, 1H, 4-H), 6.73 – 6.66 (m, 3H 2-H, 6-H and 4'-H), 4.38 (s, 2H, 1'-CH₂), 3.74 (s, 3H, 5'-OCH₃).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 159.4 (C-5'), 146.6 (C-1), 138.6 (C-1'), 133.5 (C-3'), 129.5 (C-3 and C-5), 119.1 (C-4), 115.3 (C-6'), 114.7 (C-4'), 114.2 (C-2 and C-6), 113.8 (C-2'), 55.6 (5'-OCH₃), 49.3 (1'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3418, 3048, 3003, 2932, 2836, 1600, 1504, 1466, 1432, 1294, 1265, 1240, 1159, 1053, 1015, 868, 803, 747, 691, 600.

HR-MS (EI): $m/z = [M]^{+\cdot}$ calcd for C₁₄H₁₄BrNO^{+\cdot}: 291.0253; found: 291.0253.

7-Methoxy-2-phenyl-1,2,3,4-tetrahydroisoquinoline (114c[#])C₁₆H₁₇NOM_w = 239.32 g/mol

Prepared according to **General Procedure 2** from *N*-(2-bromo-5-methoxybenzyl)aniline (**112c[#]**, 73.0 mg, 0.250 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 106 μL, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 95:5) to give product **114c[#]** (29.0 mg, 0.121 mmol, 49 %) as a colourless oil.

R_f: 0.27 (hexanes/EtOAc 95:5).

¹H-NMR

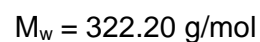
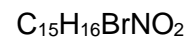
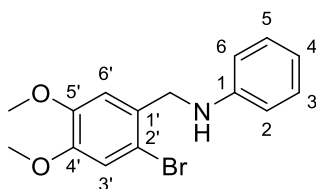
(400 MHz, CDCl₃): δ (ppm) = 7.33 – 7.27 (m, 2H, 3'-H and 5'-H), 7.08 (d, *J* = 8.3 Hz, 1H, 5-H), 6.99 (dd, *J* = 8.8, 1.1 Hz, 2H, 2'-H and 6'-H), 6.87 – 6.81 (m, 1H, 4'-H), 6.76 (dd, *J* = 8.4, 2.7 Hz, 1H, 6-H), 6.71 (d, *J* = 2.7 Hz, 1H, 8-H), 4.39 (s, 2H, 1-H), 3.81 (s, 3H, 7-OCH₃), 3.56 (t, *J* = 5.8 Hz, 2H, 3-H), 2.92 (t, *J* = 5.8 Hz, 2H, 4-H).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 158.0 (C-7), 150.7 (C-1'), 135.7 (C-8a), 129.6 (C-5), 129.3 (C-3' and C-5'), 127.1 (C-4a), 118.8 (C-4'), 115.4 (C-2' and C-6'), 112.7 (C-6), 111.4 (C-8), 55.5 (7-OCH₃), 51.1 (C-1), 47.0 (C-3), 28.3 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3023, 2907, 2832, 1598, 1501, 1461, 1382, 1319, 1253, 1234, 1147, 1119, 1038, 932, 813, 748, 691.

HR-MS (ESI): *m/z* = [M-H]⁻ calcd for C₁₆H₁₆NO⁻: 238.1232; found: 238.1226.

***N*-(2-Bromo-4,5-dimethoxybenzyl)aniline (112d[#])**

Prepared according to **General Procedure 1** from 2-bromo-4,5-dimethoxybenzaldehyde (**111d**, 147 mg, 0.600 mmol) and aniline (**4**; 45.6 μ L, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 80:20) to give product **112d[#]** (126 mg, 0.392 mmol, 78 %) as a yellow solid.

R_f: 0.36 (hexanes/EtOAc 80:20).

m.p.: 81 – 83 °C.

¹H-NMR

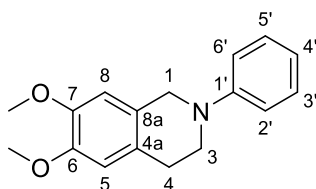
(500 MHz, CDCl₃): δ (ppm) = 7.21 – 7.16 (m, 2H, 3-H and 5-H), 7.04 (s, 1H, 3'-H), 6.97 (s, 1H, 6'-H), 6.78 – 6.73 (m, 1H, 4-H), 6.68 – 6.63 (m, 2H, 2-H and 6-H), 4.33 (s, 2H, 1'-CH₂), 3.87 (s, 3H, 4'-OCH₃ or 5'-OCH₃), 3.79 (s, 3H, 4'-OCH₃ or 5'-OCH₃).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 148.8 (C-4' or C-5'), 148.7 (C-4' or C-5'), 147.7 (C-1), 130.2 (C-1'), 129.4 (C-3 and C-5), 118.4 (C-4), 115.7 (C-3'), 113.5 (C-2 and C-6), 113.3 (C-2'), 112.4 (C-6'), 56.4 (4'-OCH₃ or 5'-OCH₃), 56.2 (4'-OCH₃ or 5'-OCH₃), 48.7 (1'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3378, 2929, 2837, 1600, 1499, 1434, 1388, 1312, 1255, 1223, 1164, 1099, 1027, 958, 851, 797, 754, 694.

HR-MS (ESI): m/z = [M]⁺ calcd for C₁₅H₁₅BrNO₂⁺: 321.0359; found: 321.0358.

6,7-Dimethoxy-2-phenyl-1,2,3,4-tetrahydroisoquinoline (114d[#])C₁₇H₁₉NO₂M_w = 269.34 g/mol

Prepared according to **General Procedure 2** from *N*-(2-bromo-4,5-dimethoxybenzyl)aniline (**112d[#]**, 80.6 mg, 0.250 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 106 μL, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 75:25) to give product **114d[#]** (36.7 mg, 0.136 mmol, 55 %) as a yellow solid.

R_f: 0.45 (hexanes/EtOAc 75:25).

m.p.: 86 – 90 °C.

¹H-NMR

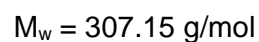
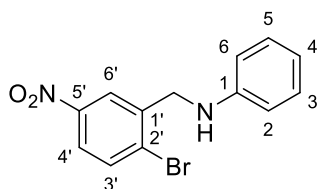
(400 MHz, CDCl₃): δ (ppm) = 7.33 – 7.27 (m, 2H, 3'-H and 5'-H), 7.03 (d, *J* = 7.9 Hz, 2H, 2'-H and 6'-H), 6.87 (t, *J* = 7.6 Hz, 1H, 4'-H), 6.65 (s, 1H, 5-H or 8-H), 6.65 (s, 1H, 5-H or 8-H), 4.35 (s, 2H, 1-H), 3.88 (s, 3H, 6-OCH₃ or 7-OCH₃), 3.87 (s, 3H, 6-OCH₃ or 7-OCH₃), 3.56 (t, *J* = 5.8 Hz, 2H, 3-H), 2.91 (t, 2H, 4-H).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 149.6 (C-1'), 147.9 (C-6 or C-7), 147.8 (C-6 or C-7), 129.4 (C-3' and C-5'), 126.5 (C-4a and C-8a), 122.2 (C-4'), 115.9 (C-2' and C-6'), 111.5 (C-5), 109.5 (C-8), 56.13 (6-OCH₃ or 7-OCH₃), 56.08 (6-OCH₃ or 7-OCH₃), 51.0 (C-1) 47.3 (C-3), 28.3 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2920, 2805, 2684, 1599, 1516, 1462, 1450, 1377, 1252, 1209, 1114, 1025, 979, 932, 855, 825, 750, 731, 693.

HR-MS (ESI): *m/z* = [M-H]⁺ calcd for C₁₇H₁₈NO₂⁺: 268.1338; found: 268.1332.

***N*-(2-Bromo-5-nitrobenzyl)aniline (112e[#])**

Prepared according to **General Procedure 1** from 2-bromo-5-nitrobenzaldehyde (**111e**, 138 mg, 0.600 mmol) and aniline (**4**, 45.6 μL, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 80:20) to give product **112e[#]** (135 mg, 0.440 mmol, 88 %) as a yellow solid.

R_f: 0.50 (hexanes/EtOAc 80:20).

m.p.: 123 – 128 °C.

¹H-NMR

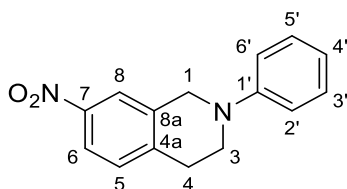
(400 MHz, CDCl₃): δ (ppm) = 8.28 (d, *J* = 2.8 Hz, 1H, 6'-H), 7.99 (dd, *J* = 8.7, 2.8 Hz, 1H, 4'-H), 7.76 (d, *J* = 8.6 Hz, 1H, 3'-H), 7.22 – 7.14 (m, 2H, 3-H and 5-H), 6.80 – 6.73 (m, 1H, 4-H), 6.61 – 6.56 (m, 2H, 2-H and 6-H), 4.48 (s, 2H, 1'-CH₂).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 147.8 (C-5'), 147.0 (C-1), 141.0 (C-1'), 133.9 (C-3'), 130.1 (C-2'), 129.6 (C-3 and C-5), 123.7 (C-6'), 123.4 (C-4'), 118.7 (C-4), 113.1 (C-2 and C-6), 48.5 (1'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3398, 3089, 2929, 2857, 1602, 1573, 1525, 1493, 1340, 1270, 1239, 1108, 1027, 987, 917, 872, 806, 749, 737, 692.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₃H₁₁BrN₂O₂⁺: 305.9998; found: 305.9999.

7-Nitro-2-phenyl-1,2,3,4-tetrahydroisoquinoline (**114e**[#])C₁₅H₁₄N₂O₂M_w = 254.29 g/mol

Prepared according to **General Procedure 2** from *N*-(2-bromo-5-nitrobenzyl)aniline (**112e**[#], 76.8 mg, 0.250 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 106 μL, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 85:15) to give product **114e**[#] (39.9 mg, 0.157 mmol, 63 %) as an orange solid.

R_f: 0.50 (hexanes/EtOAc 85:15).

m.p.: 66 – 70 °C.

¹H-NMR

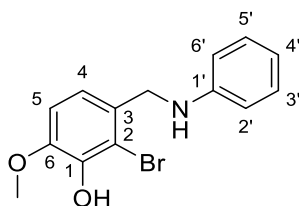
(400 MHz, CDCl₃): δ (ppm) = 8.07 – 8.02 (m, 2H, 6-H and 8-H), 7.34 – 7.28 (m, 3H, 3'-H, 5'-H and 5-H), 7.02 – 6.98 (m, 2H, 2'-H and 6'-H), 6.89 (tt, *J* = 7.3, 1.1 Hz, 1H, 4'-H), 4.48 (s, 2H, 1-H), 3.61 (t, *J* = 5.8 Hz, 2H, 3-H), 3.08 (t, *J* = 5.8 Hz, 2H, 4-H)

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 150.1 (C-1'), 146.5 (C-7), 142.8 (C-4a), 136.2 (C-8a), 129.8 (C-5), 129.5 (C-3' and C-5'), 121.9 (C-6 or C-8), 121.5 (C-6 or C-8), 119.8 (C-4'), 115.9 (C-2' and C-6'), 51.0 (C-1), 46.6 (C-3), 29.3 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2920, 2846, 2774, 1599, 1514, 1493, 1460, 1378, 1340, 1262, 1194, 1086, 931, 891, 813, 756, 733, 695.

HR-MS (ESI): *m/z* = [M-H]⁺ calcd for C₁₅H₁₃NO₂⁺: 253.0977; found: 253.0971.

2-Bromo-6-methoxy-3-((phenylamino)methyl)phenol (112f)C₁₄H₁₄BrNO₂M_w = 308.18 g/mol

Prepared according to **General Procedure 1** from 2-bromo-3-hydroxy-4-methoxybenzaldehyde (**111f**, 139 mg, 0.600 mmol) and aniline (**4**, 45.6 μ L, 0.500 mmol). The crude product was purified by FCC (DCM/hexanes 0:100 \rightarrow 65:35) to give product **112f** (137 mg, 0.445 mmol, 89 %) as a beige solid.

R_f: 0.23 (DCM/hexanes 65:35).

m.p.: 86 – 88 °C.

¹H-NMR

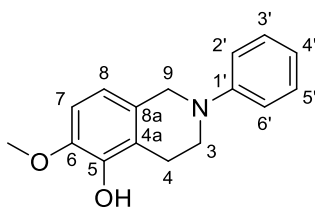
(400 MHz, CDCl₃): δ (ppm) = 7.21 – 7.15 (m, 2H, 3'-H and 5'-H), 6.98 (d, J = 8.4 Hz, 1H, 4-H), 6.79 – 6.74 (m, 2H, 4'-H and 5-H), 6.68 (d, J = 7.8 Hz, 2H, 2'-H and 6'-H), 4.38 (s, 2H, CH₂), 3.88 (s, 3H, OCH₃).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 146.8 (C-1'), 146.5 (C-6), 143.4 (C-1), 130.4 (C-3), 129.4 (C-3' and C-5'), 120.3 (C-4), 118.8 (C-4'), 114.0 (C-2 and C-6), 109.8 (C-2), 109.6 (C-5), 56.5 (OCH₃), 48.8 (CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3424, 2840, 1599, 1487, 1435, 1332, 1276, 1232, 1193, 1139, 1030, 987, 947, 810, 741, 689.

HR-MS (EI): m/z = [M]⁺ calcd for C₁₄H₁₄BrNO₂⁺: 307.0202; found: 307.0201.

6-Methoxy-2-phenyl-1,2,3,4-tetrahydroisoquinolin-5-ol (114f)C₁₆H₁₇NO₂M_w = 255.31 g/mol

Prepared according to **General Procedure 2** from compound **112f** (116 mg, 0.376 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 159 μL, 0.753 mmol). The crude product was purified by FCC (DCM/hexanes 0:100 → 70:30) to give product **114f** (28.0 mg, 0.110 mmol, 29 %) as a yellow solid.

R_f: 0.26 (DCM/hexanes 70:30).

m.p.: 84 – 86 °C.

¹H-NMR

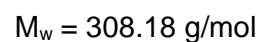
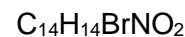
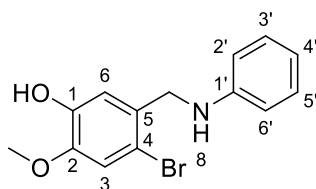
(400 MHz, CDCl₃): δ (ppm) = 7.32 – 7.27 (m, 2H, 3'-H and 5'-H), 7.03 (d, *J* = 8.1 Hz, 2H, 2'-H and 6'-H), 6.86 (t, *J* = 7.3 Hz, 1H, 4'-H), 6.75 (d, *J* = 8.3 Hz, 1H, 7-H), 6.68 (d, *J* = 8.3 Hz, 1H, 8-H), 5.70 (s, 1H, OH), 4.36 (s, 2H, 9-H), 3.88 (s, 3H, OCH₃), 3.57 (t, *J* = 6.0 Hz, 2H, 3-H), 2.95 (t, *J* = 6.0 Hz, 2H, 4-H)

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 144.6 (C-6), 143.0 (C-5), 129.3 (C-3' and C-5'), 127.9 (C-8a), 121.5 (C-4a), 119.4 (C-4'), 117.3 (C-8), 116.1 (C-2' and C-6'), 108.8 (C-7), 56.3 (OCH₃), 50.9 (C-9), 47.0 (C-3), 22.9 (C-4), C-1' not visible.

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3457, 2923, 2839, 1598, 1500, 1445, 1392, 1342, 1281, 1228, 1196, 1090, 1043, 1006, 875, 793, 747, 689.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₆H₁₇NO₂⁺: 255.1254; found: 255.1260.

4-Bromo-2-methoxy-5-((phenylamino)methyl)phenol (112g)

Prepared according to **General Procedure 1** from 2-bromo-5-hydroxy-4-methoxybenzaldehyde (**111g**, 139 mg, 0.600 mmol) and aniline (**4**, 45.6 μ L, 0.500 mmol). The crude product was purified by FCC (DCM/hexanes 0:100 \rightarrow 70:30) to give product **112g** (141 mg, 0.458 mmol, 92 %) as a brownish solid.

R_f: 0.20 (DCM/hexanes 70:30).

m.p.: 62 – 63 °C.

¹H-NMR

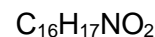
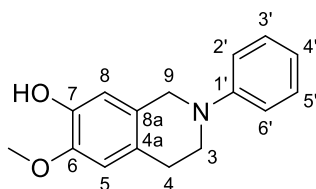
(400 MHz, CDCl₃): δ (ppm) = 7.21 – 7.15 (m, 2H, 3'-H and 5'-H), 7.05 (s, 1H, 6-H), 7.02 (s, 1H, 3-H), 6.77 (tt, J = 7.2, 0.8 Hz, 1H, 4'-H), 6.71 – 6.66 (m, 2H, 2'-H and 6'-H), 4.31 (s, 2H, CH₂), 3.87 (s, 3H, OCH₃).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 146.62 (C-2), 146.56 (C-1'), 145.3 (C-1), 130.4 (C-5), 129.4 (C-3' and C-5'), 118.9 (C-4'), 115.7 (C-6), 115.2 (C-3), 114.1 (C-2' and C-6'), 112.5 (C-4), 56.4 (OCH₃), 48.7 (CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3475, 2922, 1602, 1497, 1434, 1328, 1269, 1197, 1146, 1034, 861, 822, 797, 745, 692.

HR-MS (EI): m/z = [M]⁺ calcd for C₁₄H₁₄BrNO₂⁺: 307.0202; found: 307.0200.

6-Methoxy-2-phenyl-1,2,3,4-tetrahydroisoquinolin-7-ol (114g)

M_w = 255.31 g/mol

Prepared according to **General Procedure 2** from compound **112g** (77.0 mg, 0.250 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 106 μL, 0.500 mmol). The crude product was purified by FCC (DCM/hexanes 0:100 → 70:30) to give product **114g** (36.0 mg, 0.141 mmol, 56 %) as a brownish solid.

R_f: 0.26 (DCM/hexanes 70:30).

m.p.: 96 – 97 °C.

¹H-NMR

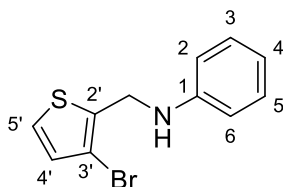
(400 MHz, CDCl₃): δ (ppm) = 7.32 – 7.27 (m, 2H, 3'-H and 5'-H), 7.02 (d, *J* = 8.1 Hz, 2H, 2'-H and 6'-H), 6.86 (t, *J* = 7.3, 6.7 Hz, 1H, 4'-H), 6.72 (s, 1H, 8-H), 6.62 (s, 1H, 5-H), 5.54 (s, 1H, OH), 4.32 (s, 2H, 9-H), 3.87 (s, 3H, OCH₃), 3.55 (t, *J* = 5.9 Hz, 2H, 3-H), 2.90 (t, *J* = 5.9 Hz, 2H, 4-H).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 150.5 (C-1'), 145.2 (C-6), 144.0 (C-7), 129.2 (C-3' and C-5'), 127.0 (C-4a or C-8a), 126.1 (C-4a or C-8a), 118.8 (C-4'), 115.4 (C-2' and C-6'), 112.2 (C-8), 110.6 (C-5), 56.0 (OCH₃), 50.4 (C-9), 46.9 (C-3), 28.6 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3231, 2942, 2825, 1595, 1529, 1501, 1458, 1376, 1274, 1219, 1203, 1110, 1025, 924, 857, 828, 758, 684.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₆H₁₇NO₂⁺: 255.1254; found: 255.1248.

***N*-((3-Bromothiophen-2-yl)methyl)aniline (112h)**C₁₁H₁₀BrNSM_w = 268.17 g/mol

Prepared according to **General Procedure 1** from 3-bromothiophene-2-carboxaldehyde (**111h**, 115 mg, 0.600 mmol) and aniline (**4**, 45.6 μ L, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 95:5) to give product **112h** (117 mg, 0.438 mmol, 88 %) as a yellow liquid.

R_f: 0.47 (hexanes/EtOAc 95:5).

¹H-NMR

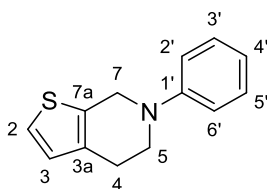
(500 MHz, CDCl₃): δ (ppm) = 7.22 – 7.17 (m, 3H, 3-H and 5-H and 5'-H), 6.96 (d, J = 5.3 Hz, 1H, 4'-H), 6.76 (tt, J = 7.4, 1.1 Hz, 1H, 4-H), 6.70 – 6.66 (m, 2H, 2-H and 6-H), 4.48 (d, J = 5.0 Hz, 2H, 2'-CH₂), 4.14 (s, 1H, NH).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 147.4 (C-1), 138.0 (C-2'), 130.2 (C-4'), 129.4 (C-3 and C-5), 124.8 (C-5'), 118.6 (C-4), 113.5 (C-2 and C-6), 108.6 (C-3'), 43.1 (2'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3410, 1600, 1501, 1312, 1259, 1180, 1152, 1095, 1067, 923, 869, 748, 690.

HR-MS (EI): m/z = [M]⁺⁺ calcd for C₁₁H₁₀BrNS⁺⁺: 266.9712; found: 266.9712.

6-Phenyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine (114h) $C_{13}H_{13}NS$ $M_w = 215.31 \text{ g/mol}$

Prepared according to **General Procedure 2** from compound **112h** (53.6 mg, 0.200 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 84.7 μL , 0.400 mmol). The crude product was purified by FCC (hexanes/EtOAc 95:5) to give product **114h** (16.4 mg, 0.0762 mmol, 38 %) as a yellow oil.

R_f: 0.26 (DCM/hexanes 25:75).

¹H-NMR

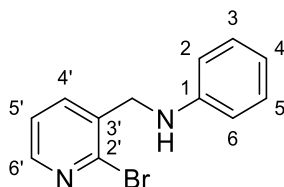
(500 MHz, CDCl₃): δ (ppm) = 7.30 – 7.25 (m, 2H, 3'-H and 5'-H), 7.14 (dt, $J = 5.0, 0.8 \text{ Hz}$, 1H, 2-H), 7.02 – 6.98 (m, 2H, 2'-H and 6'-H), 6.86 (tt, $J = 7.3, 1.1 \text{ Hz}$, 1H, 4'-H), 6.81 (d, $J = 5.0 \text{ Hz}$, 1H, 3-H), 4.47 – 4.45 (m, 2H, 7-H), 3.62 (t, $J = 5.7 \text{ Hz}$, 2H, 5-H), 2.82 (tt, $J = 5.8, 1.7 \text{ Hz}$, 2H, 4-H).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 150.6 (C-1'), 134.3 (C-3a), 132.9 (C-7a), 129.4 (C-3' and C-5'), 127.2 (C-3), 122.7 (C-2), 119.6 (C-4'), 116.4 (C-2' and C-6'), 48.3 (C-7), 47.6 (C-5), 25.5 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3318, 2920, 1598, 1497, 1381, 1317, 1244, 1228, 1183, 1127, 994, 886, 750, 690.

HR-MS (EI): $m/z = [M]^{+\bullet}$ calcd for C₁₃H₁₃NS⁺: 215.0763; found: 215.0763.

***N*-(2-Bromopyridin-3-yl)methylaniline (112i)**C₁₂H₁₁BrN₂M_w = 263.14 g/mol

Prepared according to **General Procedure 1** from 2-bromo-3-pyridinecarboxaldehyde (**111i**, 112 mg, 0.600 mmol) and aniline (**4**, 45.6 μL, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 85:15) to give product **112i** (121 mg, 0.460 mmol, 92 %) as a beige solid.

R_f: 0.26 (hexanes/EtOAc 85:15).

m.p.: 107 – 109 °C.

¹H-NMR

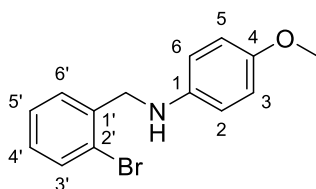
(500 MHz, CDCl₃): δ (ppm) = 8.27 (dt, *J* = 4.7, 1.3 Hz, 1H, 6'-H), 7.69 (ddt, *J* = 7.6, 1.9, 0.9 Hz, 1H, 4'-H), 7.22 (dd, *J* = 7.6, 4.7 Hz, 1H, 5'-H), 7.20 – 7.15 (m, 2H, 3-H and 5-H), 6.75 (tt, *J* = 7.3, 1.1 Hz, 1H, 4-H), 6.59 – 6.55 (m, 2H, 2-H and 6-H), 4.41 (d, *J* = 4.6 Hz, 2H, 3'-CH₂), 4.29 (s, 1H, NH).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 148.7 (C-6'), 147.2 (C-1), 142.8 (C-2'), 137.2 (C-4'), 135.8 (C-3'), 129.6 (C-3 and C-5), 123.2 (C-5'), 118.4 (C-4), 113.1 (C-2 and C-6), 47.5 (3'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3295, 2921, 1600, 1560, 1531, 1496, 1398, 1324, 1276, 1254, 1178, 1051, 987, 860, 800, 745, 689.

HR-MS (EI): *m/z* = [M]⁺⁺ calcd for C₁₂H₁₁BrN₂⁺⁺: 262.0100; found: 262.0101.

***N*-(2-Bromobenzyl)-4-methoxyaniline (112j[#])**C₁₄H₁₄BrNOM_w = 292.18 g/mol

Prepared according to **General Procedure 1** from 2-bromobenzaldehyde (**111**, 111 mg, 0.600 mmol) and *p*-anisidine (**4j**, 62.2 mg, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 90:10) to give product **112j[#]** (108 mg, 0.369 mmol, 74 %) as a dark yellow liquid.

R_f: 0.39 (hexanes/EtOAc 90:10).

¹H-NMR

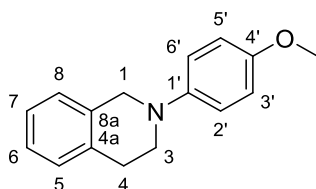
(400 MHz, CDCl₃): δ (ppm) = 7.56 (dd, *J* = 8.0, 1.3 Hz, 1H, 3'-H), 7.44 (dd, 1H, 6'-H), 7.29 – 7.23 (m, 1H, 5'-H), 7.13 (td, 1H, 4'-H), 6.80 – 6.60 (m, 4H, 2-H, 3-H, 5-H and 6-H), 4.38 (s, 2H, 1'-CH₂), 3.74 (s, 3H, OCH₃).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 153.0 (C-4), 141.1 (C-1), 138.0 (C-1'), 132.9 (C-3'), 129.7 (C-6'), 128.9 (C-4'), 127.7 (C-5'), 123.6 (C-2'), 115.1 (C-2 and C-6 or C-3 and C-5), 115.0 (C-2 and C-6 or C-3 and C-5), 55.9 (OCH₃), 49.8 (1'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3419, 3059, 2995, 2931, 2831, 1618, 1568, 1509, 1464, 1440, 1357, 1232, 1178, 1123, 1082, 1025, 818, 749, 660.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₄H₁₄BrNO⁺: 291.0253; found: 291.0253.

2-(4-Methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (114j[#])C₁₆H₁₇NOM_w = 239.32 g/mol

Prepared according to **General Procedure 2** from *N*-(2-bromobenzyl)-4-methoxyaniline (**112j[#]**, 51.0 mg, 0.175 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 74.2 μL, 0.350 mmol). The crude product was purified by FCC (hexanes/EtOAc 85:15) to give product **114j[#]** (22.9 mg, 95.7 μmol, 55 %) as a brown solid.

R_f: 0.58 (hexanes/EtOAc 85:15).

m.p.: 80 – 84 °C.

¹H-NMR

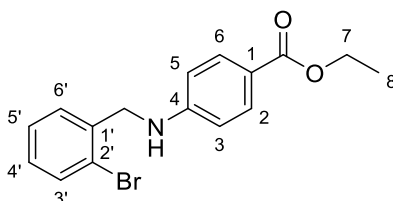
(400 MHz, CDCl₃): δ (ppm) = 7.21 – 7.11 (m, 4H, 5-H, 6-H, 7-H and 8-H), 7.05 – 6.98 (m, 2H, 2'-H and 6'-H), 6.90 – 6.85 (m, 2H, 3'-H and 5'-H), 4.31 (s, 2H, 1-H), 3.78 (s, 3H, OCH₃), 3.46 (t, *J* = 5.9 Hz, 2H, 3-H), 3.00 (t, *J* = 5.9 Hz, 2H, 4-H).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 153.6 (C-4'), 145.5 (C-1'), 134.8 (C-4a or C-8a), 134.7 (C-4a or C-8a), 128.8 (C-5), 126.7 (C-6 or C-7 or C-8), 126.4 (C-6 or C-7 or C-8), 126.0 (C-6 or C-7 or C-8), 118.2 (C-2' and C-6'), 114.7 (C-3' and C-5'), 55.8 (OCH₃), 52.8 (C-1), 48.6 (C-3), 29.2 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2920, 2808, 1509, 1459, 1442, 1385, 1272, 1240, 1206, 1188, 1151, 1111, 1035, 930, 822, 800, 754, 720, 701.

HR-MS (EI): *m/z* = [M-H]⁺ calcd for C₁₆H₁₆NO⁺: 238.1232; found: 238.1226.

Ethyl 4-((2-bromobenzyl)amino)benzoate (112k[#])C₁₆H₁₆BrNO₂M_w = 334.21 g/mol

Prepared according to **General Procedure 1** from 2-bromobenzaldehyde (**111**, 111 mg, 0.600 mmol) and ethyl-4-aminobenzoate (**4k**, 82.6 mg, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 75:25) to give product **112k[#]** (108 mg, 0.323 mmol, 65 %) as a white solid.

R_f: 0.47 (hexanes/EtOAc 75:25).

m.p.: 117 – 120 °C.

¹H-NMR

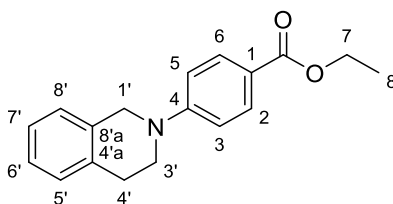
(400 MHz, CDCl₃): δ (ppm) = 7.91 – 7.85 (m, 2H, 2-H and 6-H), 7.57 (dd, *J* = 7.9, 1.3 Hz, 1H, 3'-H), 7.41 (d, 1H, 6'-H), 7.29 – 7.23 (m, 1H, 5'-H), 7.15 (td, *J* = 7.6, 1.7 Hz, 1H, 4'-H), 6.67 (d, *J* = 8.3 Hz, 2H, 3-H and 5-H), 4.49 (s, 2H, 1'-CH₂), 4.31 (q, *J* = 7.1 Hz, 2H, 7-H), 1.35 (t, *J* = 7.1 Hz, 3H, 8-H).

¹³C-NMR

(101 MHz, CDCl₃): δ (ppm) = 166.8 (1-COOEt), 150.6 (C-4), 136.9 (C-1'), 133.1 (C-3'), 131.6 (C-2 and C-6), 129.4 (C-6'), 129.3 (C-4'), 127.8 (C-5'), 123.6 (C-2'), 120.3 (C-1), 112.7 (C-3 and C-5), 60.5 (C-7), 48.4 (1'-CH₂), 14.6 (C-8).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3374, 2928, 1722, 1672, 1598, 1530, 1439, 1336, 1268, 1173, 1106, 1023, 839, 770, 752, 700.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₆H₁₆BrNO₂⁺: 333.0359; found: 333.0358.

Ethyl 4-(3,4-dihydroisoquinolin-2(1*H*)-yl)benzoate (**114k**[#])C₁₈H₁₉NO₂M_w = 281.36 g/mol

Prepared according to **General Procedure 2** from ethyl 4-((2-bromobenzyl)amino)benzoate (**112k**[#], 83.6 mg, 0.250 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 106 μL, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 85:15) to give product **114k**[#] (49.1 mg, 0.175 mmol, 70 %) as an off-white solid.

R_f: 0.32 (hexanes/EtOAc 95:5).

m.p.: 49 – 51 °C.

¹H-NMR

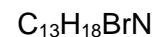
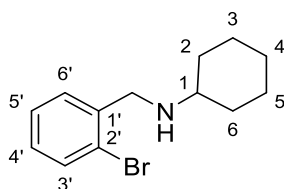
(500 MHz, CDCl₃): δ (ppm) = 7.98 – 7.94 (m, 2H, 2-H and 6-H), 7.24 – 7.16 (m, 4H, 5'-H, 6'-H, 7'-H and 8'-H), 6.91 – 6.85 (m, 2H, 3-H and 5-H), 4.52 (s, 2H, 1'-2H), 4.33 (q, *J* = 7.1 Hz, 2H, 7-H), 3.66 (t, *J* = 5.9 Hz, 2H, 3'-H), 3.00 (t, *J* = 5.9 Hz, 2H, 4'-H), 1.38 (t, *J* = 7.1 Hz, 3H, 8-H).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 167.0 (1-COOEt), 153.1 (C-4), 135.2 (C-8'a), 134.0 (C-4'a), 131.4 (C-2 and C-6), 128.4 (C-5'), 126.9 (C-6' or C-7' or C-8'), 126.7 (C-6' or C-7' or C-8'), 126.5 (C-6' or C-7' or C-8'), 118.9 (C-1), 112.3 (C-3 and C-5), 60.4 (C-7), 49.3 (C-1'), 45.0 (C-3'), 29.2 (C-4'), 14.6 (C-8).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2977, 2850, 1695, 1604, 1519, 1390, 1362, 1277, 1228, 1181, 1102, 1024, 926, 827, 767, 741, 698.

HR-MS (ESI): *m/z* = [M-H]⁺ calcd for C₁₈H₁₈NO₂⁺: 280.1338; found: 280.1332.

***N*-(2-Bromobenzyl)cyclohexanamine (112I)**

M_w = 268.20 g/mol

Prepared according to **General Procedure 1** from 2-bromobenzaldehyde (**111**, 111 mg, 0.600 mmol) and cyclohexylamine (**4I**, 57.8 μL, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc+Et₃N 90:10+1) to give product **112I** (64.0 mg, 0.239 mmol, 48 %) as a colourless oil.

R_f: 0.30 (hexanes/EtOAc+Et₃N 90:10+1).

¹H-NMR

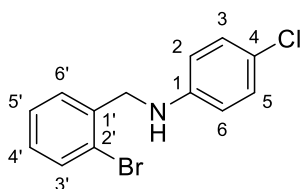
(500 MHz, CDCl₃): δ (ppm) = 7.53 (dd, *J* = 7.9, 1.3 Hz, 1H, 3'-H), 7.40 (dd, *J* = 7.6, 1.8 Hz, 1H, 6'-H), 7.27 (td, *J* = 7.5, 1.1 Hz, 1H, 5'-H), 7.11 (td, *J* = 7.7, 1.8 Hz, 1H, 4'-H), 3.88 (s, 2H, 1'-CH₂), 2.46 (tt, *J* = 10.3, 3.7 Hz, 1H, 1-H), 1.96 – 1.90 (m, 2H, 2-H and/or 6-H), 1.77 – 1.71 (m, 2H, 3-H and/or 5-H), 1.64 – 1.58 (m, 1H, 4-H), 1.31 – 1.20 (m, 2H, 2-H and/or 6-H), 1.20 – 1.10 (m, 3H, 4-H and 3-H and/or 5-H).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 140.0 (C-1'), 132.9 (C-3'), 130.5 (C-6'), 128.6 (C-4'), 127.6 (C-5'), 124.1 (C-2'), 56.2 (C-1), 51.11 (1'-CH₂), 33.7 (C-2 and C-6), 26.3 (C-4), 25.2 (C-3 and C-5).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2923, 2851, 1462, 1440, 1347, 1259, 1124, 1024, 888, 746, 656.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₃H₁₈BrN⁺: 267.0617; found: 267.0617.

N-(2-Bromobenzyl)-4-chloroaniline (112m)C₁₃H₁₁BrClNM_w = 296.59 g/mol

Prepared according to **General Procedure 1** from 2-bromobenzaldehyde **3** (**111**, 111 mg, 0.600 mmol) and 4-chloroaniline (**4m**, 65.1 mg, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc 95:5) to give product **112m** (112 mg, 0.379 mmol, 76 %) as a pale yellow oil.

R_f: 0.33 (hexanes/EtOAc 95:5).

¹H-NMR

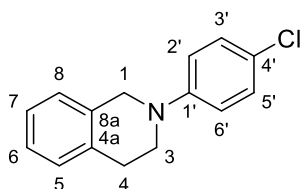
(500 MHz, CDCl₃): δ (ppm) = 7.57 (dd, *J* = 7.9, 1.2 Hz, 1H, 3'-H), 7.36 (dq, *J* = 7.7, 0.8 Hz, 1H, 6'-H), 7.26 (td, *J* = 7.5, 1.3 Hz, 1H, 5'-H), 7.18 – 7.13 (m, 1H, 4'-H), 7.13 – 7.09 (m, 2H, 3-H and 5-H), 6.55 – 6.51 (m, 2H, 2-H and 6-H), 4.38 (d, *J* = 5.7 Hz, 2H, 1'-CH₂), 4.22 (t, *J* = 5.3, 4.5 Hz, 1H, NH).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 146.4 (C-1), 137.8 (C-1'), 133.1 (C-3'), 129.3 (C-3 and C-5), 129.2 (C-6'), 129.0 (C-4'), 127.7 (C-5'), 123.4 (C-2'), 122.5 (C-4), 114.2 (C-2 and C-6), 48.6 (1'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3430, 2923, 2852, 1599, 1497, 1464, 1440, 1319, 1264, 1177, 1095, 1024, 812, 747.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₃H₁₁BrClN⁺: 294.9758; found: 294.9758.

2-(4-Chlorophenyl)-1,2,3,4-tetrahydroisoquinoline (114m)C₁₅H₁₄ClNM_w = 243.73 g/mol

Prepared according to **General Procedure 2** from **112m** (59.3 mg, 0.200 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 84.7 μ L, 0.400 mmol). The crude product was purified by FCC (hexanes/EtOAc 98:2) to give product **114m** (33.6 mg, 0.138 mmol, 69 %) as a beige solid.

R_f: 0.36 (hexanes/EtOAc 98:2).

m.p.: 59 – 62 °C.

¹H-NMR

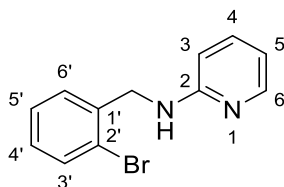
(500 MHz, CDCl₃): δ (ppm) = 7.24 – 7.21 (m, 2H, 3'-H and 5'-H), 7.21 – 7.14 (m, 4H, 5-H, 6-H, 7-H and 8-H), 6.90 – 6.87 (m, 2H, 2'-H and 6'-H), 4.38 (s, 2H, 1-H), 3.53 (t, J = 5.9 Hz, 2H, 3-H), 2.98 (t, J = 5.9 Hz, 2H, 4-H).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 149.2 (C-1'), 134.8 (C-4a), 134.2 (C-8a), 129.2 (C-3' and C-5'), 128.7 (C-5), 126.7 (C-6 or C-7 or C-8), 126.6 (C-6 or C-7 or C-8), 126.3 (C-6 or C-7 or C-8), 123.5 (C-4'), 116.3 (C-2' and C-6'), 50.8 (C-1), 46.7 (C-3), 29.1 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3316, 2915, 2842, 1595, 1494, 1459, 1430, 1384, 1341, 1300, 1224, 1156, 1093, 928, 806, 738, 720.

HR-MS (EI): m/z = [M-H]⁺ calcd for C₁₅H₁₃ClN⁺: 242.0737; found: 242.0730.

***N*-(2-Bromobenzyl)pyridin-2-amine (112n)**C₁₂H₁₁BrN₂M_w = 263.14 g/mol

Prepared according to **General Procedure 1** from 2-bromobenzaldehyde (**111**, 111 mg, 0.600 mmol) and 2-aminopyridine (**4n**, 47.1 mg, 0.500 mmol). The crude product was purified by FCC (hexanes/EtOAc+Et₃N 90:10+1) to give product **112n** (62.0 mg, 0.236 mmol, 47 %) as a yellow solid.

R_f: 0.25 (hexanes/EtOAc+Et₃N 90:10+1).

m.p.: 109 – 112 °C.

¹H-NMR

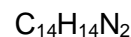
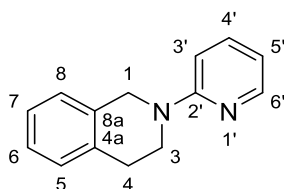
(500 MHz, CDCl₃): δ (ppm) = 8.11 (ddd, *J* = 5.1, 1.9, 0.9 Hz, 1H, 6-H), 7.56 (dd, *J* = 7.9, 1.2 Hz, 1H, 3'-H), 7.45 – 7.39 (m, 1H, 6'-H), 7.40 (ddd, *J* = 8.7, 7.1, 1.9 Hz, 1H, 4-H), 7.28 – 7.24 (m, 1H, 5'-H), 7.15 – 7.10 (m, 1H, 4'-H), 6.60 (ddd, *J* = 7.1, 5.0, 1.0 Hz, 1H, 5-H), 6.36 (dt, *J* = 8.3, 0.9 Hz, 1H, 3-H), 5.06 – 5.00 (m, 1H, NH), 4.59 (d, *J* = 6.3 Hz, 2H, 1'-CH₂).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 158.5 (C-2), 148.3 (C-6), 138.2 (C-1'), 137.7 (C-4), 132.9 (C-3'), 129.4 (C-6'), 128.9 (C-4'), 127.7 (C-5'), 123.6 (C-2'), 113.5 (C-5), 107.0 (C-3), 46.6 (1'-CH₂).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3235, 3020, 1600, 1580, 1537, 1448, 1425, 1331, 1277, 1152, 1023, 767, 750.

HR-MS (EI): *m/z* = [M]⁺ calcd for C₁₂H₁₁BrN₂⁺: 262.0100; found: 262.0100.

2-(Pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline (114n)

$$M_w = 210.28 \text{ g/mol}$$

Prepared according to **General Procedure 2** from *N*-(2-bromobenzyl)pyridin-2-amine (**112n**, 52.6 mg, 0.200 mmol) and (*E*)-2-ethoxyvinylboronic acid pinacol ester (**110**, 84.7 μL , 0.400 mmol). The crude product was purified by FCC (hexanes/EtOAc+Et₃N 90:10+1) to give product **114n** (10.1 mg, 0.0480 mmol, 24 %) as an off-white oily solid.

R_f: 0.40 (hexanes/EtOAc+Et₃N 95:5+1).

¹H-NMR

(500 MHz, CDCl₃): δ (ppm) = 8.22 (ddd, $J = 5.0, 2.0, 0.9$ Hz, 1H, 6'-H), 7.50 (ddd, $J = 8.9, 7.1, 2.0$ Hz, 1H, 4'-H), 7.21 (dp, $J = 3.7, 1.9$ Hz, 2H, 6-H and/or 7-H and/or 8-H), 7.18 (td, $J = 4.7, 4.1, 2.1$ Hz, 2H, 5-H and 6-H or 7-H or 8-H), 6.68 (dt, $J = 8.6, 1.0$ Hz, 1H, 3'-H), 6.60 (ddd, $J = 7.1, 5.0, 0.9$ Hz, 1H, 5'-H), 4.71 (s, 2H, 1-H), 3.85 (t, $J = 6.0$ Hz, 2H, 3-H), 2.98 (t, $J = 5.9$ Hz, 2H, 4-H).

¹³C-NMR

(126 MHz, CDCl₃): δ (ppm) = 158.9 (C-2'), 148.1 (C-6'), 137.6 (C-4'), 135.6 (C-4a), 134.5 (C-8a), 128.5 (C-5), 126.7 (C-8), 126.5 (C-6 or C-7), 126.3 (C-6 or C-7), 112.6 (C-5'), 106.8 (C-3'), 47.3 (C-1), 42.7 (C-3), 29.2 (C-4).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 3006, 2922, 2837, 1666, 1592, 1562, 1480, 1435, 1387, 1312, 1299, 1228, 1157, 978, 937, 764, 741.

HR-MS (EI): $m/z = [M]^{+}$ calcd for C₁₄H₁₄N₂⁺: 210.1151; found: 210.1150.

7. Abbreviations

5-ASA	5-aminosalicylic acid
Ac	acetyl
acetyl-CoA	acetyl coenzyme A
AcOH	acetic acid
ADP	adenosine diphosphate
AFU	absolute fluorescence units
AIBN	azobisisobutyronitrile
AMC	7-amino-4-methylcoumarin
aq.	aqueous
Ar	aryl
Arg	arginine
ATR	attenuated total reflection
B ₂ pin ₂	bis(pinacolato)diboron
Bn	benzyl
Boc	<i>tert</i> -butyloxycarbonyl
BPO	benzoyl peroxide
BRD	bromodomain
Bu	butyl
calcd	calculated
CAM	ceric ammonium molybdate
CDI	carbonyldiimidazole
cf.	confer (compare)
conc.	concentrated
CP	compound pull-down
CPSI	carbamoylphosphate synthetase I
ctrl	control
d	doublet
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane

Abbreviations

DMEM	Dulbecco's modified eagle medium
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNPH	2,4-dinitrophenylhydrazine
dppf	1,1'-bis(diphenylphosphino)ferrocene
DTT	dithiothreitol
e.g.	exempli gratia (for example)
EC ₅₀	half maximal effective concentration
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDG	electron donating group
EI	electron ionisation
eq	equivalent
ESI	electrospray ionisation
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
F	fluorescence units
FA	formic acid
FAD	flavin adenine dinucleotide
FBS	fetal bovine serum
FCC	flash column chromatography
G6PD	glucose-6-phosphate dehydrogenase
GCD	glutaryl-CoA dehydrogenase
glac.	glacial
Glu	glutamine
Gly	glycine
HATU	1-[bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-oxide hexafluorophosphate
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HBPIn	pinacolborane (4,4,5,5-tetramethyl-1,3,2-dioxaborolane)
HDAC	histone deacetylase/histone deacylase
Het	hetero
HMGCS2	hydroxymethylglutaryl-CoA synthase

Abbreviations

HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromatography
HR-MS	high resolution mass spectrometry
i.a.	inter alia
IBX	2-iodoxybenzoic acid
IC ₅₀	half maximal inhibitory concentration
IDH2	isocitrate dehydrogenase 2
inh	inhibitor
IR	infrared spectroscopy
KAT	lysine acetyltransferase/lysine acyltransferase
KDAC	lysine deacetylase/lysine deacylase
KO ^t BU	potassium <i>tert</i> -butoxide
LC-MS	liquid chromatography-mass spectrometry
M	molarity
<i>m</i> -CPBA	<i>m</i> -chloroperbenzoic acid
Me	methyl
m.p.	melting point
m/z	mass-to-charge ratio
MCE	microchip electrophoresis
MD	molecular dynamics
MeCN	acetonitrile
MeOH	methanol
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid
Mw	molecular weight
N	normality
NAD	nicotinamide adenine dinucleotide
NBS	<i>N</i> -bromosuccinimide
NDPK	nucleoside diphosphate kinase
NHS	<i>N</i> -hydroxysuccinimide
n.i.	no inhibition
NMR	nuclear magnetic resonance
<i>o</i>	<i>ortho</i>
OAADPr	<i>O</i> -acetyl-ADP-ribose/ <i>O</i> -acyl-ADP-ribose
<i>p</i>	<i>para</i>

PAINS	pan-assay interference compounds
PBS	phosphate buffered saline
PDB	protein data bank
PEG	polyethylene glycol
pK_a	acid dissociation constant
PTM	post translational modification
q	quartet
QM/MM	quantum mechanics/molecular mechanics
quant.	quantitative
R_f	retardation factor
RNA	ribonucleic acid
ROS	reactive oxygen species
RP-HPLC	reversed phase high performance liquid chromatography
rpm	revolutions per minute
rt	room temperature
s	singlet
SA	salicylic acid
SAR	structure-activity relationship
sat.	saturated
SIR2	silent information regulator 2
Sirt	sirtuin
SOD1	superoxide dismutase 1
SPE	solid phase extraction
t	triplet
<i>t</i>	tertiary
TBAF	tetra- <i>n</i> -butylammonium fluoride
TCA	tricarboxylic acid
temp.	temperature
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofurane
TLC	thin layer chromatography
TMEDA	<i>N,N,N',N'</i> -tetramethylethylenediamine
TMS	trimethylsilyl
Tyr	tyrosine

Abbreviations

UV	ultraviolet
Val	valine
vol.	volume
vs.	versus
ZKsA	Z-Lys(succinyl)-7-amino-4-methylcoumarin
ZMAL	Z-Lys(acetyl)-AMC
$\tilde{\nu}$	wavenumber

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