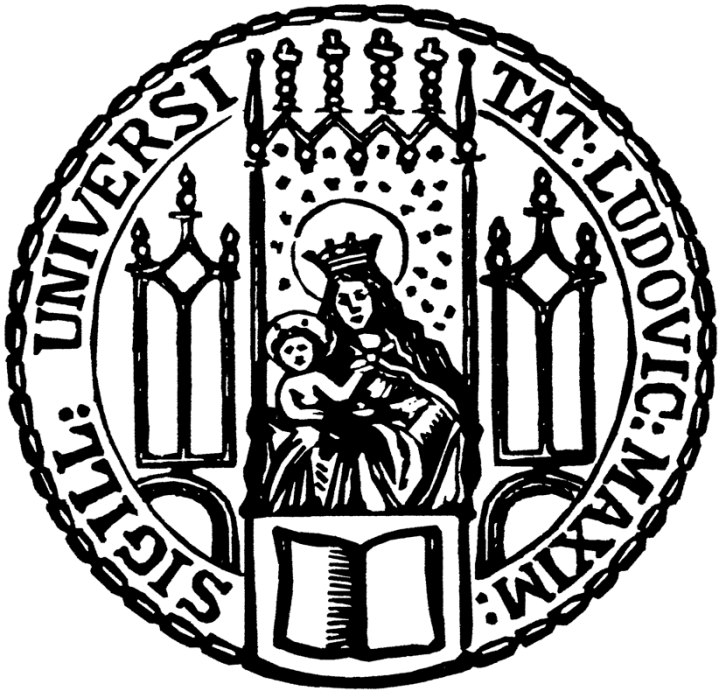
Dissertation zur Erlangung des Doktorgrades der Fakultät für Chemie und Pharmazie der Ludwig-Maximilians-Universität München



Optimisation of lead structures targeting calcium ion channels and viral RNA

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Erklärung

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GRAZIE.

TO MY FAMILY

Preface

The present thesis addresses two distinct and independent research topics on which I had the opportunity to work during the course of my doctoral studies.

The first topic regards the dimeric chloroquinoline Lys05 (**1**), which has been found to be a promising inhibitor of endolysosomal two pore channel 2 (TPC2). The second topic focuses on the implementation of a benzothiazole structure targeting the RNA of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

Both topics consist in the optimisation of a lead compound and structure-activity relationship (SAR) studies. The results are presented as monograph, since they have not been published yet.

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1 Introduction

1.1 Endolysosomal system and two pore channels

The endolysosomal system is pivotal in intracellular processes, including the transport, sorting, recycling, and degradation of macromolecules, thereby maintaining cellular homeostasis[1]. Through endocytosis, cells internalise materials such as plasma membrane components, particles, fluids, and macromolecules, either directly or *via* receptor-mediated mechanisms. Once internalised, these vesicles fuse with early endosomes, directing their contents toward recycling, degradation, or retrograde pathways[2-4]. Interactions with other compartments, such as trans-Golgi network, are crucial for the maturation of early endosomes into late endosomes[5]. The latter are distinguished by an acidic luminal environment of approximately pH 5.5[6], essential condition for lysosomal hydrolase activity, intraluminal vesicles (IVLs), and multi-vesicular bodies (MVBs)[7]. In case of degradation, late endosomes fuse with lysosomes to form endolysosomes – a hybrid structure in which acidic luminal environment from endosomes activates the many acid hydrolases of the lysosome to break down the various molecules, including proteins, lipids, nucleic acids, and carbohydrates, into reusable building blocks[8]. The efficient functioning of the endolysosomal system is regulated by various signalling pathways, molecular regulators, and ion channels. Membrane fusion, pH balance, and enzyme activities are modulated by ion currents, which are, in turn, regulated by different ion channels, such as vacuolar-type ATPase (V-ATPase)[9], mucolipin receptor family (TRPML)[10], purinergic P2X4 receptors[11], transmembrane proteins (TMEMs)[12], and two pore channels (TPCs)[13].

The TPCs are voltage-gated ion channels largely present in endolysosomal membranes. The human genome encodes two TPC isoforms: TPC1, which is distributed across various endolysosomal organelles, in particular early endosomes, and TPC2, mainly localised in late endosomes and lysosomes. Structurally, each TPC subunit forms a homodimer, with each monomer consisting of two homologous six-transmembrane (6-TM) domains. The cryo-EM structure of TPC2, elucidated by She *et al*.[14], highlights its unique architecture, supporting its complex regulatory mechanisms. Unlike conventional ion channels with a single gating mechanism, TPC2 exhibits multimodal regulation, responding to distinct physiological ligands. Upon activation by nicotinic acid adenine dinucleotide phosphate (NAADP), TPC2 mediates robust Ca^{2+} release from lysosomes, influencing calcium-dependent processes such as vesicle trafficking, exocytosis, and autophagy. However, when activated by phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$), TPC2 functions as a highly selective Na^+ channel, contributing to lysosomal membrane potential and ionic balance. This dual activation mechanism, extensively discussed by Gerndt *et al*.[15], suggests that TPC2 dynamically adapts to cellular needs,

integrating multiple upstream signals to control endolysosomal function. Beyond ion transport, TPCs are a key regulator of several signalling cascades, including mTOR and TFEB pathways[16], which control lysosomal biogenesis, metabolism, and cellular stress responses, as well as endolysosomal trafficking, fusion processes, and autophagy[17]. Over the past decade, significant progress has been made in understanding the activation mechanisms of TPCs, as summarised by Patel *et al*.[18]. However, a comprehensive understanding of all the involved pathways and their implications for cellular signalling and physiological processes remains an on-going area of research.

1.2 TPCs and related diseases

Understandably, given their aforementioned crucial role in the endolysosomal system, the link between TPCs and many diseases has been established over the past years. TPC2 dysfunction has been implicated in lysosomal storage disorders (LSDs) linked to impaired lysosomal degradation and exocytosis, stating a rescue effect of TPC2 activation[19, 20]. Similarly, the lysosomal dysfunction in non-alcoholic fatty liver disease (NAFLD) was related to loss of TPC2 function[21]. On the other hand, impaired autophagy in Parkinson disease was proven to be linked to TPC2 activity, where lysosomal defects caused by LRRK2 mutations were normalised by silencing TPC2[22, 23]. In addition, the role played by TPC2 was highlighted in case of cardiac disfunctions[17, 24, 25] and diabetes[26]. Furthermore, TPCs function is exploited by certain viruses, to facilitate their entry and trafficking within host cells. For instance, Ebola virus hijacks the endolysosomal system *via* TPC2-dependent endosomal escape, inducing membrane destabilisation to promote viral genome release[27]. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) entry and endolysosomal trafficking were proven to be supported by TPCs activity[28], and SARS-CoV-2 entry was strongly inhibited blocking the kinase PIKfyve and TPC2[29]. Moreover, tumours and cancer hallmarks have been also correlated to TPCs, even though the link appears complex and to be context dependent. Müller *et al.* stated that TPC2 knockout has an impact on cancer cell proliferation, metabolism, and tumour growth[30]. Cancer cell migration and adhesion were impaired by silencing TPCs[31], and vascular endothelial growth factors (VEGF)-induced neoangiogenesis was blocked by TPCs inhibition[32, 33], both *in vitro* and *in vivo*. Overexpression of TPCs was registered in human breast cancer[34] and oral squamous cell carcinoma cell lines[35], while, conversely, it correlated with an increased survival in bladder cancer[36].

Given their involvement in numerous physiological processes and several distinct diseases, TPCs have become a focal point in cation channel research, increasing the need for more in-depth studies on the mechanism of action and, therefore, on their potential as therapeutic targets.

1.3 Pharmacological inhibition of TPCs

Recent studies have increasingly highlighted the pharmacological inhibition of TPCs, particularly TPC2, as a promising strategy for modulating cellular processes in various pathological conditions. Given its central role in lysosomal function, autophagy, homeostasis, and intracellular signalling, TPC2 has emerged as a potential drug target for diseases such as cancer, viral infections, and neurodegenerative disorders. Different flavonoids emerged as TPC2 inhibitors: naringenin was shown to block NAADP-dependent TPC2 calcium signalling at high dosage, leading to the suppression of VEGF-induced angiogenesis[32]. Given the crucial role of angiogenesis in tumour growth and metastasis, naringenin's ability to modulate TPC2 suggests its potential as an anti-cancer agent. These results were further confirmed by Netcharoensirisuk *et al.*[37], extending the pool to the flavonoids pratensein and duartin, since they were proven to reduce melanoma cell proliferation, migration, and invasion through TPC2 inhibition. Alkaloids have also been identified as potent inhibitors of TPCs activity, with significant implications in both infectious diseases and cancer. The bisbenzylisoquinoline alkaloid tetrandrine was identified by Sakurai *et al.*[27] as inhibitor of both TPC1 and TPC2. Tetrandrine effectively impairs viral entry by disrupting TPC2-dependent endosomal escape mechanisms, which are critical for the infection cycle of several enveloped viruses, along with the less active verapamil and Ned19[27]. Subsequent studies have extended its inhibitory effects to other viral infections, including MERS[28] and SARS-CoV-2[29]. Beyond its antiviral properties, tetrandrine has also been investigated for its role in cancer treatment, as it has been shown to inhibit tumour cell migration and invasion[31]. Furthermore, the truncated tetrandrine variants SG-005 and SG-094 were validated in our group as TPCs inhibitors[30], and a recent study on enantiomerically pure (S)-SG-094 highlighted the impact on calcium signalling and lysosomal autophagosomal-lysosomal fusion[38]. Additionally, the phosphoinositide kinase inhibitors YM201636 and PI-103 have been shown to inhibit TPC2, possibly contributing to the respectively proven antiviral effect and antitumour activity[39].

Despite the growing interest in TPC2 pharmacology, no known inhibitor has been specifically designed or studied to selectively target only one of its two activation mechanisms – NAADP-dependent Ca^{2+} release or $PI(3,5)P_2$-mediated Na^+ transport. The need for more precise modulators that can selectively inhibit only one of them lies in the possibility to further clarifying the role of TPC2 in various signalling pathways and its pathological implications. In this context, Lys05 (**1**), initially introduced by McAfee *et al.*[40] as an autophagy inhibitor and antitumor agent, emerged from a high-throughput screening (HTS) campaign as suitable basis for a potential selective TPC2 inhibitor, as described in more detail in Chapter 2.1.

1.4 SARS-CoV-2: characteristics and spread

Pathogens of the *Coronaviridae* family are able to infect animals as well as humans, causing respiratory, digestive, and neurological diseases[41]. The earliest coronavirus respiratory infection of domestic chickens was documented in North America in 1920s[42]. Since then, many different coronaviruses were identified and classified into the subfamily *Orthocoronavirinae* divided in turn into four different genera: *Alpha*-, *Beta*-, *Gamma*-, and *Deltacoronavirus*[41, 43]. The list of different pathogens, belonging to the human coronaviruses and, in particular, to the *Betacoronavirus,* include the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), whose outbreak was registered in 2002-2003[44], MERS-CoV, identified in 2012[45], and SARS-CoV-2, well known as public health concerns worldwide[46]. The novel SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus with a spherical shape of about 60 – 140 nm in diameter[47]. The spike (S) protein, which covers the surface of the virus and gives the crown-like appearance that inspired its name, is one of the most important structural proteins, since it's involved in the viral entry mechanism into host cells. The S-protein engages the human angiotensin-converting enzyme 2 (ACE2) receptor[48] and is cleaved by host serine protease TMPRSS2, allowing membrane fusion and viral entry into human lung cells[49, 50]. Upon entry, the viral RNA is realised into the cytoplasm and translated into viral proteins by the host ribosome system. A complex programme of gene expression leads to the production of structural viral proteins, that are then assembled in the host endoplasmic reticulum and Golgi apparatus. The new virions are secreted *via* exocytosis from the infected cells[51].

SARS-CoV-2 was first identified in late 2019 in Wuhan, China, and its rapid spread from human-to-human lead to a pandemic of acute respiratory disease[52, 53]. The most accredited hypothesis for SARS-CoV-2 spread regards the zoonotic spillover from bats, considered the original reservoirs, which allow the virus to replicate without causing a severe disease. This theory is supported by phylogenetic analysis of SARS-CoV-2, which demonstrated a close relationship to bat coronaviruses, even more than to SARS-CoV and MERS[54, 55], with its genetic sequence approximately 96% identical to the bat-coronavirus RaTG13[48]. Moreover, the early cases of infection were registered in the Huanan Seafood Wholesale Market in Wuhan, where wild animals were sold[56], supporting the zoonotic transmission. Pangolins are other species probably linked with SARS-CoV-2, since the Guangdong strains of pangolin viruses exhibited approximately 92% sequence identity with SARS-CoV-2[57]. Despite these findings, the exact zoonotic origin and path, including other animals as intermediate reservoirs, remain unclear. Another theory, which could explain the origin and diffusion of SARS-CoV-2, is the leakage from the Wuhan Institute of Virology, where recent studies on bat coronaviruses

were conducted[58]. No strong direct evidence was provided to support this thesis, especially under the light of the clear epidemiological link with the market in Wuhan[56].

The novel SARS-CoV-2 causes the 'coronavirus disease 2019' (COVID-19), which primary affects the respiratory tract. The common symptoms are fever, fatigue, and dry cough, along with shortness of breath and diarrhea. In severe cases it can progress to pneumonia and acute respiratory distress syndrome, especially in elderly patients[59]. The human-to-human transmission spreads through respiratory droplets, when an infected person cough, sneezes, or talks in close person-to-person contacts[60]. The transmission also occurs in case of mild symptomatic or asymptomatic infected people[61], a factor that made extremely difficult to control the spread. Moreover, a prolonged risk of infection is linked to the ability of SARS-CoV-2 to persist on inanimate surfaces for days[62]. The extreme transmissibility and the underestimation of the sanitary emergency played a crucial role in SARS-CoV-2 global spread. The pandemic status was declared by the World Health Organization (WHO) on March 11th, 2020. From January 2020 to the present date, roughly 230 countries and territories were affected, more than 777 million people infected, and about 7 million deaths reported. The first approach to limit the massive and quick spread of COVID19 consisted in social distancing policies[63, 64], as neither prophylactic vaccinations nor pharmaceuticals for infected patients were available. The state of pandemic prompted extensive research into vaccines and drug development. Among the vaccines, different strategies were applied, including inactivated, nucleic acid, adenovirus-based vector, and recombinant subunit vaccines[65]. The primary target is the S-protein due to its ability to induce neutralising antibodies and T-cell immune response[66-68]. A great threat of this approach is represented by new viral variants of SARS-CoV-2, which could develop mutations of the S protein able to escape the immune system recognition, having as main consequence the need to constantly redesign vaccines on the new variants. On the other hand, several drug candidates, which impair the SARS-CoV-2 life cycle on various stages, have been reproposed and tested. Many replication inhibitors have been approved for the treatment of COVID19, such as: remdesivir, a small-molecule adenine nucleotide antiviral drug used before against Ebola virus infection, which interferes with RNA-dependent RNA polymerase (RdRp) and was proved to inhibit *in vivo* SARS-CoV-2 replication[69]; a combination of nirmatrelvir, an antiviral 3CL protease inhibitor, and ritonavir, a known pharmacokinetic enhancer that slows the metabolism of nirmatrelvir, which was effective in treating COVID19[70]; molnupiravir, a ribonucleoside prodrug which interferes with RdRp[71]. Inhibitors of viral entry, targeting TMPRSS2 serine protease and ACE2, were also proved to block the entry of SARS-CoV-2 into human lung cells, as for camostat mesylate[49]. Another approach is the utilisation of immune modulators that can reduce the inflammatory response, which is responsible for the organ damage due to the cytosine storm. As adjunctive therapy against COVID19, dexamethasone, a corticosteroid largely used for its anti-

inflammatory effects, and interleukin-6 receptor antagonists, such as tocilizumab, showed effectiveness in clinical trials[72, 73]. Currently, no drug, specifically designed to target SARS-CoV-2 and treat COVID19, has been approved.

1.5 SARS-CoV-2 genome and target identification

As aforementioned, SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus, whose genome can be directly translated into viral proteins by the host's ribosome system. The viral genome, composed of around 29.9 kilobases, contains open reading frames (ORF) between the two untranslated regions (UTRs) – the capped 5'-untranslated region and the 3'-poly-A tail. The ORFs encode for non-structural proteins, 4 structural proteins - spike (S), membrane (M), envelope (E), and nucleocapsid (N) -, and 8 accessory proteins[74]. The secondary structure of the 5'-UTR of SARS-CoV-2 RNA is composed by several stem-loops (SL1, SL2, SL3, SL4, S5, SL5A, SL5B, SL5C, SL6, SL7, and SL8)[75]. The structure of the 29 nucleotides-RNA construct 5_SL1 was determined by NMR spectroscopy by Richter and Hohmann *et al.*[76] and Toews and Wacker *et al.*[77]. It consists of two A-form helical structures, an asymmetrical internal loop, and a pyrimidine-rich apical loop (Figure 1).

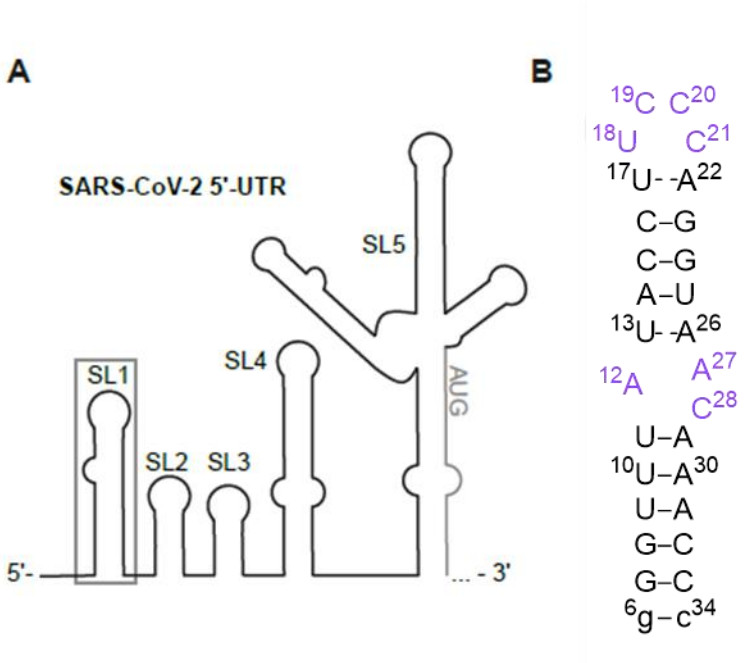


Figure 1: (**A**) Secondary structure of 5'-UTR region of SARS-CoV-2 RNA. (**B**) Truncated 5_SL1 structure composed of 29 nucleotides, stabilised with a terminal G-C base pair (lowercase letters)[76, 77]. The asymmetrical internal loop and apical loop are highlighted in violet.

The interaction of 5_SL1 with the non-structural protein 1 (Nsp1), translated from the ORF1a of SARS-CoV-2 genome and one of the first proteins produced, is crucial for viral replication of *Betacoronaviruses*. The C-terminal domain of Nsp1 binds the 40S host's ribosome subunit competing with host mRNA for the mRNA entry channel[78-80]. Tidu *et al.*[81] showed that the

5_SL1 harpin is responsible for Nsp1 evasion, interacting with the N-terminal domain of Nsp1 while the C-terminal domain of Nsp1 is still bound to the host's ribosome[79], allowing selective viral translation and interfering with cellular host translation. The truncated 5'-UTR, without the first 40 nucleotides containing 5_SL1, and mutants of 5_SL1 brought to complete abrogation of the Nsp1 evasion mechanism, impairing viral translation[81]. Therefore, Nsp1 can be considered a gatekeeper that selectively allows viral translation (Figure 2).

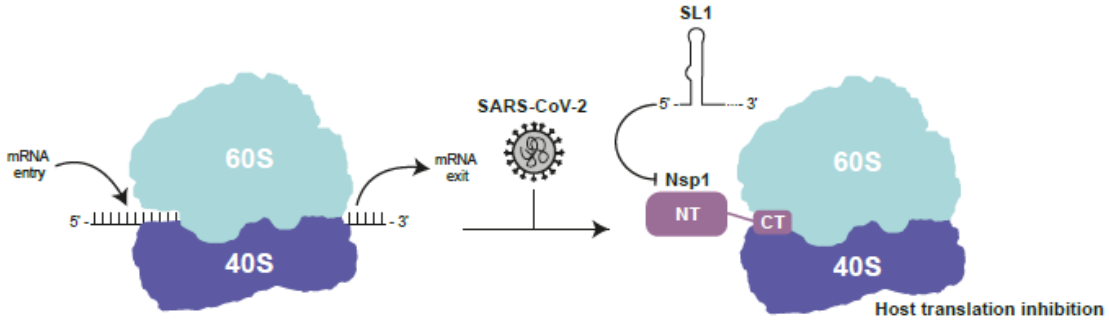


Figure 2: Nsp1 interaction with the mRNA entry channel of host's ribosome system, responsible for mRNA translation inhibition. In the proposed model, 5_SL1 interacts with Nsp1 evading the blockage of Nsp1 thus allowing viral RNA translation.

Moreover, all the 9 subgenomic RNAs produced in the late phase of infection contain the capped 5' leader sequence that forms SL1, SL2, and SL3[82], confirming the great potential of 5_SL1 as a target for blocking genomic RNA translation in the early stage of infection and subgenomic RNA translation in the late stage of infection. Targeting the 5_SL1 region to inhibit SARS-CoV-2 replication has been successfully proven in the last years. Peptide-conjugated morpholino oligomers (P-PMOs), single-stranded nucleic acid analogues, inhibit viral proliferation by interfering with the RNA structure *via* steric hindrance. Their antiviral effect was reported in 2005 by Neuman *et al.*[83] by targeting the 5'-UTR of SARS-CoV-1. More recently, P-PMOs were found to effectively inhibit SARS-CoV-2 growth when targeting the 5'-UTR more than the translation start site region, underlining the crucial role of this structural element. Viral translation was effectively disrupted by antisense oligonucleotides (ASOs) targeting 5_SL1[79] and binding of this structured RNA element was shown to prevent viral growth *in vitro* and *in vivo* by Zhu *et al.*[84]. In this study, locked nucleic acid (LNA) ASOs, synthetic nucleic acids, were utilised to bind specific complementary SARS-CoV-2 RNA, resulting in RNA degradation, accomplished by the recruitment of RNase, or preventing the interaction with the host's translational machinery by steric hindrance mechanisms. Administration of intranasal LNA ASO, specifically targeting the 5' leader sequence, to the COVID-19 mouse models, significantly reduced the viral load in the lungs[84]. Moreover, the 5_SL1 is highly conserved

among the many different *Betacoronaviruses*[85-87] and the virus viability strongly depends on this sequence, making it less prone to mutations and broadening the potential application of drugs that target this structure.

2 Objectives

2.1 TPC2 inhibitors

As mentioned in the Introduction (see Chapter 1.2), TPC2 is involved in many diseases and is a central topic of research nowadays. A HTS campaign with about 7,000 compounds (drugs, natural substances, and toxins) to explore potential TPCs inhibitors was performed by Nicole Urban, Schaefer group (University of Leipzig), in collaboration with Prof. Christian Grimm (LMU Munich), leading to the identification of Lys05 (**1**) as a promising TPC2 inhibitor. The screening was performed by means of a fluorometric calcium assay using a HEK293 cell line stably expressing plasma membrane TPC2$^{L11A/L12A}$-RFP and the two known synthetic low-molecular activators TPC2-A1-N (**2**) and TPC2-A1-P (**3**), depicted in Figure 3 and described by Gerndt *et al.*[15], which mimic respectively each of the two natural activation pathways, NAADP and PI(3,5)P_2, previously mentioned in the Introduction (see Chapter 1.1). Moreover, Lys05 (**1**) was identified as a promising selective inhibitor of PI(3,5)P_2 activation of TPC2 with a 9-fold stronger inhibitory effect on TPC2-A1-P (**3**) mediated activation compared to TPC2-A1-N (**2**) activation (Figure 3), suggesting Lys05 (**1**) and derivatives thereof as potential modality specific inhibitors of TPC2.

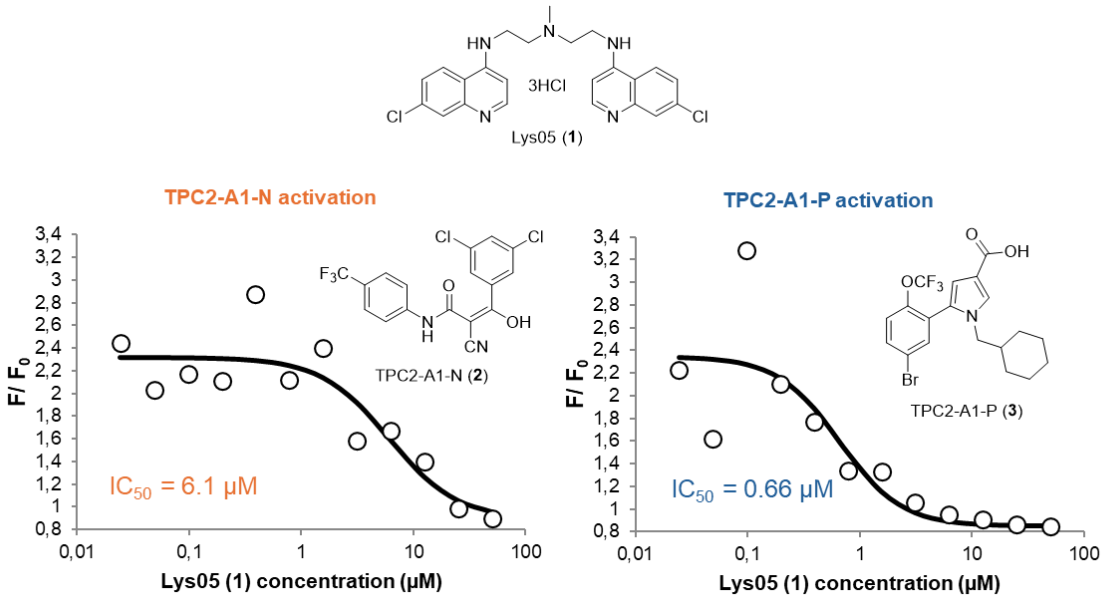


Figure 3: Dose–response curves of the primary screening hit Lys05 (**1**) after TPC2 activation with TPC2-A1-N (**2**, left) and TPC2-A1-P (**3**, right). Half-maximal inhibitory concentrations of the newly identified TPC2 inhibitor are indicated as IC_{50} for the two different modes of activation.

The Lys05 (**1**, the trihydrochloride of Lys01 (**4**)), a dimeric chloroquinoline with a triamino linker methylated on the central amine[40], is structurally related to the antimalarial drug chloroquine (CQ). Its distinct antimalarial activity against both CQ-resistant K1 and CQ-sensitive FCR-3 *Plasmodium falciparum* has been proven *in vitro*, even though the therapeutic effect *in vivo*

was not better than the one of the novel antimalarial drug artesunate in *P. berghei*-infected mice[88]. On the other hand, Lys05 (**1**) accumulates in lysosomes and displays significant antitumor activity, related to its autophagy modulation. McAfee *et al.*[40] demonstrated the superior efficacy of Lys05 (**1**) over hydroxychloroquine (HCQ) in terms of lysosomal accumulation and autophagy inhibition, impairing tumour growth *in vivo*, features that are in line with described effects of TPC2 inhibition, knockdown or knockout. Lys05 (**1**) is thus nowadays considered a lysosomal autophagy inhibitor, whose antitumor activity has been tested in various cancer models[40, 89-91]. Importantly, Lys05 (**1**) has been described to cause lysosomal dysfunction by deacidifying the lysosome after accumulation in them, likely through inhibition of an unknown lysosomal protein target[40]. Based on the available literature, TPC2 has not yet been explored as a possible target for the activity of Lys05 (**1**). In this work, the characterisation of Lys05 (**1**) as a TPC2 inhibitor and a first SAR investigation of Lys05 (**1**) and its derivatives is described.

For an in-depth characterisation, systematic structure modifications of this compound were performed, in order to get meaningful information regarding the SARs of Lys01 (**4**) and related TPC2 inhibitors. The first modifications on Lys01 (**4**) involved methylation on the two aromatic amines (Figure 4, in red), substitutions on the central tertiary aliphatic amine, including the *N*-nor-Lys01 and various substituents (residue R, in green), and a set of monomers (in blue), to explore the actual need of its symmetrical structure.

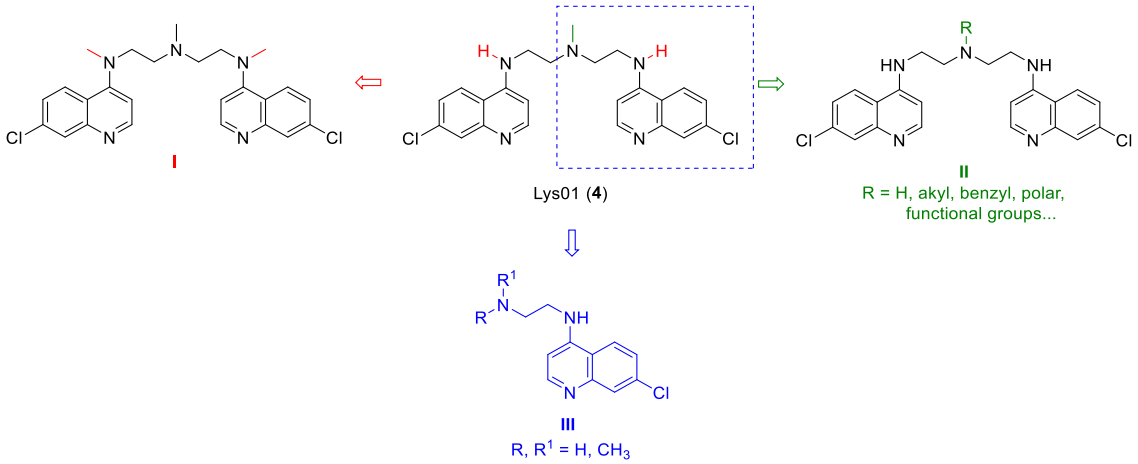


Figure 4: Firsts planned modifications of Lys01 (**4**) structure. Lys05 (**1**) is the trihydrochloride of Lys01 (**4**).

The lysosomal patch-clamp technique allows to analyse TPC inhibitors right after activation with the endogenous TPC2 activators (NAADP and $PI(3,5)P_2$) but it's a very delicate and complex technique. Therefore, more accessible calcium-imaging experiments, using Fura-2 (**5**) and Fluo-4 (**6**) as dyes, were conducted. These techniques allow to detect changes in the calcium levels of the cells and, consequently, to efficiently assess the potential activity of Lys01 (**4**) derivatives as TPC2 inhibitors.

2.2 SARS-CoV-2 RNA ligands

As mentioned in the Introduction (see Chapter 1.5), the structural 5_SL1 RNA element of SARS-CoV-2 genome was highlighted by recent studies as crucial viral target[78, 81, 84]. The fragment lead, taken as starting point of this work and identified by Sreeramulu and Richter *et al.*[92] by NMR spectroscopy, consists of a benzothiazole scaffold, modified at the amino group as *N*-methylurea (Figure 6, compound **7**). The study of fragment interactions with biomolecules using NMR measurements was first addressed by the work of Shuker *et al.*[93] as a powerful tool for fragment lead detection and SAR analysis. It allows the identification of interactions between small molecules and biomolecules, such as RNA, with the added advantage of testing the ligand and the target in a solution that is close to the physiological state. Small fragments, potentially part of drug candidates, can be screened against the biological target detecting even weak target-fragment interactions in a process known as fragment-based screening. This NMR-based screening consisted in three different independent NMR experiments, based on the change in the hydrogen signals of the ligand in the presence and absence of the RNA[92], determined by 1D-^{1}H NMR. First, chemical shift perturbations (CSPs), induced by the binding, were identified: when a compound binds to a target, shifts or changes in the NMR spectra can be detected. Second, water-ligand observed *via* gradient spectroscopy (wLOGSY)[94] was utilised, measuring the transfer of magnetisation from water to the ligand. In the event of a binding between the RNA and the ligand, a positive signal is recorded, while, on the other hand, a negative signal states for a non-binding event. As third and last parameter, relaxation times (T_2) were determined by means of Carr-Purcell-Meiboom-Gill (CPMG) experiments[95]. This technique exploits the transverse T_2 using a pulse sequence, which correlates with the molecular rotational correlation time: high molecular weight complexes, due to ligand-RNA interactions, show broad NMR signals in comparison to small molecules, in this case the free ligand, whose faster relaxation gives sharper peaks. These three parameters were determined for each compound, as shown in Figure 5 for the lead fragment **7** (CSP = 3.96 Hz, wLOGSY = 0.78, T_2 = 66.95%). According to these results, compounds that respected at least two of the following statements were considered as hits: CSP ≥3 Hz, wLOGSY ≥0.7, and T_2 ~40%.

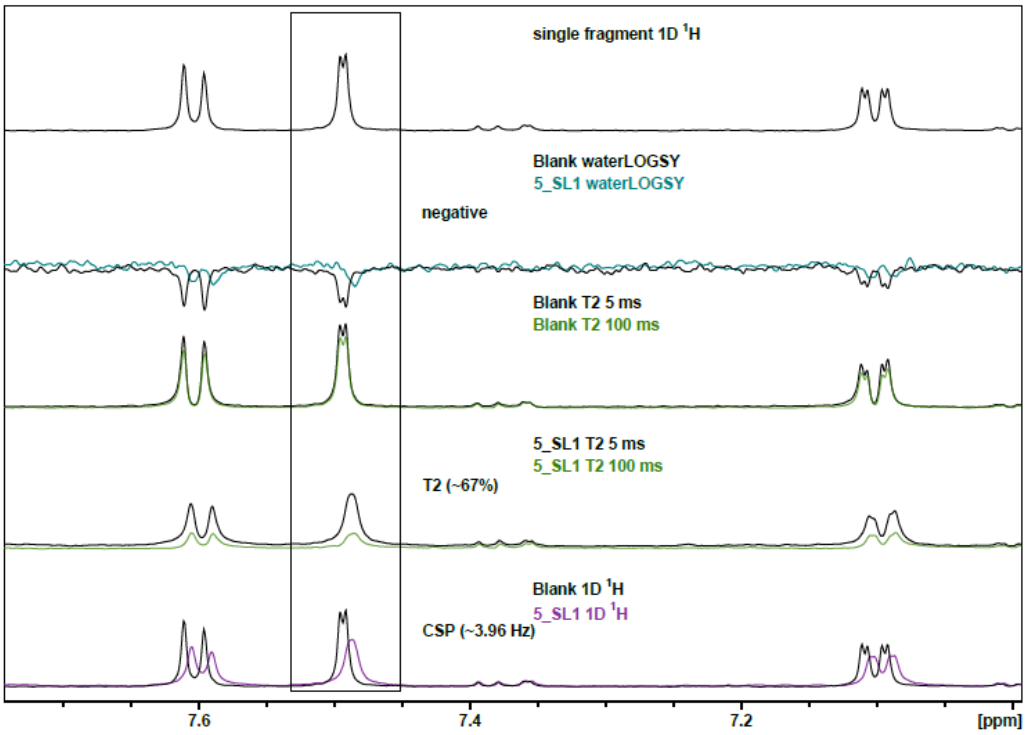


Figure 5: Determination of the three parameters for lead fragment **7**. From top to bottom: ^{1}H NMR of the free ligand **7**; wLOGSY spectra in absence of 5_SL1 (black) and in presence of 5_SL1 (light blue); T_2 respectively in absence (black) and presence of 5_SL1 (green); ^{1}H NMR in absence of 5_SL1 (black) and in presence of 5_SL1 (violet). NMR spectra were recorded at 298 K, 600 Hz, 10 µM RNA, and 200 µM **7** in NMR screening buffer (25 mM KPi, pH 6.2, 50 mM KCl, 5% DMSO-d_6).

Systematic modifications of the structure of **7** were planned in order to increase the affinity towards the target and its selectivity for the 5_SL1 region, as shown in Figure 6: different functionalisations of the 2-amino group, as guanidine derivatives, ureas, and amides (structures **I**, in blue); various substitution patterns of the aromatic region (structures **II**, in red); replacement of the benzothiazole moiety with other bicyclic heteroaromatic rings (structures **III** in green).

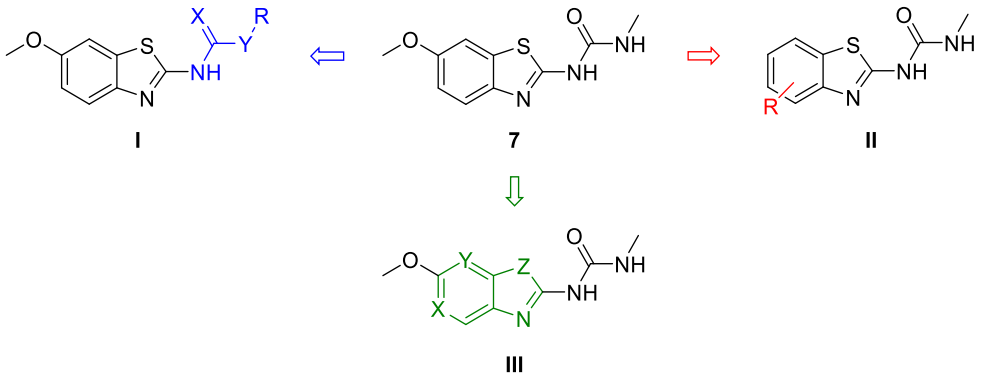


Figure 6: Planned modifications of the fragment lead **7**: 2-amino functionalisation (structures **I**, in blue), substitution pattern of the aromatic region (structures **II**, in red), and replacement of benzothiazole with bicyclic heteroaromatic rings (structures **III** in green).

The final products synthesised during the course of this work have been regularly tested with NMR-based screening, as previously described in this Chapter.

3 Results and discussion

3.1 TPC2 inhibitors

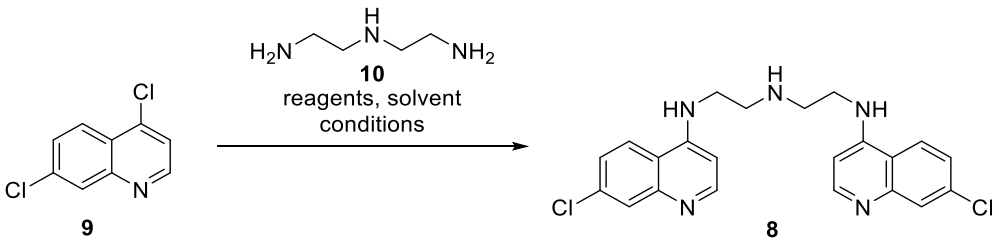
Staring from Lys01 (**4**), systematic modifications to the structure were performed, and the final compounds were tested at first by single cell calcium imaging (see Chapters 3.1.1.3 and 3.1.2.2). Further experiments were then conducted on the most promising compounds to determine IC_{50} values, toxicity, and the electrophysiological behaviour, as described in the following chapters.

3.1.1 First generation of compounds

In this Chapter, the syntheses and the single cell calcium imaging results of the first set of compounds based on the lead structure Lys01 (**4**) are presented.

3.1.1.1 Synthesis of analogues variously substituted on the amino groups

As starting point of the amine substitutions, *N*-demethylated analogue of Lys01 (**4**), compound **8**, was synthesised as depicted in Table 1. At first, literature-known methods were carried out, leading to disappointing results. The synthesis was performed *via* nucleophilic aromatic substitution of 4,7-dichloroquinoline (**9**) with diethylene triamine (**10**). The mesomeric and inductive effects of the quinoline nitrogen, creating an electron deficiency at position C-2 and C-4, and the presence of Cl at C-4 allow a regioselective substitution of the aromatic ring. The first protocol attempted, presented by Van Heerden *et al.*[96] with 3.0 eq of 4,7-dichloroquinoline (**9**) and no solvent, led to a 16% yield (Table 1, entry 1). The reaction was then attempted following Girault *et al.*[97] protocol with only 0.2 eq of **10** and potassium carbonate in *N*,*N*-dimethylformamide (DMF), with a slightly better yield of 24% (entry 2), and with Et_3N in *N*-methylpyrrolidone[98] (entry 3) leading to the same result. It was possible to increase the yield by reducing the temperature to 110 °C, therefore prolonging the reaction time (entry 4). A moderate yield of 48% was obtained by reducing the equivalents of **10** to 0.5 (entry 5).

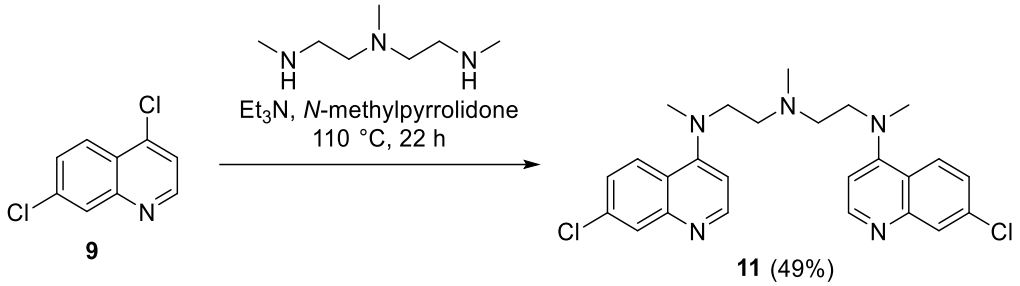


Entry	Reagent	Solvent	Conditions	Yield
1	**10** (3.0 eq)	-	135 °C, 16 h	16%

2	**10** (0.2 eq), K_2CO_3	DMF	120 °C, 8.0 h	24%
3	**10** (1.0 eq), Et_3N	*N*-methylpyrrolidone	130 °C, 6.5 h	24%
4	**10** (1.0 eq), Et_3N	*N*-methylpyrrolidone	110 °C, 11 h	37%
5	**10** (0.5 eq), Et_3N	*N*-methylpyrrolidone	110 °C, 22 h	48%

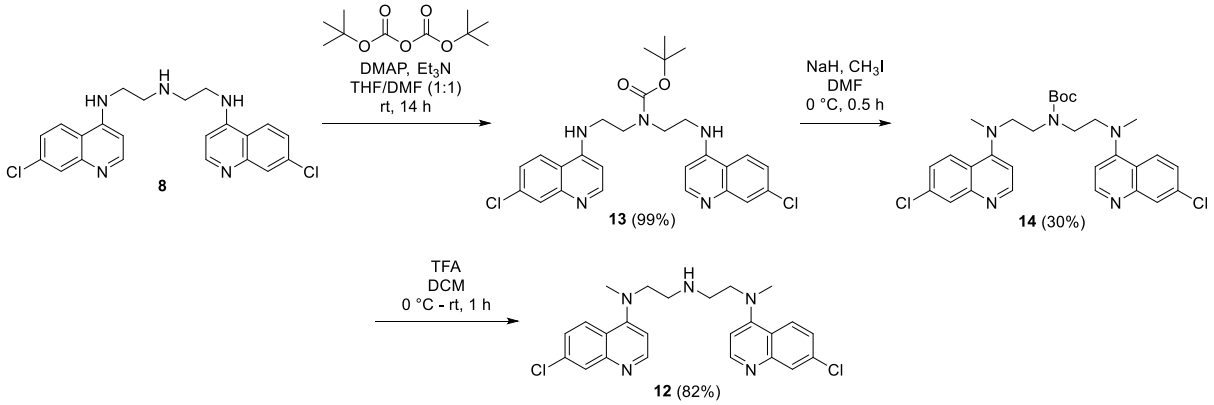
Table 1: Implementation of the synthesis of *N*-demethylated compound **8**.

The *N*,*N*',*N*''-trimethylated analogue **11** was synthesised from *N*,*N*',*N*''-trimethyldiethylene triamine using the protocol of Table 1, entry 5 with a similar outcome of 49% yield, as depicted in Scheme 1.



Scheme 1: Synthesis of *N*,*N*',*N*''-trimethylated analogue **11**.

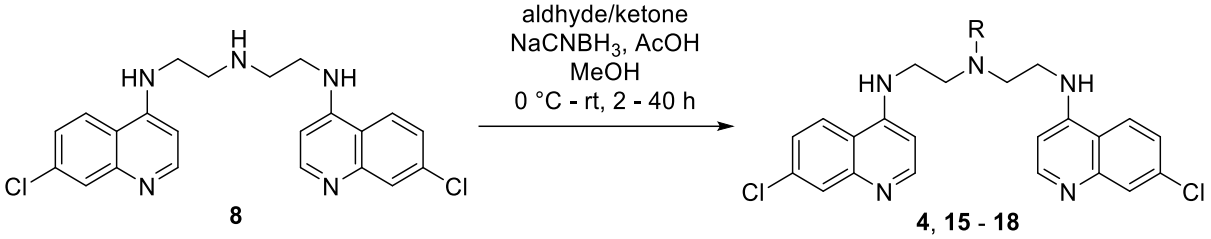
A different approach was used to synthesise the *N*,*N*''-dimethyl analogue **12**, selectively methylated only on the two aromatic amines (Scheme 2). Due to the weaker nucleophilicity of *N*-aryl amines, the treatment with a strong methylating agent was needed, upon protection of the central aliphatic amine. Triamine **8** was chemoselectively Boc-protected at the aliphatic amino group with *tert*-butoxycarbonyl anhydride and 4-dimethylaminopyridine (DMAP) to give compound **13**, which was then treated with NaH and iodomethane. The dimethylated product **14** underwent the TFA-mediated Boc deprotection to release the free triamine **12**.

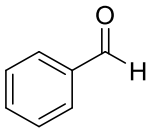
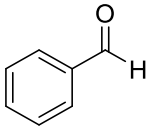
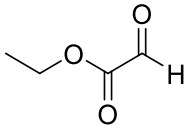


Scheme 2: Synthesis of *N*,*N*''-dimethylated analogue **12**.

The alkylation of the secondary aliphatic amine **8** was, without a need for protection of the secondary aromatic amino groups, achieved selectively *via* reductive *N*-alkylation, due to its

stronger nucleophilicity in comparison with *N*-aryl amines. *N*-Methylation was performed utilising formaldehyde and sodium triacetoxyborohydride as reducing agent with 64% yield (Table 2, entry 1), while with benzaldehyde derivative **15** was isolated with a very poor yield (entry 2, 3%). Using the stronger reducing reagent sodium cyanoborohydride, *N*-benzylated analogue **15** was synthesised with a 57% yield (entry 3) and the protocol was applied with acetaldehyde, acetone, and ethyl glyoxalate, to obtain respectively compounds **16** – **18** (entries 4 – 6).



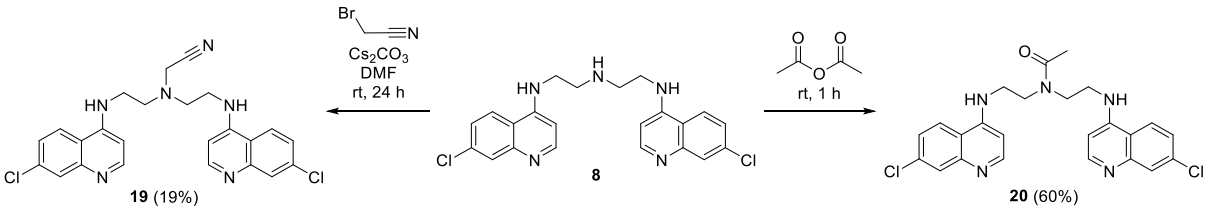
Entry	Aldehyde/ketone	R	Yield (%)
1		CH_3	Lys01 (**4**, 64%)*
2		CH_2Ph	**15** (3%)*
3		CH_2Ph	**15** (57%)
4		CH_2CH_3	**16** (64%)
5		$CH(CH_3)_2$	**17** (81%)
6		CH_2COOEt	**18** (46%)

*Conditions: $NaBH(OAc)_3$, Et_3N, MeOH, rt, 24 h.

Table 2: Synthesis of *N*-alkylated derivatives of oligoamine **8**.

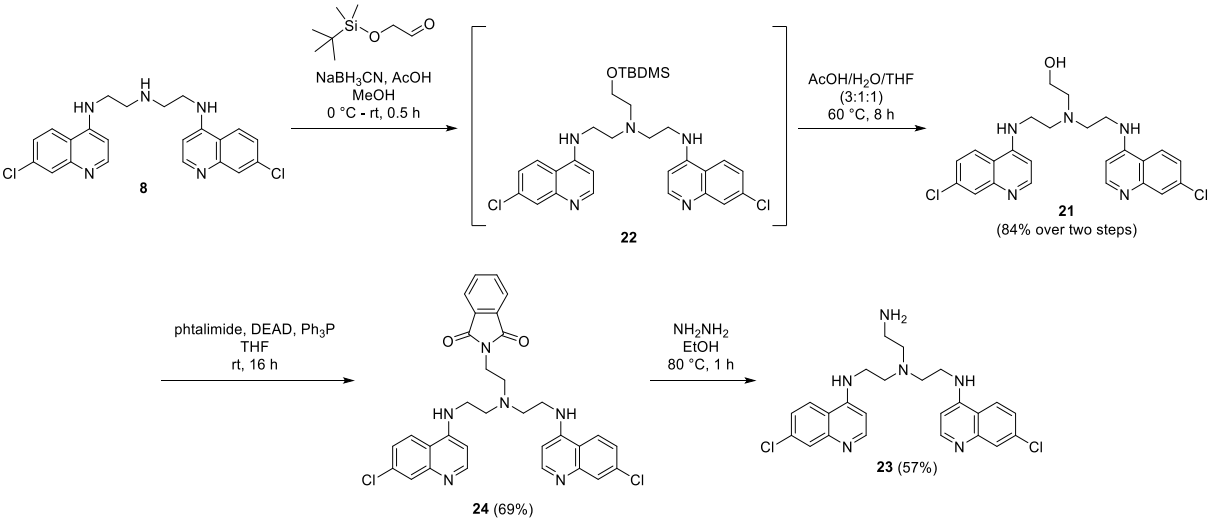
N-Substituted derivatives of amine **8** containing polar functional groups were synthesised as follows. Nucleophilic substitution on 2-bromoacetonitrile with Cs_2CO_3 as base gave *N*-cyanomethyl derivative **19**, while *N*-acetylation with acetic anhydride gave *N*-acetyl derivative

20, as an example of a derivative missing basicity at the central position of the oligoamine linker (Scheme 3).



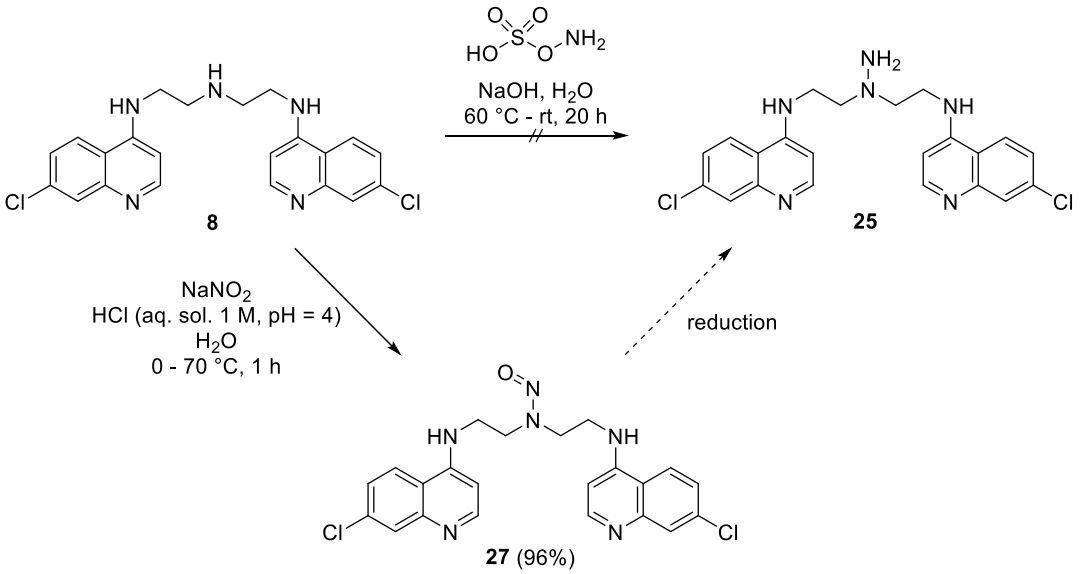
Scheme 3: Synthesis of *N*-cyanomethyl derivative **19** and *N*-acetyl derivative **20**.

To obtain *N*-hydroxyethyl derivative **21** (Scheme 4), alkylation of amine **8** was performed *via* reductive alkylation, as previously described in Table 2, entries 3 – 6, using glycolaldehyde protected as *tert*-butyldimethylsilyl (TBDMS) ether as alkylating agent to give TBDMS-protected *N*-hydroxyethyl derivative **22**. The deprotection was conducted in aq. acetic acid[99] without isolating the intermediate, giving *N*-hydroxyethyl derivative **21** with an overall 84% yield. *N*-Aminoethyl derivative **23** was synthesised by conversion of alcohol **21** into phthalimide derivative **24**, performing a Mitsunobu reaction with phthalimide and diethyl azodicarboxylate (DEAD), followed by hydrazinolysis to give the free tetraamine **23**.



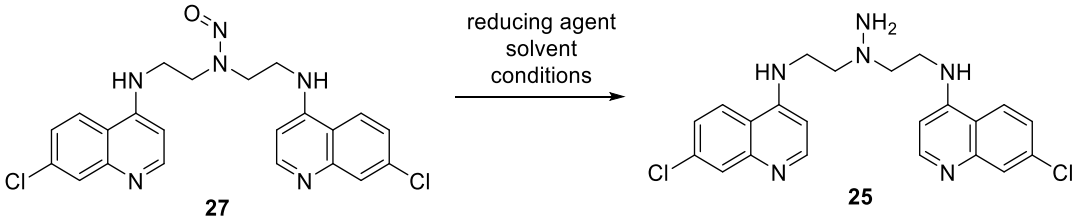
Scheme 4: Synthesis of *N*-hydroxyethyl derivative **21** and *N*-aminoethyl derivative **23**.

For the synthesis of hydrazine derivatives **25** and **26**, two possible pathways were considered, as presented in the work of Lodo *et al.*[100]. The one-step synthesis starting from secondary amine **8**, using hydroxylamine-*O*-sulfonic acid and sodium hydroxide, to directly obtain hydrazine **25**, which failed, and the *N*-nitrosation of secondary amine **8**, which proceeded smoothly to nitrosamine intermediate **27** (Scheme 5). The additional step of reduction to hydrazine derivative **25**, whose results are listed in Table 3, was on the other hand troublesome.



Scheme 5: Initial attempts to synthesise hydrazine derivative **25**.

The reduction of nitrosamine intermediate **27** to hydrazine derivative **25** was at first attempted with zinc powder in aq. acetic acid[100] and in conc. aq. HCl[101] with no reaction occurring (Table 3, entries 1 and 2). The same result was obtained with lithium aluminium hydride[101] and titanium(III) chloride[102] (entries 3 and 4). Another attempt was performed with thiourea dioxide (TDO), as described by Chaudhary *et al.*[103]. Despite the method was developed and implemented on aryl nitrosamines, aliphatic nitrosamines were reduced to hydrazine derivatives with 22 – 30% yield[103]. Applying the same reaction conditions, no reduction was observed on **27** as substrate (entry 5). Surprisingly, product **25** was isolated in a 25% yield using diisobutylaluminium hydride (DIBAL-H) as a reducing agent (entry 6), even being a weaker reducing agent than $LiAlH_4$. Hypothetically, interactions between the quinoline and the reducing agents could prevent this reaction, which is perhaps favoured with DIBAL-H due to the steric hindrance of the reagent. The increases of the amount of DIBAL-H to 3.5 eq and the temperature to 40 °C led to a 62% yield (entry 7), while 4.0 eq of DIBAL-H at room temperature with slightly longer reaction time (3.0 h vs 1.5 h) gave the best result of 74% yield (entry 8).



Entry	Reducing agent	Solvent	Conditions	Yield
1	Zn	H_2O/AcOH (50% v/v)	50 °C, 22 h	0%
2	Zn, conc. HCl	THF	rt, 23 h	0%

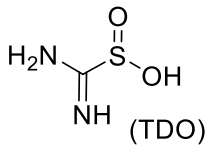
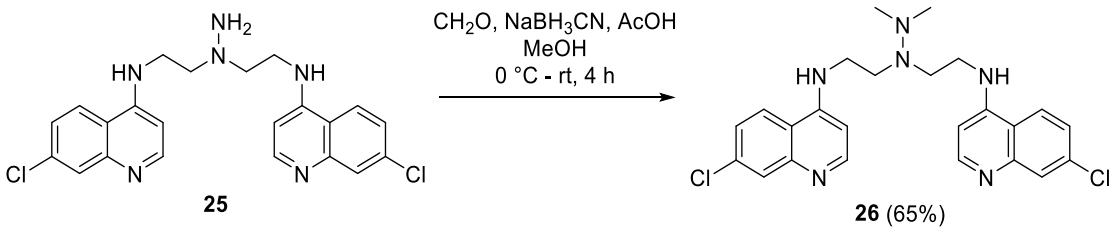
3	$LiAlH_4$	THF	0 °C – rt, 24 h	0%
4	$TiCl_3$	MeOH	rt, 48 h	0%
5	 (TDO) NaOH	MeOH	50 – 80 °C, 15 h	0%
6	DIBAL-H (2.0 eq, 1 M in DCM)	DCM	0 °C – rt, 3.0 h	25%
7	DIBAL-H (3.5 eq, 1 M in DCM)	DCM	0 – 40 °C, 1.5 h	62%
8	DIBAL-H (4.0 eq, 1 M in DCM)	DCM	0 °C – rt, 3.0 h	74%

Table 3: Reduction of nitrosamine intermediate **27** to hydrazine derivative **25**.

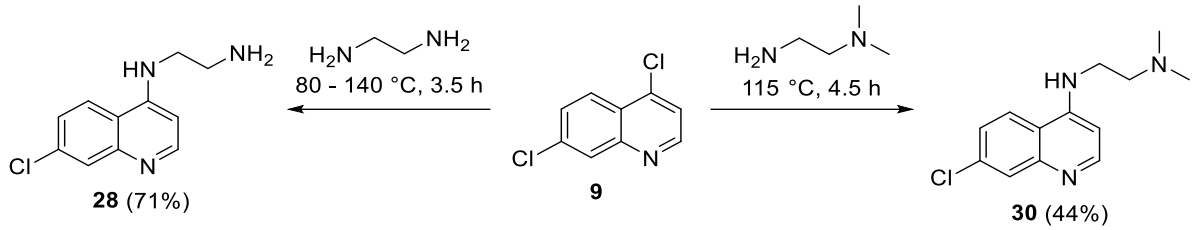
N,*N*-Dimethylated hydrazine derivative **26** was then obtained *via* reductive methylation of hydrazine derivative **25** with a 65% yield (Scheme 6).



Scheme 6: Synthesis of *N*,*N*-dimethylated hydrazine derivative **26** from hydrazine derivative **25**.

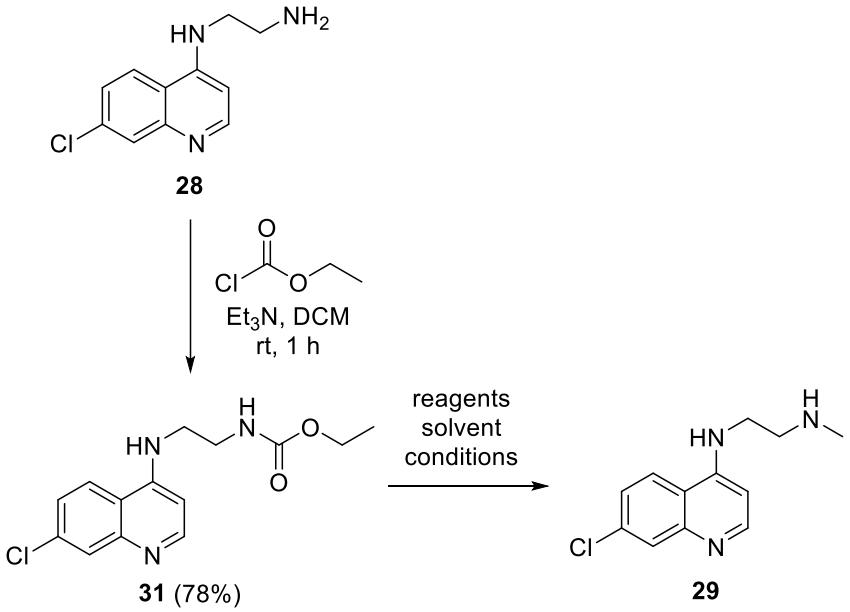
3.1.1.2 Synthesis of monomers

Monomers **28** – **30** were synthesised as depicted in Schemes 7 and 8. The 2-aminoethylamino derivative **28**[104] and (*N*,*N*-dimethylamino)ethylamino derivative **30**[105] were obtained *via* regioselective nucleophilic aromatic substitution of 4,7-dichloroquinoline (**9**) with respectively ethylendiamine and *N*,*N*-dimethylethylenediamine (Scheme 7). In comparison with the synthesis of compounds **8** and **11** (see Chapter 3.1.1.1, Table 1 and Scheme 1), a large excess of the respective diamines fulfils the role of reagent, auxiliary base, and solvent.



Scheme 7: Synthesis of monomers **28** and **30**.

A first approach for the synthesis of the *N*-monomethyl analogue **29** was attempted *via* conversion of primary amine **28** into ethyl carbamate **31** and subsequent reduction, a common and secure way to obtain *N*-monomethylated amines. As expected, the synthesis of ethyl carbamate derivative **31** with ethyl chloroformate proceeded smoothly, while the reduction step was troublesome (Table 4). The reduction was attempted with lithium aluminium hydride[106] (Table 4, entry 1) and borane tetrahydrofuran complex[107] (entry 2) as reducing agents. The desired product **29** was obtained with unsatisfactory yields.

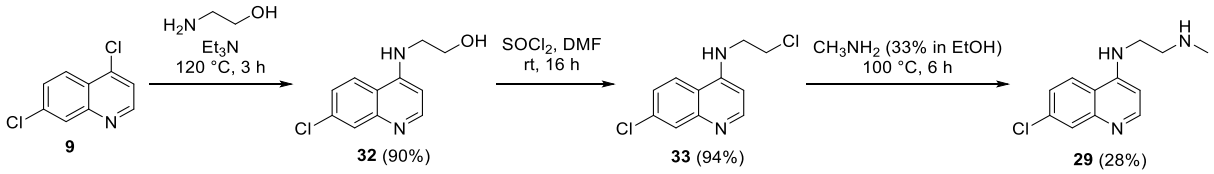


Entry	Reagent, solvent, conditions	Yield
1	$LiAlH_4$ (11 eq), THF, 0 – 75 °C, 22 h	16%
2	1. BH_3·THF (8.0 eq), THF, 0 – 75 °C, 24 h 2. HCl (4 N in dioxane), MeOH, 0 – 65 °C, 15 min	18%

Table 4: First attempts to synthesise **29** *via* reduction of carbamate **31**.

To avoid the reduction step, which already proved to be particularly time consuming for the nitrosamine derivative **27** (see Chapter 3.1.1.1, Table 3), a completely different pathway was followed. Interestingly, the poor outcome of this common reduction might enforce the previously mentioned idea of interactions between the quinoline and the reducing agents,

preventing the efficient reduction in both cases, for nitrosamine **27** and carbamate **31**. Following the alternative pathway, described in literature by de Souza *et al.*[104], 4,7-dichloroquinoline (**9**) was aminated with 90% yield with ethanolamine to intermediate **32**, whose hydroxy group was then easily converted into alkyl chloride **33** by treatment with thionyl chloride (Scheme 8). The final amination with methylamine *via* nucleophilic substitution gave the desired monomethylated product **29** in moderate yield.



Scheme 8: Synthesis of monomethylated monomer **29**.

3.1.1.3 First screening *via* single cell calcium imaging

In a first step, lead compound Lys01 (**4**) was tested with Fura-2-based single cell calcium imaging, performed by myself in Prof. Grimm's lab. The cell wall permeable dye Fura-2/AM (**34**) detects subtle changes in intracellular Ca^{+2} levels in adherent cells. Upon entering the cell, the lipophilic "double ester" Fura-2/AM (**34**) is hydrolysed by esterases to the corresponding tetra-carboxylate Fura-2 (**5**), which absorbs at 380 nm. Calcium ions enter cells *via* TPC2 activation, and the subsequent formation of Fura-2 calcium complexes leads to a shift of absorption from 380 to 340 nm with emission at 510 nm (Figure 7).

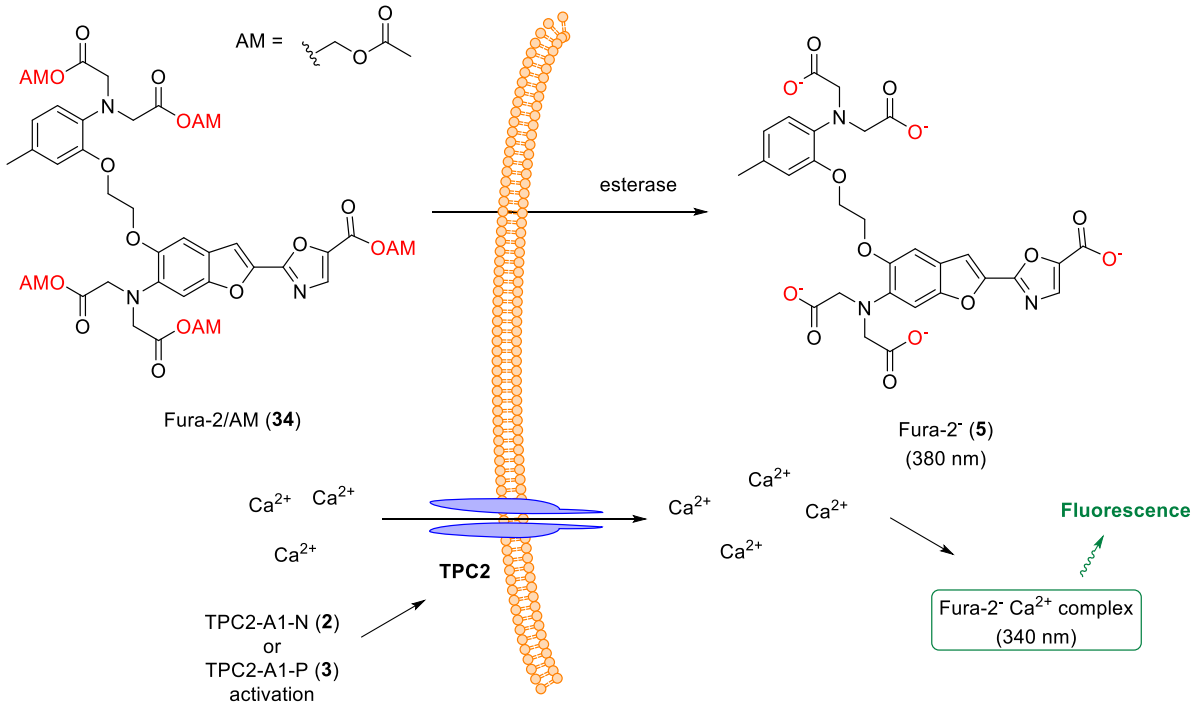


Figure 7: Schematic representation of Fura-2-based single cell calcium imaging. The lipophilic Fura-2/AM ester (**34**) enters the cell, and the free Fura-2 (**5**) tetracarboxylate is released (excitation wavelength 380 nm) *via*

esterase-mediated cleavage. Activation with TPC2-A1-N (**2**) or TPC2-A1-P (**3**) leads to TPC2 opening, increasing intracellular calcium level. The excitation wavelength of Fura-2 (**5**) shifts then to 340 nm upon complexation of calcium ions, and the emission is detected at 510 nm.

The inhibitor was added first, before stimulating the cells with the synthetic agonists (TPC2-A1-N (**2**) and TPC2-A1-P (**3**)), and the effect was monitored over time (Figure 8, **B** and **D**), as described in the work of Müller *et al.*[30]. In the control experiment, DMSO was added first, followed by agonist application. The difference in activation levels, measured after 400 s (Figure 8, **A** and **C**), in the presence of the inhibitor or DMSO allowed to determine the inhibitory activity. The inhibitory effect of Lys01 (**4**) was significant (***) for both activators, confirming the results obtained for Lys05 (**1**) with the HTS (see Chapter 2.1, Figure 3).

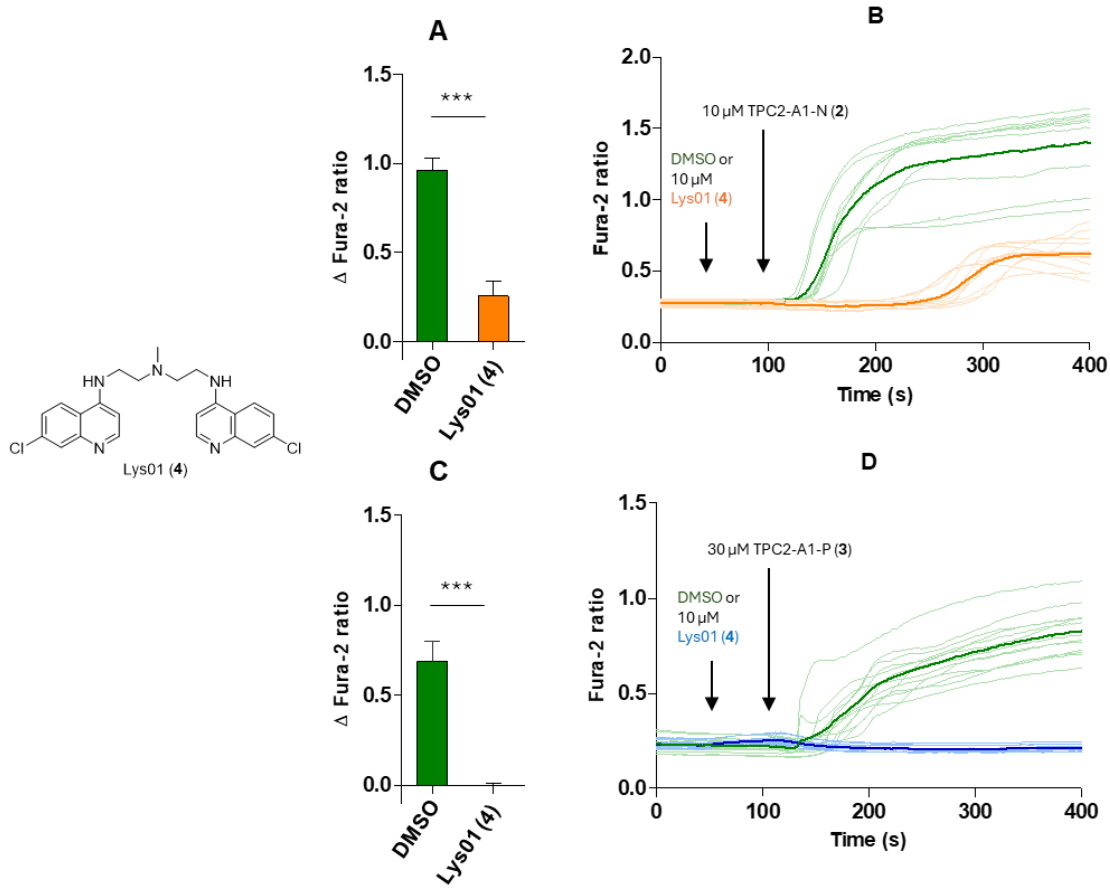


Figure 8: Single cell calcium imaging experiments to confirm Lys01 (**4**) as inhibitor. Measurements recorded from HEK293 cells stably expressing TPC2$^{L11A/L12A}$-RFP. (**A**) Cells were treated with DMSO control (0.5% DMSO in HBS) or Lys01 (**4**, 10 µM), and then activated with TPC2-A1-N (**2**, 10 µM). Mean values, measured at 400 s, normalised to basal ± SEM of three independent experiments with 8–10 cells each are shown. ***p <0.001, using unpaired t test. (**B**) Representative single experiment recording Ca^{+2} currents as in (A). Green lines represent the control measurements with DMSO, orange lines with Lys01 (**4**). The thicker lines represent the mean response of the cell population. (**C**) Experiments as in (A), using TPC2-A1-P (**3**, 30 µM) as agonist. (**D**) Experiment as in (B), representative single experiment recording Ca^{+2} currents as in (C). Blue lines represent the measurement with Lys01 (**4**).

Next, the experiments described above (see Figure 8, graphs **B** and **D**), were conducted in three replicates for each Lys01 (**4**) analogue. The results are summarised in Figure 9 and represent the activations with TPC2-A1-N (**2**, in orange) and TPC2-A1-P (**3**, in blue) after inhibition at 400 s, normalised to baseline. In this first, rather qualitative screening, the *N*-demethylated analogue **8** gave similar results compared to Lys01 (**4**), displaying significant inhibition (***) after stimulation with both TPC2-A1-N (**2**) and TPC2-A1-P (**3**). Analogues **11** and **12**, which structurally share the methylation on both aromatic amines, displayed a loss in inhibitory effect on both activators, especially with TPC2-A1-P (**3**) activation, where no significant inhibitory effect was registered. *N*-Ethyl analogue **16** and *N*-benzyl analogue **15** were identified as promising selective blockers: in comparison with Lys01 (**4**), compound **16** showed a lower inhibition effect (**) for TPC2-A1-P (**3**), along with no significant effect with TPC2-A1-N (**2**) activation; on the other hand, **15** maintained the inhibitory effect with TPC2-A1-P (**3**), with loss of inhibition with TPC2-A1-N (**2**, **). *N*-Isopropyl analogue **17** did not show the same trend, keeping the inhibitory effect on TPC2-A1-N (**2**, ***) and losing it on TPC2-A1-P (**3**, ns). Among the other analogues, variously substituted on the central amino group, *N*-cyanomethyl derivative **19**, *N*-acetyl derivative **20**, ester **18**, *N*-hydroxyethyl derivative **21**, *N*-aminoethyl derivative **23**, and the two hydrazine derivatives **25** and **26** showed a loss of inhibition with both activators. The only exception was the *N*-aminoethyl analogue **23**, displaying an inhibitory effect on TPC2-A1-N (**2**, **). In contrast to most of the dimeric analogues, the monomers **29** and **30** showed a less significant inhibition on TPC2-A1-N (**2**) activation, compared to Lys01 (**4**), and had no significant inhibitory effect on TPC2-A1-P (**3**) activation. Interestingly, monomer **28** showed an opposite selectivity trend, maintaining a significant inhibition on TPC2-A1-N (**2**) activation (***) compared to a complete loss of inhibition on TPC2-A1-P (**3**).

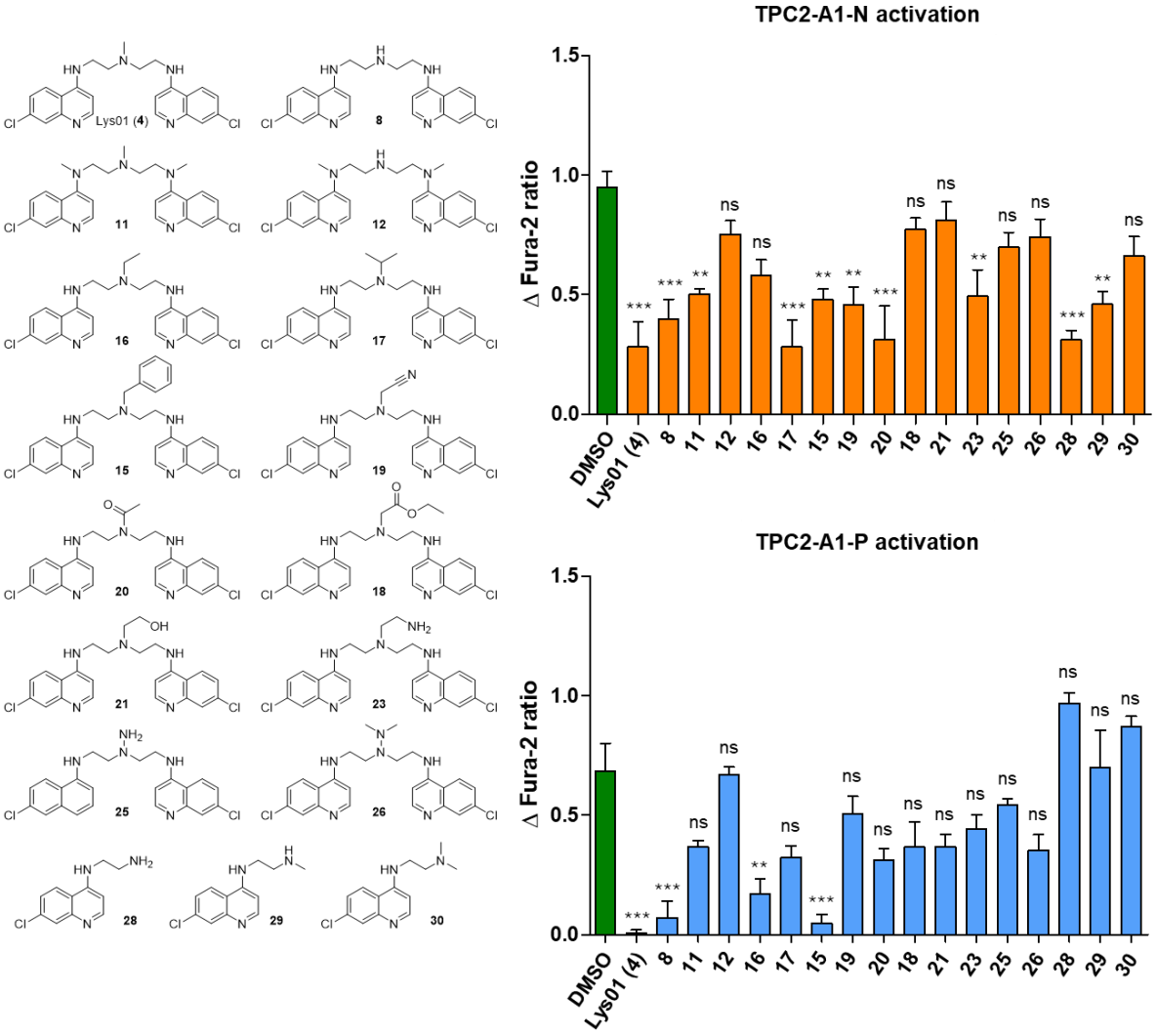


Figure 9: Single cell calcium imaging experiments for test compounds Lys01 (**4**), **8**, **11**, **12**, **15** – **21**, **23**, **25**, **26**, **28** – **30** as inhibitors. Measurements recorded from HEK293 cells stably expressing TPC2$^{L11A/L12A}$-RFP. **TPC2-A1-N**: Cells were treated with compounds Lys01 (**4**), **8**, **11**, **12**, **15** – **21**, **23**, **25**, **26**, **28** – **30** at 10 µM concentration, followed by activation with TPC2-A1-N (**2**, 10 µM). **TPC2-A1-P**: Cells were stimulated with compounds Lys01 (**4**), **8**, **11**, **12**, **15** – **21**, **23**, **25**, **26**, **28** – **30** at 10 µM concentration, followed by activation with TPC2-A1-P (**3**, 30 µM). For all graphs, mean values, measured at 400 s, normalised to basal ± SEM of three independent experiments with 8–10 cells each are shown. ***p <0.001, **p <0.01, *p <0.05, ns = not significant, using one-way ANOVA followed by Tukey's post hoc test. Orange was used for TPC2-A1-N (**2**) activation and blue for TPC2-A1-P (**3**) activation, while in green are depicted DMSO controls.

From these results it was possible to get a first insight into the SARs of Lys01 (**4**). The results obtained with *N*-methylation of both aromatic amino groups, as in compounds **11** and **12**, suggests that even small modifications on this site result in a rather strong decrease of inhibitory potency. Regarding the substitution on the central aliphatic amine, modifications with apolar substituent were more tolerated, with the best results obtained for *N*-demethylated derivative **8**, *N*-ethyl analogue **16**, and *N*-benzyl analogue **15**. Based on the results displayed

by the monomers, the dimeric structure (two chloroquinoline units connected by an oligoamine chain) of the molecule is crucial to maintain the inhibitory effect.

After testing this first series of Lys01 (**4**) derivatives providing no superiority over the lead structure, more structural modifications were carried out, as described in the next Chapter.

3.1.2 Second generation of compounds

The second batch of analogues focused on a different set of modifications, regarding the length and type of the aliphatic linker chain and the role of the chloro substituents on the quinoline ring, as shown in Figure 10, maintaining the free amino group or the methylation on the central amine as in respectively **8** and Lys01 (**4**), given the results obtained with single cell calcium imaging analysis of the previous series of compounds (see Chapter 3.1.1.3, Figure 9).

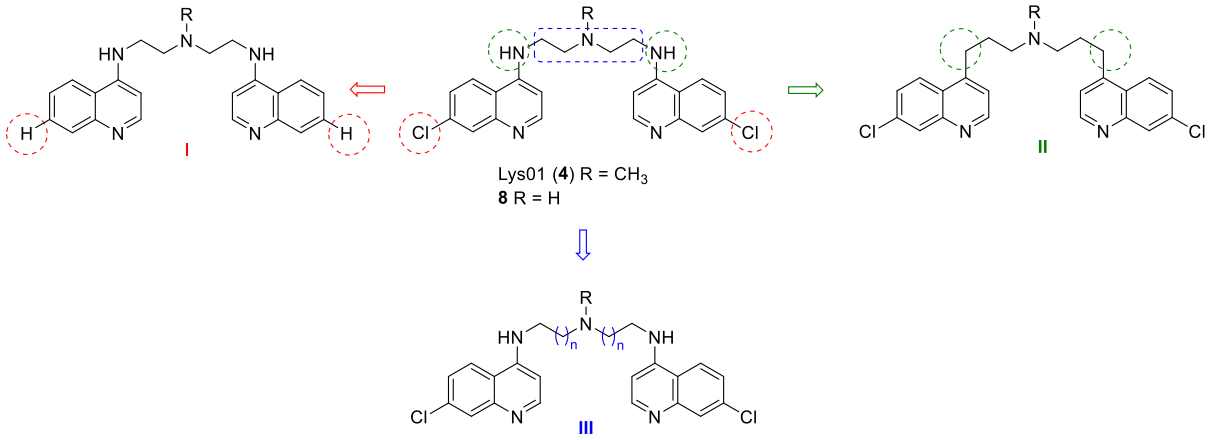
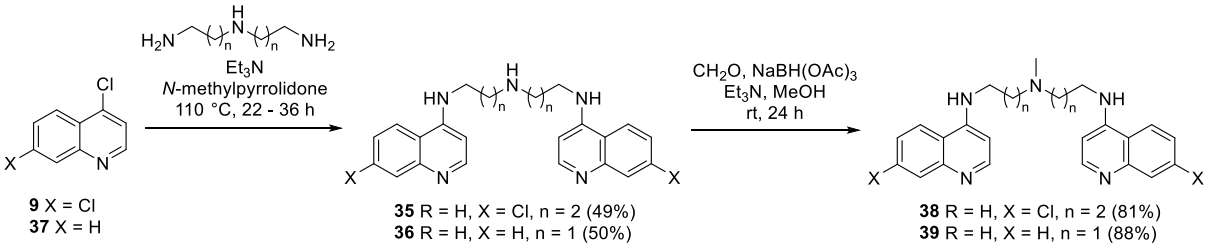


Figure 10: Second set of planned modifications of Lys01 (**4**) and *N*-nor-Lys01 **8**.

The syntheses and the single cell calcium imaging results are described in the following Chapters.

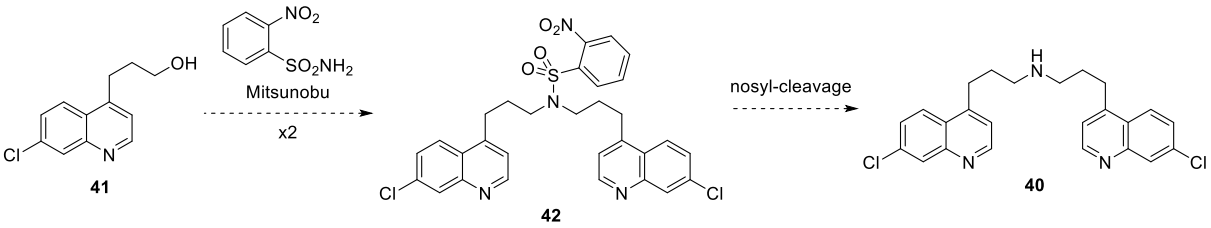
3.1.2.1 Synthesis

Homologue **35** and bis-dechlorinated Lys01 (**4**) analogue **36** (with unmodified chain length) were synthesised applying the same protocol already described for **8** and **11** (see Chapter 3.1.1.1, Table 1 and Scheme 1) *via* regioselective nucleophilic aromatic substitution, using respectively 4,7-dichloroquinoline (**9**) and 4-chloroquinoline (**37**) and the corresponding oligoamines (Scheme 9). The longer reaction time needed for derivative **36** (36 h vs 22 h for **35**) might be due to its very poor solubility in the solvent. The two *N*-methylated derivatives, homologue **38** and bis-dechlorinated analogue **39**, were synthesised *via* reductive *N*-methylation (Scheme 9), as previously described for **4** (see Chapter 3.1.1.1, Table 2).



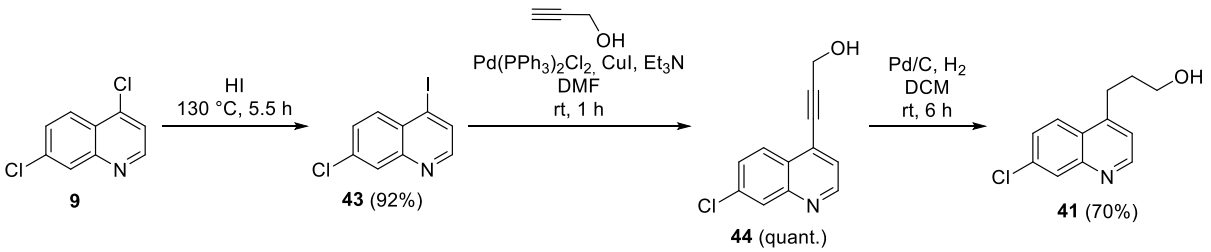
Scheme 9: Synthesis of homologue **35**, bis-dechlorinated analogue **36**, and the corresponding *N*-methylated derivatives **38** and **39**.

To further evaluate the role of the aromatic amino groups, in addition to the results obtained with **11** and **12** (see Chapter 3.1.1.3, Figure 9), in which *N*,*N*'-dimethylation was shown to have a strong negative impact on the activity, the synthesis of the bis-desaza analogue **40** was planned. The initial synthetic plan, depicted in Scheme 10, consists of a Mitsunobu reaction performed two times on the hydroxypropyl derivative **41** with 2-nitrobenzenesulfonamide (nosyl amide), inspired by the work of Guisado *et al.*[108], followed by cleavage of the nosyl group.



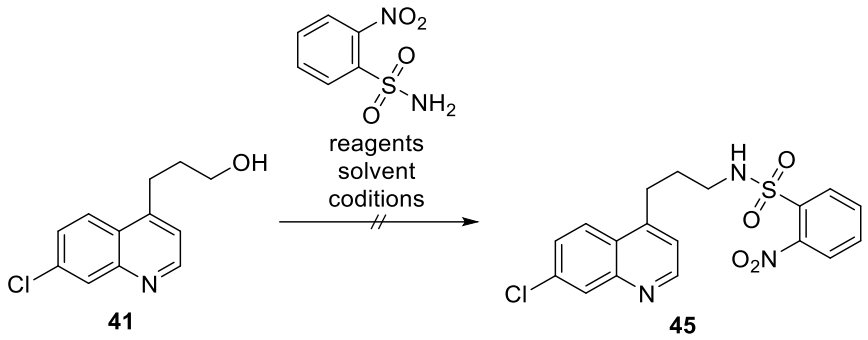
Scheme 10: Initial synthetic plan for bis-desaza analogue **40**.

For the synthesis of 4-(3-hydroxypropyl)quinoline **41** (Scheme 11), an exchange of 4-chloro substituent of 4,7-dichloroquinoline (**9**) for iodine to give 7-chloro-4-iodoquinoline (**43**)[109] was performed in order to guarantee high yields for the following palladium-catalysed cross-coupling. Sonogashira coupling with prop-2-yn-1-ol led to alkyne **44** in a quantitative yield, which was then reduced *via* palladium-catalysed hydrogenation to hydroxypropyl derivative **41**.



Scheme 11: Synthesis of hydroxypropyl quinoline derivative **41**.

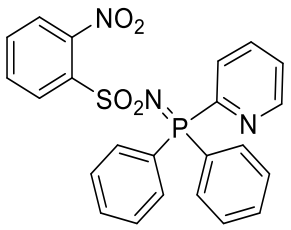
The attempts for the Mitsunobu reaction on the hydroxypropyl derivative **41** with nosyl amide are listed in Table 5. The reaction was performed using diphenyl-2-pyridyl-phopshine and di-*tert*-butyl azodicarboxylate (DBAD) (Table 5, entry 1)[108] and triphenylphosphine and DEAD (entry 2)[110], with no reaction occurring.



Entry	Reagents	Solvent	Conditions
1	diphenyl-2-pyridyl-phopshine, DBAD	DCM	rt, 24 h
2	Ph_3P, DEAD	THF	rt, 48 h

Table 5: Failed attempts of Mitsunobu reaction on the hydroxypropyl derivative **41** with nosyl amide.

The only by-product that was possible to isolate in traces, according to the set of aromatic signals integrated for 18 H and the mass/charge ratio by HRMS (calc. $M+H^+$: 464.0829, found: 464.0827), was the iminophosphorane **46** (Figure 11), whose formation could impair the Mitsunobu reaction.

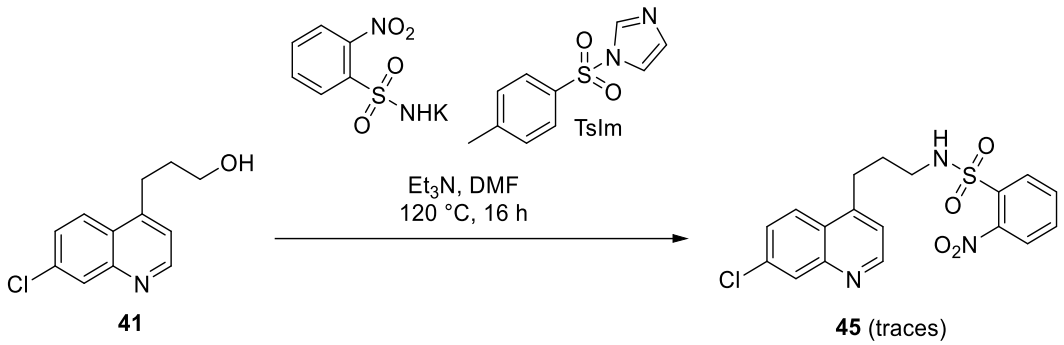


46

Figure 11: Only by-product (conditions: Table 5, entry 1) isolated in traces during the failed Mitsunobu attempts.

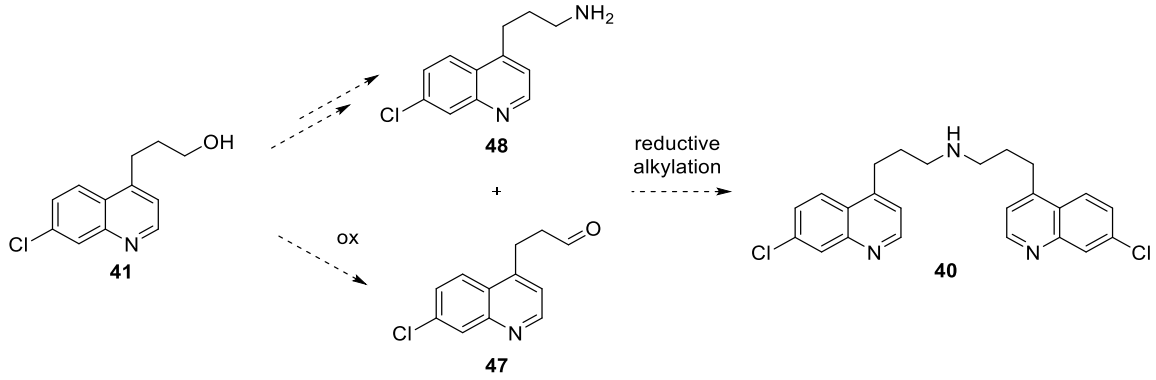
Although by-product **46** is not literature known, the formation of closely related iminophosphoranes has been described for *N*-(triphenylphosphoranylidene)benzenesulfonamides variously substituted on the aromatic ring of the sulfonamide moiety[111-114].

To confirm or confute this hypothesis, the synthesis of the *N*-monosubstituted nosyl amide derivative **45** was attempted following a different protocol, involving the use of *N*-(*p*-toluenesulfonyl) imidazole (TsIm) and the potassium salt of nosyl amide[115], with the idea of performing the Mitsunobu reaction on the intermediate **45**. As shown in Scheme 12, this reaction led to nosyl amide derivative **45** only in traces and, therefore, the amount obtained was not sufficient to go further.



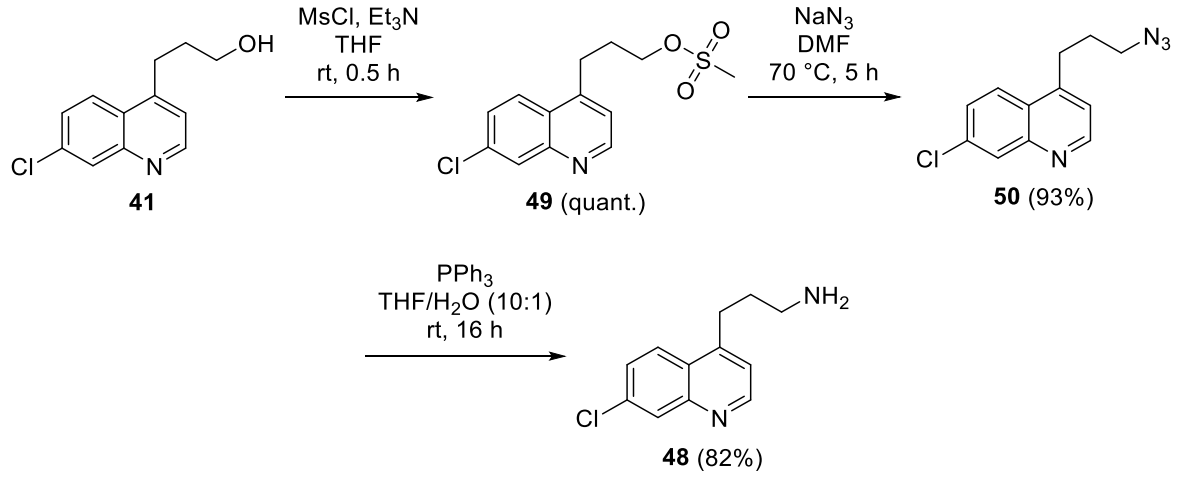
Scheme 12: Attempt to synthesise intermediate **45** with potassium salt of nosyl amide and TsIm.

In the meantime, a completely different pathway was tried out, as depicted in Scheme 13. The idea was to convert the hydroxypropyl intermediate **41** into two complementary reactive groups: on the one hand the corresponding aldehyde **47** and on the other the primary amine **48** to then perform a reductive alkylation between the two intermediates.



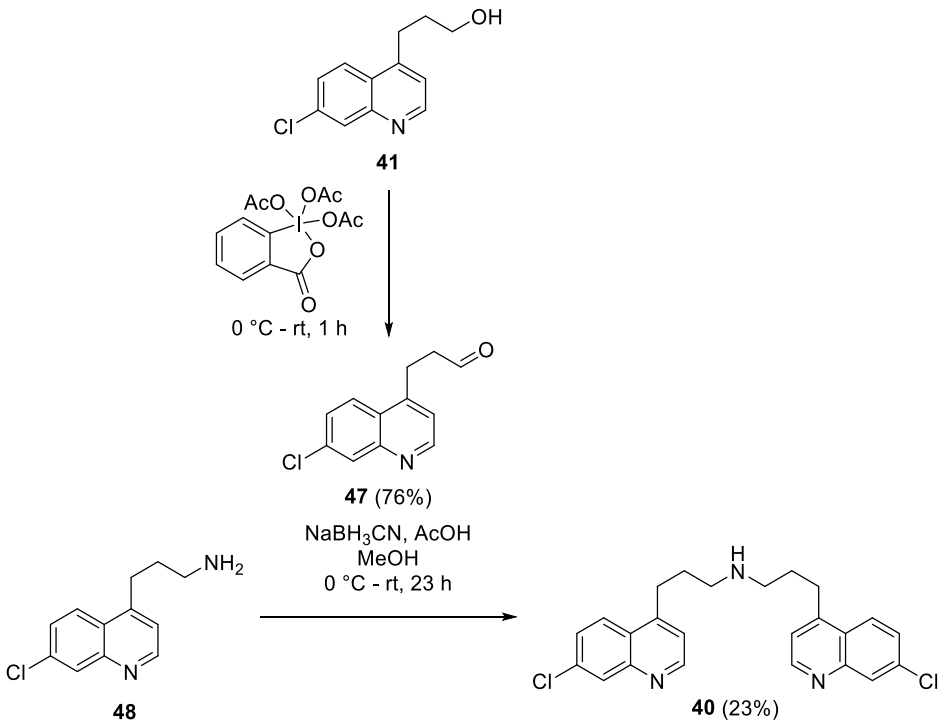
Scheme 13: Alternative synthetic plan for the synthesis of bis-desaza analogue **40**.

For the synthesis of primary amine **48**, the hydroxy group of compound **41** was first activated to mesylate derivative **49** to undergo the nucleophilic substitution with sodium azide to azide derivative **50** (Scheme 14). The desired amine **48** was then obtained by Staudinger reduction of azide derivative **50** with triphenylphosphine in excellent overall yield.



Scheme 14: Synthesis of amine **48** starting from hydroxypropyl derivative **41**.

On the other hand, aldehyde **47** was quickly (1 h) obtained *via* oxidation using Dess-Martin periodinane of hydroxypropyl derivative **41** (Scheme 15), which was then used to reductively alkylate amine **48** with sodium cyanoborohydride to obtain the desired bis-desaza analogue **40**.



Scheme 15: Synthesis of bis-desaza analogue **40**.

3.1.2.2 First screening *via* single cell calcium imaging

Again, Fura-2-based single cell calcium imaging experiments were performed. Results obtained for this second series of compounds are depicted in Figure 12. The two homologues **35** and **36** showed, compared to compound **8**, a comparable inhibitory effect on TPC2-A1-N (**2**) activation but no significant effect on TPC2-A1-P (**3**) activation. The two dechlorinated analogues **36** and **39** and bis-desaza analogue **40** showed no inhibition neither of TPC2-A1-N (**2**) nor TPC2-A1-P (**3**) activation.

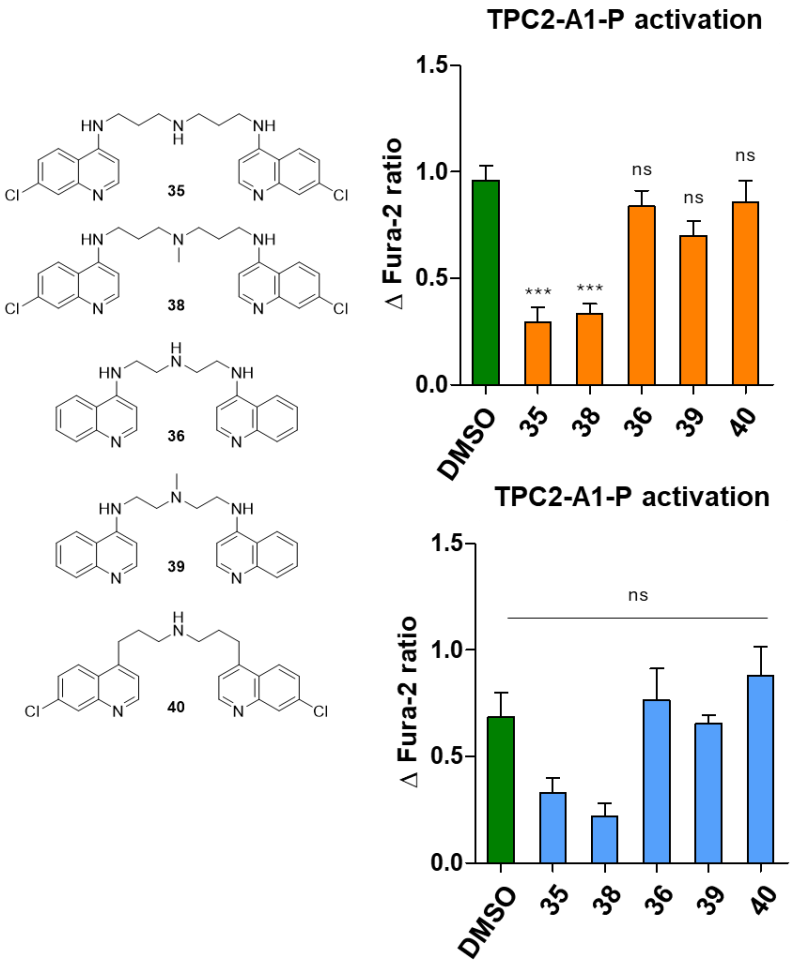


Figure 12: Single cell calcium imaging experiments to test compound **35**, **36**, **38** – **40** as inhibitors. Measurements recorded from HEK293 cells stably expressing TPC2$^{L11A/L12A}$-RFP. **TPC2-A1-N**: Cells were treated with compounds **35**, **36**, **38** – **40** at 10 µM concentration, followed by activation with TPC2-A1-N (**2**, 10 µM). **TPC2-A1-P**: Cells were stimulated with compounds **35**, **36**, **38** – **40** at 10 µM concentration, followed by activation with TPC2-A1-P (**3**, 30 µM). For all graphs, mean values, measured at 400 s, normalised to basal ± SEM of three independent experiments with 8–10 cells each are shown. ***p <0.001, **p <0.01, *p <0.05, ns = not significant, using one-way ANOVA followed by Tukey's post hoc test. Orange was used for TPC2-A1-N (**2**) activation and blue for TPC2-A1-P (**3**) activation, while in green are depicted DMSO controls.

The results obtained with the two homologues **35** and **38** underlined the crucial role of the linker length, while bis-desaza analogue **40** confirmed the conclusion drawn with the analogues **11** and **12** about the crucial role of the secondary aromatic amines in the lead structure in TPC2 inhibition. Also, the chlorination on C-7 of the quinoline, as in compounds **36** and **39**, has a fundamental impact on the activity. Due to the results obtained, no more analogues of this series were substituted additionally with the promising *N*-ethyl and *N*-benzyl substituents, emerged from the previous batch tested (see Chapter 3.1.1.3, Figure 9, respectively compounds **16** and **15**).

3.1.3 Further biological characterisation

The compounds previously presented were further analysed. To get a quantitative description of the inhibitory activity of the analogues previously tested by single cell calcium imaging, dose-response relationships were determined by Fluo-4-based calcium imaging method, performed by Nicole Urban, Schaefer group (University of Leipzig). To assess the toxicity and the microbial activity on various model microorganisms, in-house MTT assay and agar diffusion test, respectively, were performed by Martina Stadler. Patch-clamp experiments were planned to explore the electrophysiological behaviour of the most promising compounds. As these results are still preliminary, they are not presented in this work.

3.1.3.1 Dose–response relationships and toxicity

Full analysis of dose–response relationships was performed by Nicole Urban, using the Fluo-4-based calcium imaging method on compounds that showed noteworthy activity in the single cell calcium imaging experiments (see Chapters 3.1.1.3, Figure 9 and 3.1.2.2, Figure 12). The dye Fluo-4/AM (**51**) detects rapid and large changes in intracellular Ca^{+2} levels. Similarly to Fura-2/AM (**34**) mechanisms (see Chapter 3.1.1.3, Figure 7), the Fluo-4/AM ester (**51**) enters the cells and is hydrolysed by esterases to tetracarboxylate Fluo-4 (**6**). The complexation of calcium ions increases the fluorescence of 100 times, absorbing at 488 nm with 516 nm emission (Figure 13).

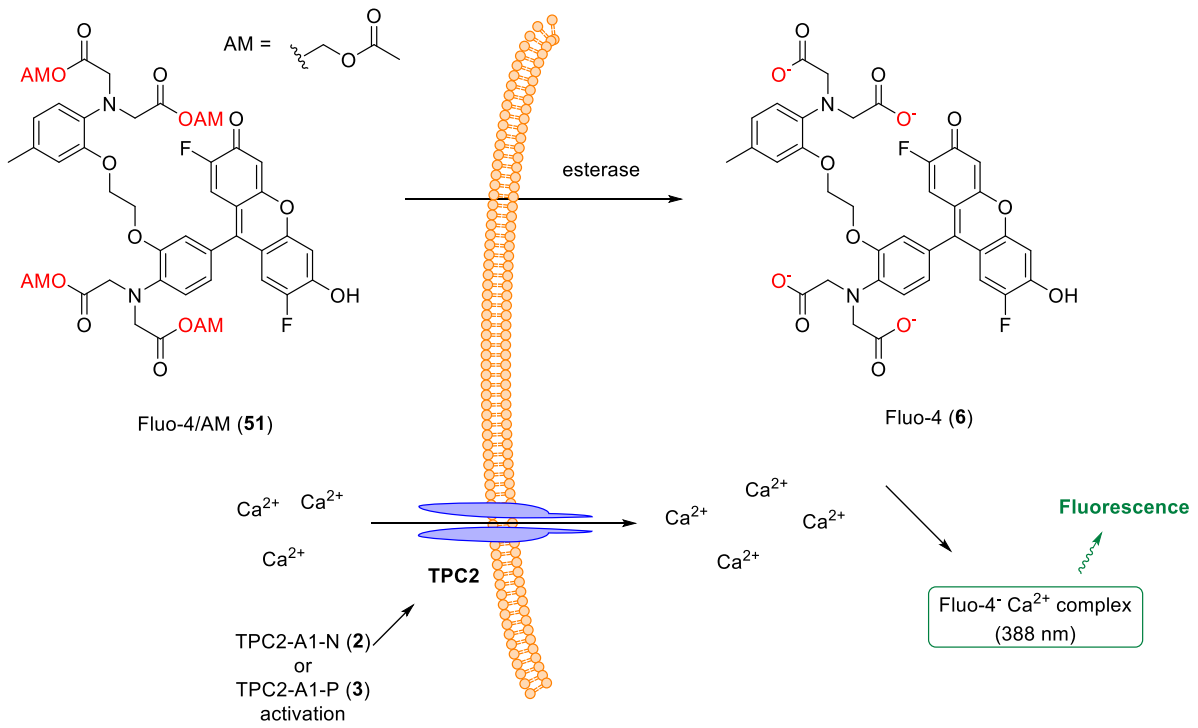


Figure 13: Schematic representation of Fluo-4-based calcium imaging. The lipophilic Fluo-4/AM ester (**51**) enters the cell, and the free Fluo-4 tetracarboxylate (**6**) is released *via* esterases cleavage. Activation with TPC2-A1-N

(**2**) or TPC2-A1-P (**3**) leads to TPC2 opening, increasing intracellular calcium level. The excitation wavelength of Fluo-4 (**6**) shifts then to 388 nm upon complexation of calcium ions, and the emission is detected at 516 nm.

The experiments were performed by sequential application of inhibitors at different concentrations followed by addition of TPC2-A1-N (**2**, 10 µM) or TPC2-A1-P (**3**, 30 µM) in cell suspensions, using the calcium indicator Fluo-4 (**6**) and performing a multiwell plate fluorescence imaging technique. The dose–response curves are shown in Figure 14.

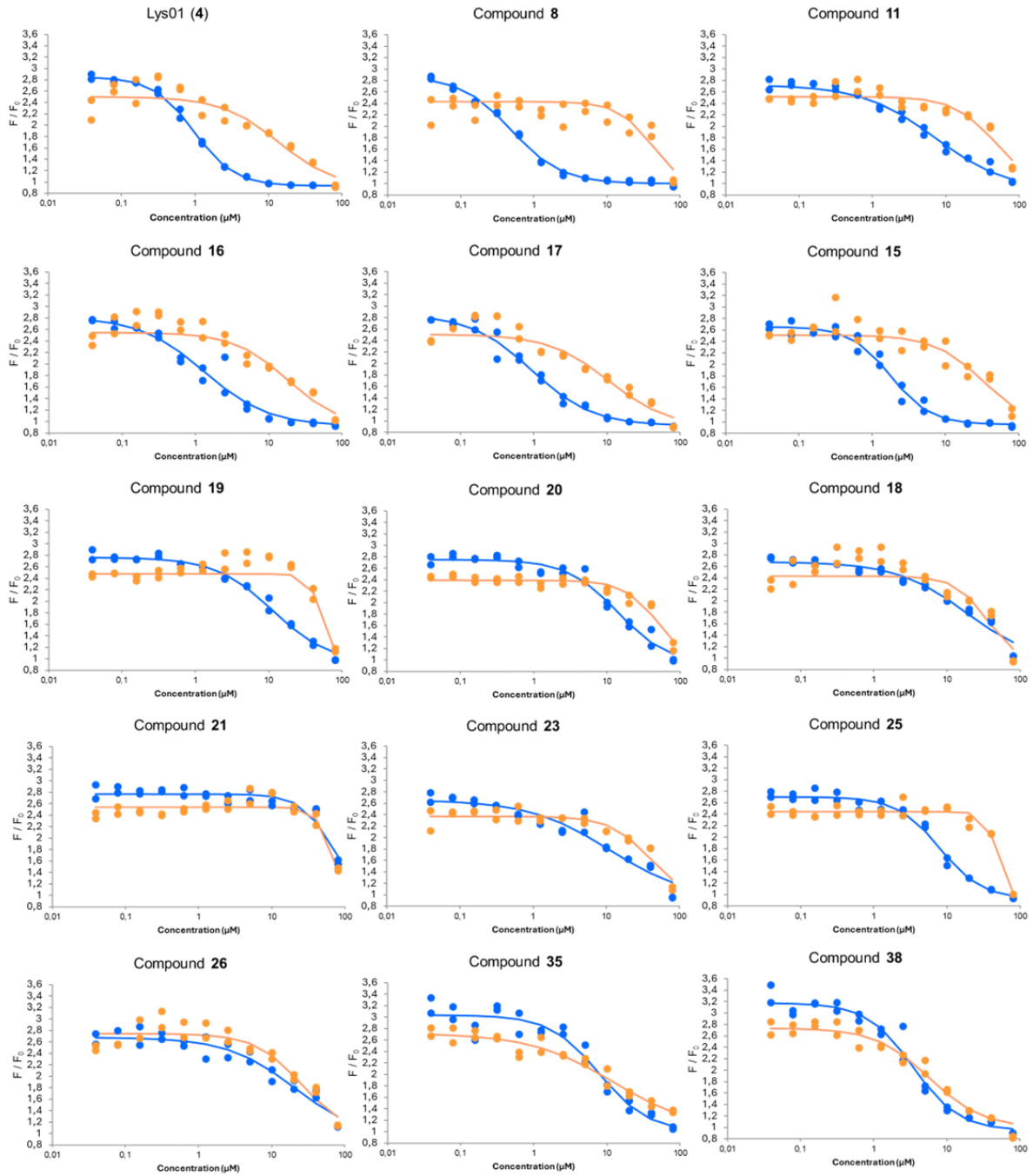
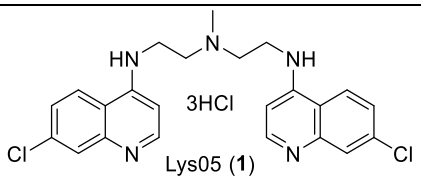
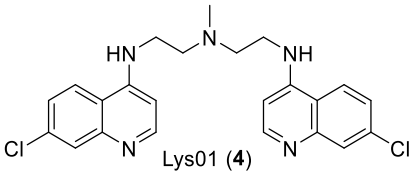
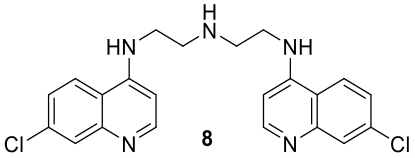
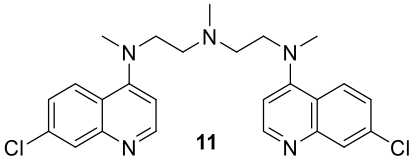
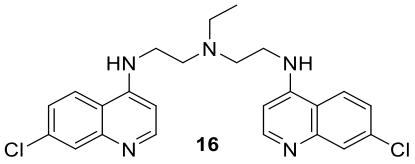
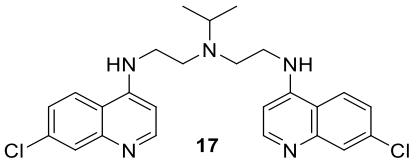
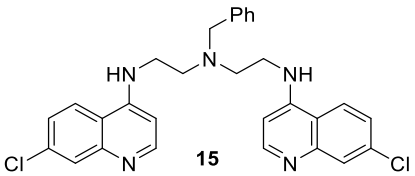
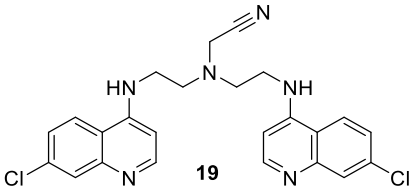
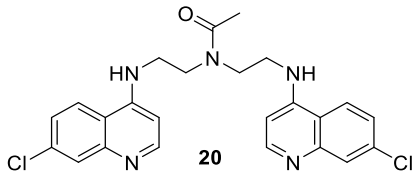
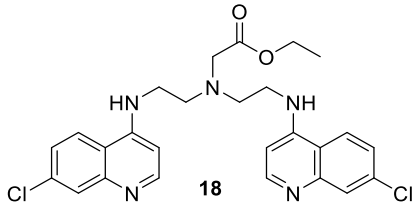
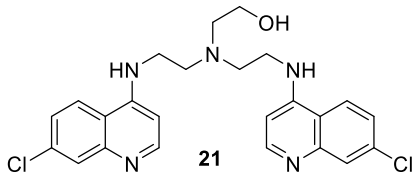
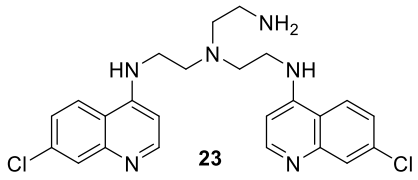
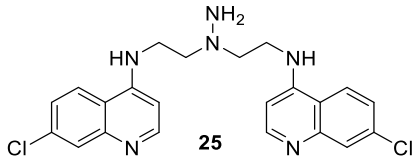
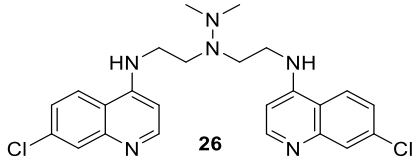
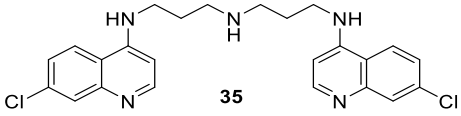
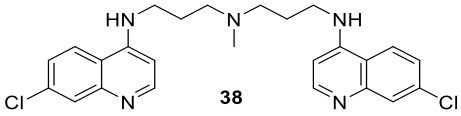


Figure 14: Dose–response curves obtained by Fluo-4 calcium imaging technique using HEK293 stably expressing plasma membrane TPC2$^{L11A/L12A}$-RFP. Cells were incubated with the respective inhibitor, followed by TPC2-A1-N (**2**, 10 µM), depicted in orange, or TPC2-A1-P (**3**, 30 µM), depicted in blue, activation.

IC_{50} values of Lys01 (**4**) and analogues **8**, **11**, **15** – **21**, **23**, **25**, **26**, **35**, and **38** were determined from the dose–response curves obtained by Fluo-4 calcium imaging. Moreover, in-house MTT assay was performed by Martina Stadler (Bracher group) to determine metabolic vitality of the cells as a measure for cytotoxicity, using human leukemia cells HL-60. The method is based on the soluble yellow tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which is reduced to an insoluble blue formazan. This reduction occurs solely *in vivo*, in metabolic active cells, as the reducing agents are NADH and NADPH. Photometric measurements determine the amount of formazan formed, which correlates with cell viability, allowing the calculation of IC_{50} values to identify potential cytotoxic substances. Triton X-100 was incubated as positive control. No statement on the mode of action can be undertaken with this method. The metabolic cell activity was analysed and compounds with IC_{50} values smaller than 50 µM were considered cytotoxic. IC_{50} values of inhibition after TPC2-A1-N (**2**) and TPC2-A1-P (**3**) activations and of MTT assay are listed in Table 6. All the analogues maintained the initial trend observed for Lys05 (**1**) and Lys01 (**4**) (Table 6, entries 1 and 2) with a lower IC_{50} after TPC2-A1-P (**3**) activation, with the best result for the *N*-demethylated Lys01 (**4**) analogue **8** (entry 3), which maintains a comparable IC_{50} for TPC2-A1-P (**3**) activation of 0.69 µM and a significantly higher IC_{50} for TPC2-A1-N (**2**, factor of 72; none of the other test compounds showed a factor >9), accompanied by no disruptive cytotoxicity (MTT). *N*-Methylation of both aromatic amino groups, as in compound **11** (entry 4), led to slightly higher IC_{50} values with TPC2-A1-P (**3**) activation and significantly higher IC_{50} with TPC2-A1-N (**2**) activation, in comparison with the lead structure Lys01 (**4**), suggesting that even small modifications on this site result in a lower inhibitory potency. Regarding the substitutions on the central aliphatic amine, *N*-alkyl substituted compounds **16**, **17**, and **15** (entries 5 – 7) displayed a less effective inhibition with TPC2-A1-N (**2**) activation and no significant difference with TPC2-A1-P (**3**) activation (respectively IC_{50} of 1.3, 1.0, and 1.6 µM), maintaining a similar factor between TPC2-A1-N (**2**) and TPC2-A1-P (**3**) inhibition as for Lys01 (**4**). While compounds **19**, **20**, **18**, **21**, **23**, **25**, and **26**, *N*-substituted on the central amine with polar functional groups (entries 8 – 14), displayed a significantly weaker inhibitory effect (>21 µM for TPC2-A1-N (**2**) and >7 µM for TPC2-A1-P (**3**) activation). In particular, cyanomethyl derivative **19**, *N*-acetyl derivative **20**, ester **18**, and the two hydrazine derivatives **25** and **26** (entries 8 – 10, 13, and 14) displayed a loss of activity towards both TPC2-A1-N (**2**) and TPC2-A1-P (**3**) activation. *N*-Hydroxyethyl derivative **21** (entry 11) showed IC_{50} >47 µM towards both TPC2-A1-N (**2**) and TPC2-A1-P (**3**) activation, while *N*-aminoethyl derivative **23** (entry 12) gave in comparison a lower IC_{50}, with a stronger effect with TPC2-A1-P (**3**) activation. Homologue **38** (entry 16) led to a strong loss of potency on both types of activation. The *N*-demethylated homologue **35** (entry 15) gave a similar result, without leading to the same effect seen for *N*-demethylated **8** in comparison with Lys01 (**4**).

Entry	Compound	IC_{50} (TPC2-A1-N activation)	IC_{50} (TPC2-A1-P activation)	Factor $\frac{IC_{50}\ (TPC2-A1-}{IC_{50}\ (TPC2-A1-}$	IC_{50} (MTT)
1*	 3HCl Lys05 (**1**)	6.1 µM	0.66 µM	9.3	-
2	 Lys01 (**4**)	9.3 µM	0.84 µM	11	37 µM
3	 **8**	49 µM	0.69 µM	72	>50 µM
4	 **11**	37 µM	8.7 µM	4.2	35 µM
5	 **16**	13 µM	1.3 µM	10	20 µM
6	 **17**	7.1 µM	1.0 µM	3.8	37 µM
7	 **15**	17 µM	1.6 µM	11	10 µM
8	 **19**	45 µM	12 µM	3.8	35 µM

9	 **20**	38 µM	9.0 µM	4.2	13 µM
10	 **18**	21 µM	15 µM	1.5	22 µM
11	 **21**	72 µM	47 µM	1.5	>50 µM
12	 **23**	38 µM	11 µM	3.6	26 µM
13	 **25**	40 µM	7.0 µM	5.8	29 µM
14	 **26**	22 µM	18 µM	1.2	>50 µM
15	 **35**	16 µM	11 µM	1.5	5.9 µM
16	 **38**	7.0 µM	3.5 µM	2.0	3.3 µM

*Results already presented in Chapter 2.1, Figure 3.

Table 6: IC_{50} determined by Fluo-4 calcium imaging technique using HEK293 stably expressing plasma membrane $TPC2^{L11A/L12A}$-RFP. Cells were incubated with the respective inhibitor, followed by TPC2-A1-N (**2**, 10 µM) or TPC2-A1-P (**3**, 30 µM) activation. The selectivity factor was obtained as ratio between the IC_{50} values respectively after TPC2-A1-N (**2**) activation and TPC2-A1-P (**3**) activation. IC_{50} values of acute toxicity screening were determined by MTT assay.

To summarise, the dimeric structure (two chloroquinoline units connected by an oligoamine chain) of the molecule is crucial for its inhibitory effect on TPC2 after activation with TPC2-A1-N (**2**) and TPC2-A1-P (**3**). The triamine linker with two ethylidene spacers displayed a crucial role, as all modifications performed resulted in a loss of activity: *N*-methylation of aromatic amine groups, as in **11** and **12**; bis-desaza analogue **40**; homologues **35** and **38**. Also, the chlorination on C-7 of the quinoline has a fundamental impact on the activity. The only modification performed in this position was, however, the dechlorination, leaving space for further modifications. Regarding the central aliphatic amino group of the linker, modifications with apolar substituents are tolerated without losing activity, with the best results obtained for the *N*-demethylated analogue **8**.

To further validate the results obtained for Lys01 (**4**) and the *N*-demethylated analogue **8** on an electrophysiological level, endolysosomal patch-clamp experiments were planned. Since there were contradictory results, probably due to the fact that irrelevant concentrations were used in the first attempt, new measurements had to be carried out again. It was not possible to obtain complete and reliable results before the finalisation of the present work. Therefore, the patch-clamp experiments are on-going and not discussed in this work.

3.1.3.2 Agar diffusion assay

The antimicrobial activity was assessed against various model microorganisms. The agar diffusion assay (see Experimental section, Chapter 5.6) was performed on all the final compounds (Lys01 (**4**), **8**, **11**, **12**, **15** – **21**, **23**, **25**, **26**, **28** – **30**, **35**, **36**, **38** – **40**) synthesised. In this assay, the semi-quantitative antimicrobial property of tested compounds is correlated with inhibition zones on the agar medium as results of inhibition of microbial growth. Various model germs growing on medium containing agar were used: gram-negative bacteria *Escherichia coli* and *Pseudomonas marginalis*, gram-positive bacteria *Staphylococcus equorum* and *Streptococcus entericus*, and yeasts *Saccharomyces cerevisiae* and *Yarrowia lipolytica*. Tetracycline hydrochloride was used as control for the antibacterial effect, along with clotrimazole for the antimycotic effect. None of the compounds tested showed an inhibition zone, and, therefore, no antimicrobial effects were registered.

3.2 SARS-CoV-2 RNA ligands

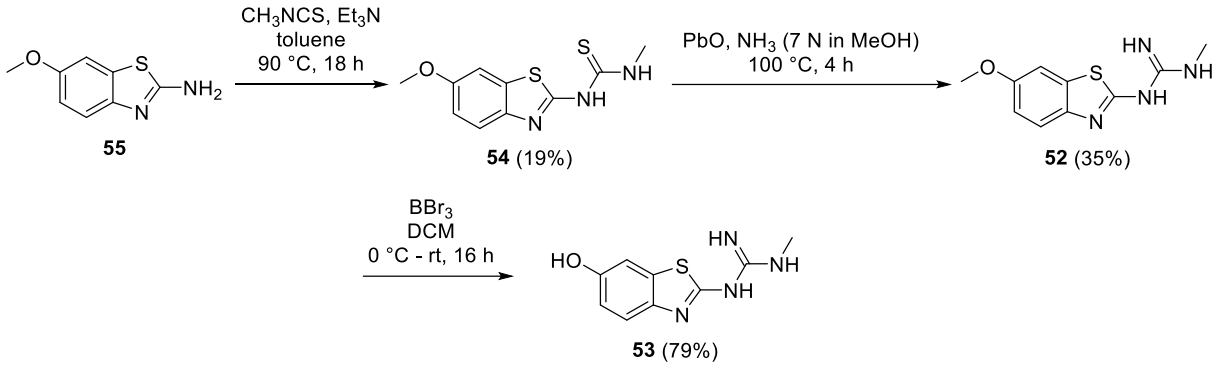
Once compound **7** was chosen as fragment lead for SARS-CoV-2 RNA-binding structures, systematic modifications were performed, and the synthesised compounds were regularly tested by NMR measurements for affinity to the target 5_SL1, as described in Chapter 2.2. The synthesised compounds are grouped according to their structural characteristics. The synthetic pathway is presented in detail for each group along with the NMR-based screening results, performed by Sabrina Toews, Goethe University Frankfurt am Main, Schwalbe group, which regularly and deeply influenced the course of this work. Before being tested, all compounds underwent an NMR-based quality control (QC, see Experimental section, Chapter 5.3), in which purity and solubility in the same condition used for the NMR measurements were proven at the very beginning, narrowing down qualitative and quantitative experiments only to the compounds that passed the QC test. The compounds described in Chapters 3.2.1 and 3.2.2 were synthesised by the technicians Karl Sauvageot-Witzku and Anja Rausch and the undergraduate student Korbinian Lohr, under my supervision. The syntheses of 2^{nd}-site binders with a linear linker (see Chapter 3.2.6.3) were performed by Ludovica Bellino during her master thesis as Erasmus student from Sapienza University of Rome, under my supervision. Further experiments examining the effects of the modifications on affinity and selectivity on the 5_SL1 region, along with potential future developments, are presented in subsequent Chapters.

3.2.1 Variations of the 2-amino group of compound 7

In this Chapter, the synthesis of lead compound **7** is described, along with analogues bearing urea moiety, as in **7**, or different functional groups, such as guanidines, thioureas, and amides. Afterwards, NMR-based screening results are presented.

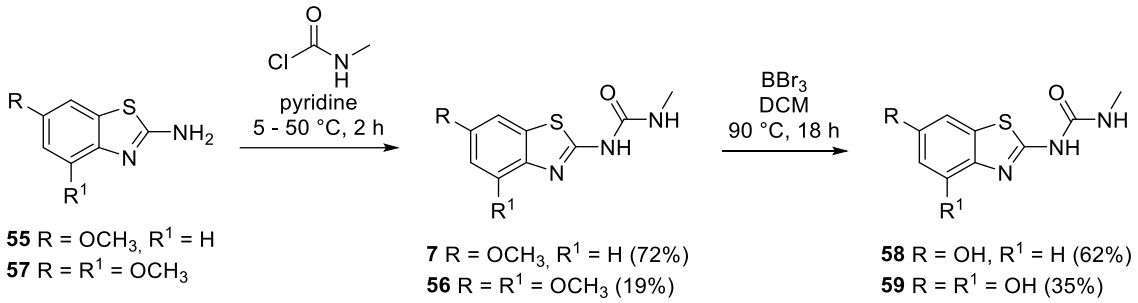
3.2.1.1 Synthesis

The first modifications performed starting from **7** focused on the replacement of the urea moiety with various functional groups, as guanidines **52** and **53**. With this purpose, thiourea **54** was obtained from 2-amino-6-methoxybenzothiazole (**55**) and methyl isothiocyanate, which was then converted into guanidine derivative **52** by reaction with PbO and ammonia in methanol (Scheme 16). The 6-methoxy group at the benzothiazole was then in turn selectively *O*-demethylated with BBr_3 to give **53**.



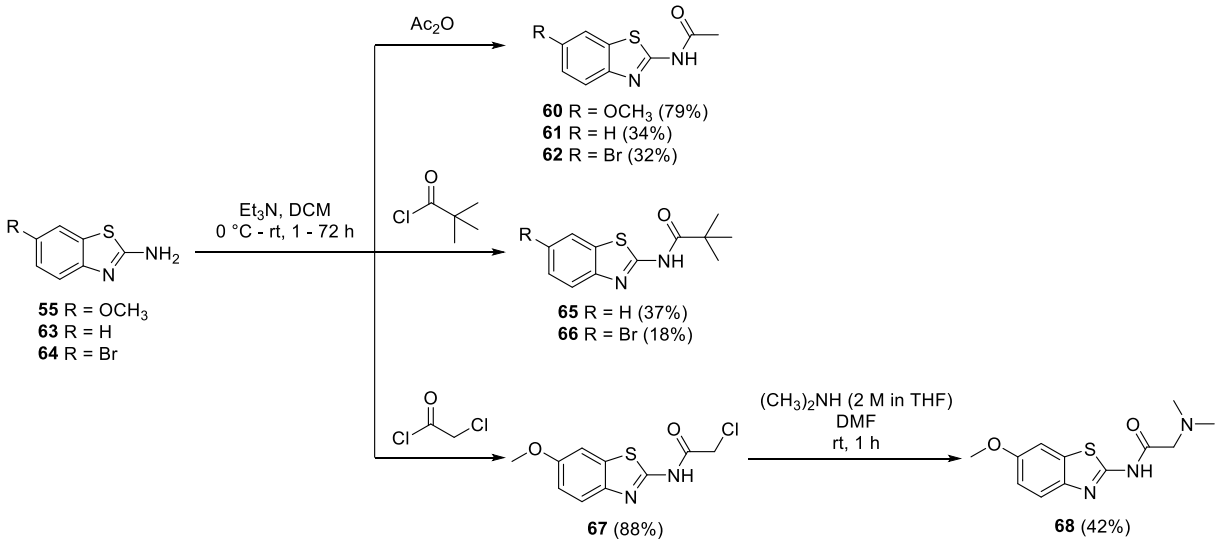
Scheme 16: Synthesis of ureas **52** and **53**.

The lead fragment **7** and urea **56** were obtained respectively from 2-aminobenzothiazoles **55** and **57** *via* urea formation using methylaminoformyl chloride (Scheme 17). *O*-Demethylations, as described in Scheme 16, were performed on both products providing respectively *O*-demethylated analogues **58** and **59**.



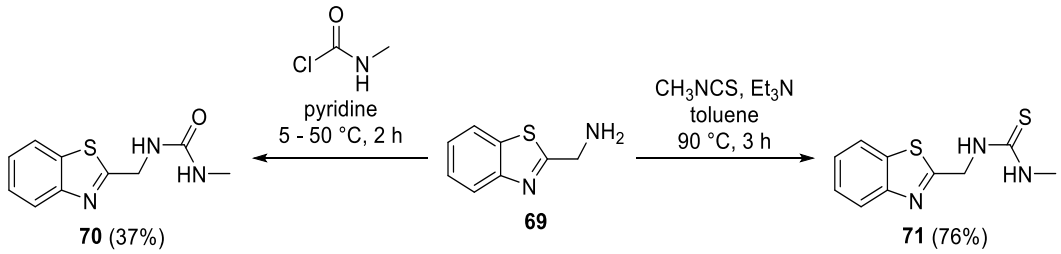
Scheme 17: Synthesis of ureas **7** and **56** and *O*-demethylated analogues **58** and **59**.

As shown in Scheme 18, to synthesise amides **60** – **62**, 2-methoxybenzothiazoles **55**, **63**, and **64** were *N*-acetylated with acetic anhydride, while **65** and **66** were obtained by acylation of **63** and **64** with pivaloyl chloride, using the same reaction conditions (Scheme 18). The choice of using pivaloyl chloride instead of pivaloyl anhydride was based on the purification process, as in one attempt the product could not be successfully separated from pivalic acid, inevitable product of this reaction. Glycinamide **68** was in turn obtained replacing the chloride with 2-chloroacetyl chloride, followed by substitution of the chloroacetamide **67** with *N*,*N*-dimethylamine. The rationale behind this compound was that due to the newly introduced aliphatic amino group we expected improved solubility, which was a limiting factor, compared to (thio)ureas and neutral amides, and possible additional interactions with phosphate groups of RNA.



Scheme 18: Synthesis of amides **60** – **62**, **65**, **66**, and **68**.

Further, analogues containing a methylene group between the benzothiazole ring and the derivatised amino group, were synthesised. As depicted in Scheme 19, benzothiazolemethanamine **69** was derivatised to obtain urea **70** with methylaminoformyl chloride and thiourea **71** using methyl isothiocyanate.

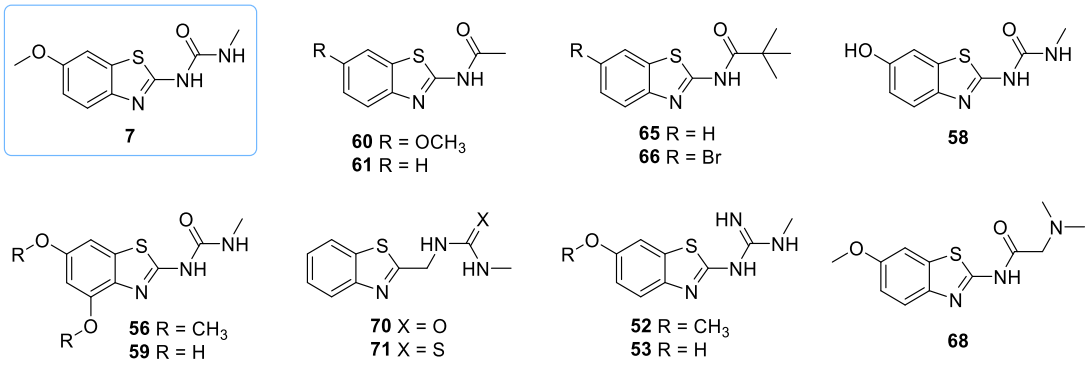


Scheme 19: Synthesis of benzothiazolemethanamine derivatives **70** and **71**.

3.2.1.2 NMR-based results

The compounds synthesised in this campaign were evaluated on the three parameters introduced in Chapter 2.2: CSP, wLOGSY, and T_2. The results for each parameter are presented in Table 7, along with the combined rank score (Table 7, ranking). To determine this final ranking, the compounds considered in a series were ranked separately for each parameter, with position 1 assigned to the highest value and the last position to the lowest. The rankings obtained across the three parameters were then summed for each molecule. Finally, the compounds were ordered in ascending order based on their total score (sum), with the lowest sum corresponding to the highest position in the final ranking. Lead fragment **7** is highlighted in light blue (Table 7, entry 1), and compounds with the best results among the series in green. Compounds **54** and **62** did not pass the solubility test and were therefore excluded. Amides **60**, **61**, **65**, and **66** (entries 2 – 5) showed a very small effect for all the three parameters taken into consideration, with 6-methoxy substituted derivative **60** displaying

higher values for CSP and T_2 than **61**, and 6-bromo substituted derivative **66** for all three parameters compared to **65**, which suggest a possible role of the aromatic decoration in the bonding effect. Comparable to **7**, were the results obtained for urea **58** (entry 6) and improved for ureas **56** and **59** (entries 7 and 8), with higher CSP and T_2 for 4,6-substituted **56** and **59** in comparison with urea **7**. The two methanamine analogues **70** and **71** (entries 9 and 10) showed no binding or very little effects. Guanidine **52** gave outstanding results for CSP (entry 11), being among the best overall in this series, while, surprisingly, the *O*-demethylated analogue **53** (entry 12) was not binding, reinforcing the concept of the possible influence of aromatic substituents. Glycinamide **68** (entry 13), on the other hand, showed promising results and was one of the best in terms of combined rank score.



Entry	Compound	CSP [Hz]	wLOGSY	T_2 reduction [%]	Highest CSP	Highest wLOGSY	Highest T_2	Sum	Ranking
1	**7**	3.96	0.78	66.95	5	4	6	15	5
2	**60**	2.23	0.21	17.52	8	10	7	25	8
3	**61**	1.11	0.26	7.31	10	9	9	28	9
4	**65**	0.60	0.29	-5.00	11	8	13	32	11
5	**66**	1.43	3.37	8.96	9	1	8	18	7
6	**58**	3.67	0.66	72.21	6	6	5	17	6
7	**56**	4.50	1.51	93.00	3	2	2	7	1
8	**59**	4.11	0.33	100.00	4	7	1	12	4
9	**70**	0.00	0.00	0.00	12	12	10	34	12
10	**71**	2.70	0.17	0.00	7	11	10	28	9
11	**52**	16.14	1.21	77.83	1	3	4	8	2
12	**53**	0.00	0.00	0.00	12	12	10	34	12
13	**68**	12.23	0.72	79.26	2	5	3	10	3

Table 7: NMR-based results of 2-aminobenzothiazole derivatised compounds.

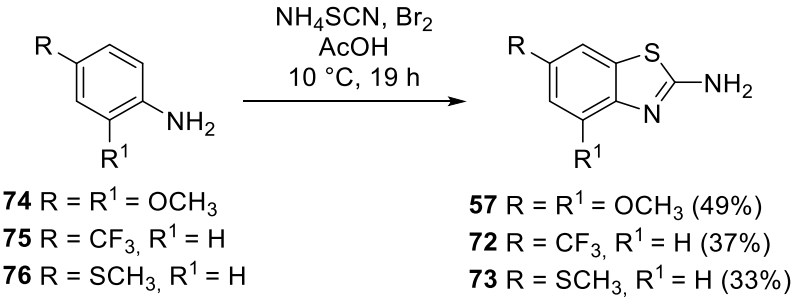
The comparison of **68** with the other synthesised amides **60**, **61**, **65**, and **66** suggested the possible promising additional interactions due of the basic *N*,*N*-dimethylamine group. Moreover, the basic *N*,*N*-dimethylamine group was crucial in gaining solubility in the buffer conditions, and thus physiological conditions, in comparison to the neutral amides (**60**, **61**, **65**, and **66**) and (thio)ureas (**7**, **58**, **56**, **59**, **70**, and **71**). Compounds **68** was therefore taken as starting point for further modifications.

3.2.2 Variations of the substitution pattern of the benzothiazole

Starting from the previous results obtained, a series of benzothiazoles, variously substituted on the benzenoid ring, were synthesised, maintaining the *N*,*N*-dimethylglycinamide lateral chain as in compound **68**.

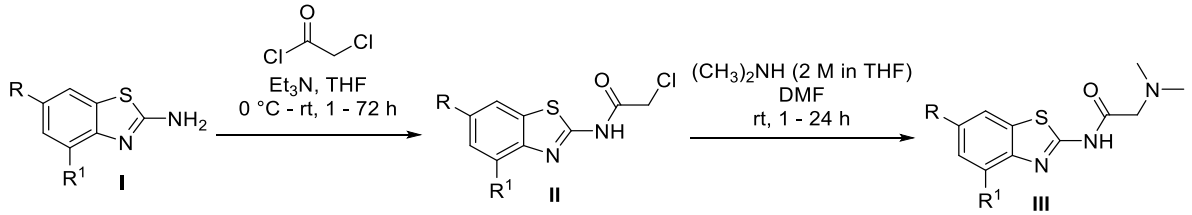
3.2.2.1 Synthesis

A diverse range of ring-substituted 2-aminobenzothiazoles were utilised as starting materials for this series of compounds. Apart from those readily available in the lab at the time, 4,6-dimethoxy derivative **57**[116] and 6-trifluoromethyl derivative **72**[117] were synthesised from appropriate anilines, as described in literature, using ammonium thiocyanate and bromine in acetic acid. The same protocol was applied to obtain 6-methylthio derivative **73**, as depicted in Scheme 20.



Scheme 20: Synthesis of 2-aminobenzothiazoles **57**, **72**, and **73**.

Following the same synthetic pathway already introduced for glycinamide **68** *via* chloroacetamide synthesis and subsequent substitution with *N*,*N*-dimethylamine, various analogues, shown in Table 8, were obtained.

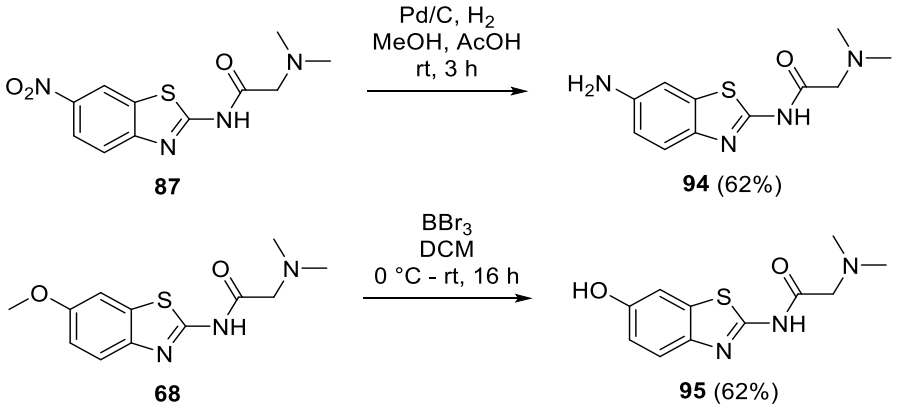


Entry	R	R1	Starting material (I)	Products (yield)

				II	III
1	NO_2	H	**77**	**80** (92%)	**87** (87%)
2	Br	H	**64**	**81** (81%)	**88** (37%)
3	OCH_3	OCH_3	**57**	**82** (89%)	**89** (69%)
4	CF_3	H	**72**	**83** (76%)	**90** (27%)
5	SCH_3	H	**73**	**84** (91%)	**91** (69%)
6	OCF_3	H	**78**	**85** (71%)	**92** (53%)
7	SO_2CH_3	H	**79**	**86** (64%)	**93** (64%)

Table 8: Synthesis of *N*,*N*-dimethylglycinamides **87** – **93**, variously substituted on the benzothiazole.

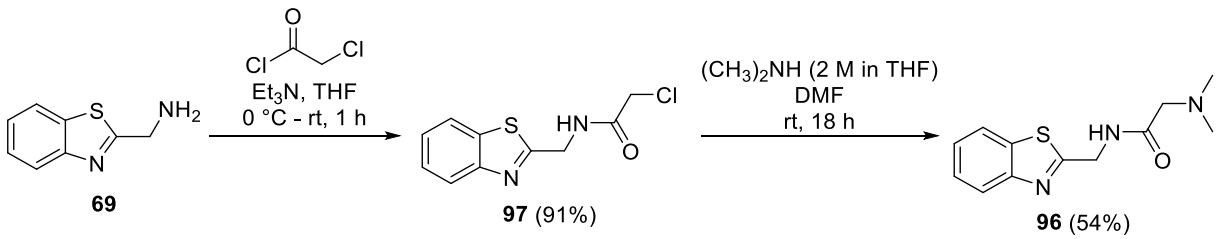
To enlarge the pool explored with more polar analogues with a potential for forming additional hydrogen bonds with RNA, nitroarene **87** was reduced to amino derivative **94** *via* catalytic hydrogenation, while phenolic **95** was obtained by *O*-demethylation of aryl ether **68** (Scheme 21).



87 **94** (62%) **68** **95** (62%)

Scheme 21: Reduction of **87** and *O*-demethylation of **68** to obtain respectively **94** and **95** with polar substituents at the benzene ring.

Benzothiazolemethanamine **69** was also derivatised to obtain *N*,*N*-dimethylglycinamide **96** (Scheme 22), in order to confirm the importance of direct connection between the amide and the benzothiazole moiety.

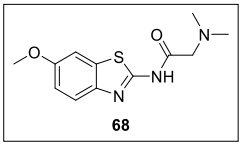


69 **97** (91%) **96** (54%)

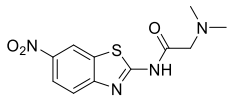
Scheme 22: Synthesis of homologous compound **96**.

3.2.2.2 NMR-based results

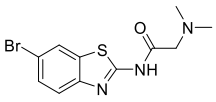
The NMR-based results for this second batch of compounds are shown in Table 9, with glycinamide **68** taken as reference (Table 9, entry 1, in yellow). All the 2-aminobenzothiazole derivatives showed binding effects (entries 2 – 10), meaning that different substituents are tolerated without complete loss of activity and confirming the determining role of the *N*,*N*-dimethylamine moiety. On the other hand, compound **96**, with the additional methylene unit between the amide group and benzothiazole, was classified as not binding (entry 11), confirming the central role of the 2-aminobenzothiazole subunit. The most promising results were displayed by nitroarene **87**, 6-bromo substituted **88**, 4,6-dimethoxy substituted **89**, and 6-(methylthio)benzothiazole **91** (entries 2 – 4 and 6), with a CSP two to three times higher in comparison to **68**. Among the other analogues, 6-(trifluoromethyl)benzothiazole **90** and 6-(trifluoromethoxy)benzothiazole **92** (entries 5 and 7) gave slightly higher results compared to **68** for CSP and T_2, while compounds bearing H-donors, 6-aminobenzothiazole **94** and 6-hydroxybenzothiazole **95** (entries 9 and 10), displayed similar results for CSP, with lower wLOGSY and T_2. Lower scores were registered for 6-(methylsulfonyl)benzothiazole **93** (entry 8) for all the three parameters.



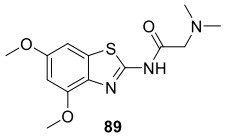
68



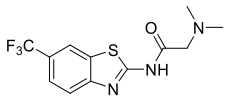
87



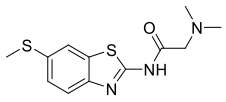
88



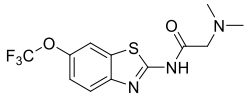
89



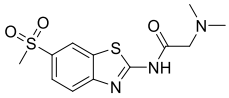
90



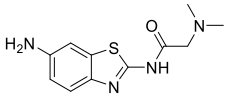
91



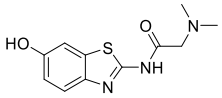
92



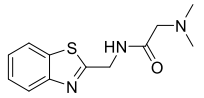
93



94



95



96

Entry	Compound	CSP [Hz]	wLOGSY	T_2 reduction [%]	Highest CSP	Highest wLOGSY	Highest T_2	Sum	Ranking
1	**68**	12.23	0.72	79.26	7	4	6	17	6
2	**87**	22.20	0.46	100.00	4	6	1	11	2
3	**88**	27.60	0.74	83.00	2	3	5	10	1
4	**89**	31.92	2.66	36.00	1	1	10	12	3
5	**90**	15.84	0.00	89.41	5	10	2	17	6
6	**91**	25.08	0.46	87.00	3	6	3	12	3
7	**92**	13.56	1.01	85.67	6	2	4	12	3

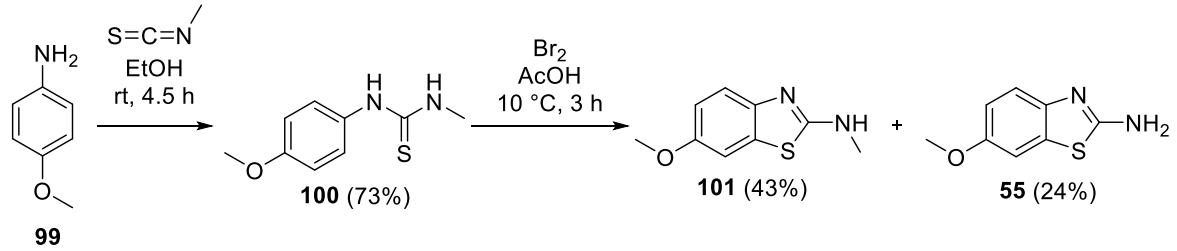
8	**93**	6.54	0.22	46.43	10	8	9	27	10
9	**94**	11.40	0.49	69.42	8	5	7	20	8
10	**95**	10.02	0.15	69.00	9	9	8	26	9
11	**96**	2.20	0.00	0.00	11	10	11	32	11

Table 9: NMR-based results of benzothiazole with various substitution pattern.

Taking a closer look at the four best compounds of this series, the poor solubility in the buffer system led to exclude nitroarene **87** from further development, while 4,6-dimethoxy derivative **89** was chosen as the best option, having a higher score of two out of three parameters compared to **88** and **91**.

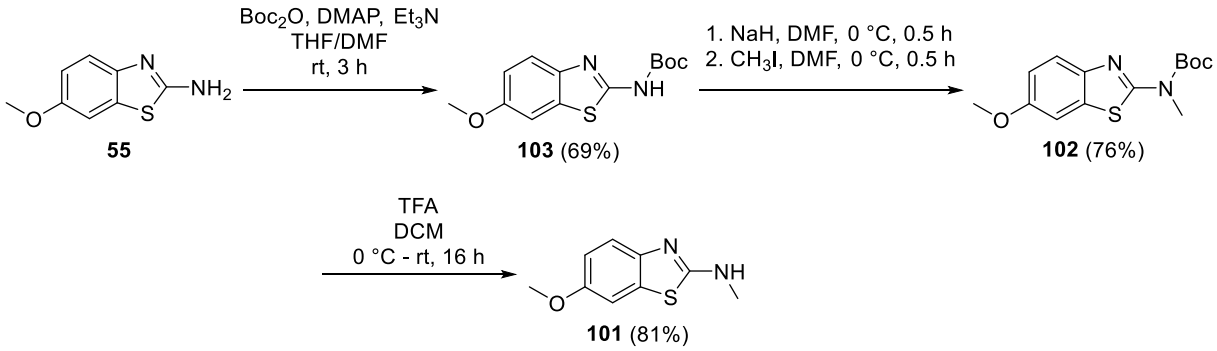
3.2.3 *N*-Methylated analogue of compound 68

Another modification involved *N*-methylation of the amide group of **68**. For the synthesis of *N*-methyl analogue **98**, in order to avoid possible competing *N*-methylation of the glycinamide, the methyl group was introduced at the very beginning performing the first step of thiourea synthesis between *p*-anisidine (**99**) and methyl isothiocyanate, as depicted in Scheme 23. Thiourea **100** was then cyclised with bromine in acetic acid to 2-(methylamino)benzothiazole **101**. The latter step gave the desired product **101** along with the unexpected demethylated analogue **55** with considerable yield (24%).



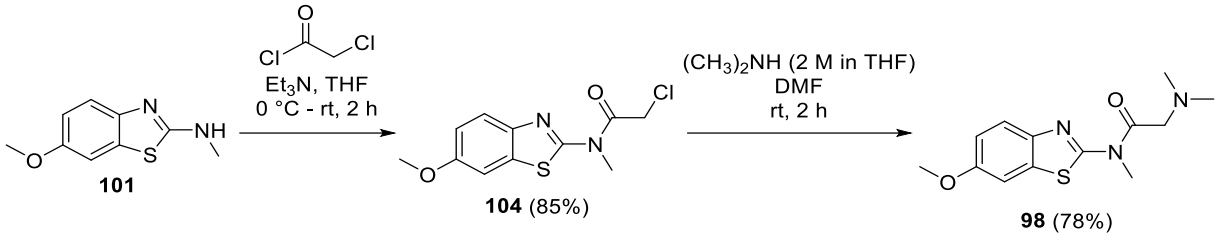
Scheme 23: Synthesis of *N*-methyl 2-aminobenzothiazole **101**.

In parallel, another synthetic pathway was attempted starting from 2-aminobenzothiazole **55**, performing a Boc protection of the aromatic amine, followed by *N*-methylation with iodomethane to *N*-methyl analogue **102**, and subsequent deprotection to release **101** (Scheme 24). This pathway gave a higher overall yield, despite the additional synthetic steps of protection and deprotection, and the possibility of functionalising the amino group with different lateral chains, using various alkyl halides, concurring in a greater expansion possibility of the chemical pool.



Scheme 24: Alternative synthesis of *N*-methyl-2-aminobenzothiazole **101**.

The last two steps of amide formation with chloroacetyl chloride to chloroacetamide **104**, followed by *N*,*N*-dimethylamino substitution to give final product **98**, are depicted in Scheme 25.



Scheme 25: Lateral functionalisation of **101** to give the final product **98**.

Compound **98** was not tested due to instability issue. The HPLC measurements of benzothiazole **98** uncovered the instability of this compound, as shown in Figure 15. Two HPLC measurements are shown: above, the measurement was conducted 10 minutes after dissolving **98** in MeOH; below, the HPLC of the same solution was measured again after 24 h. Due to its instability issue, compound **98** was not tested and no further analogues methylated on the amide were synthesised.

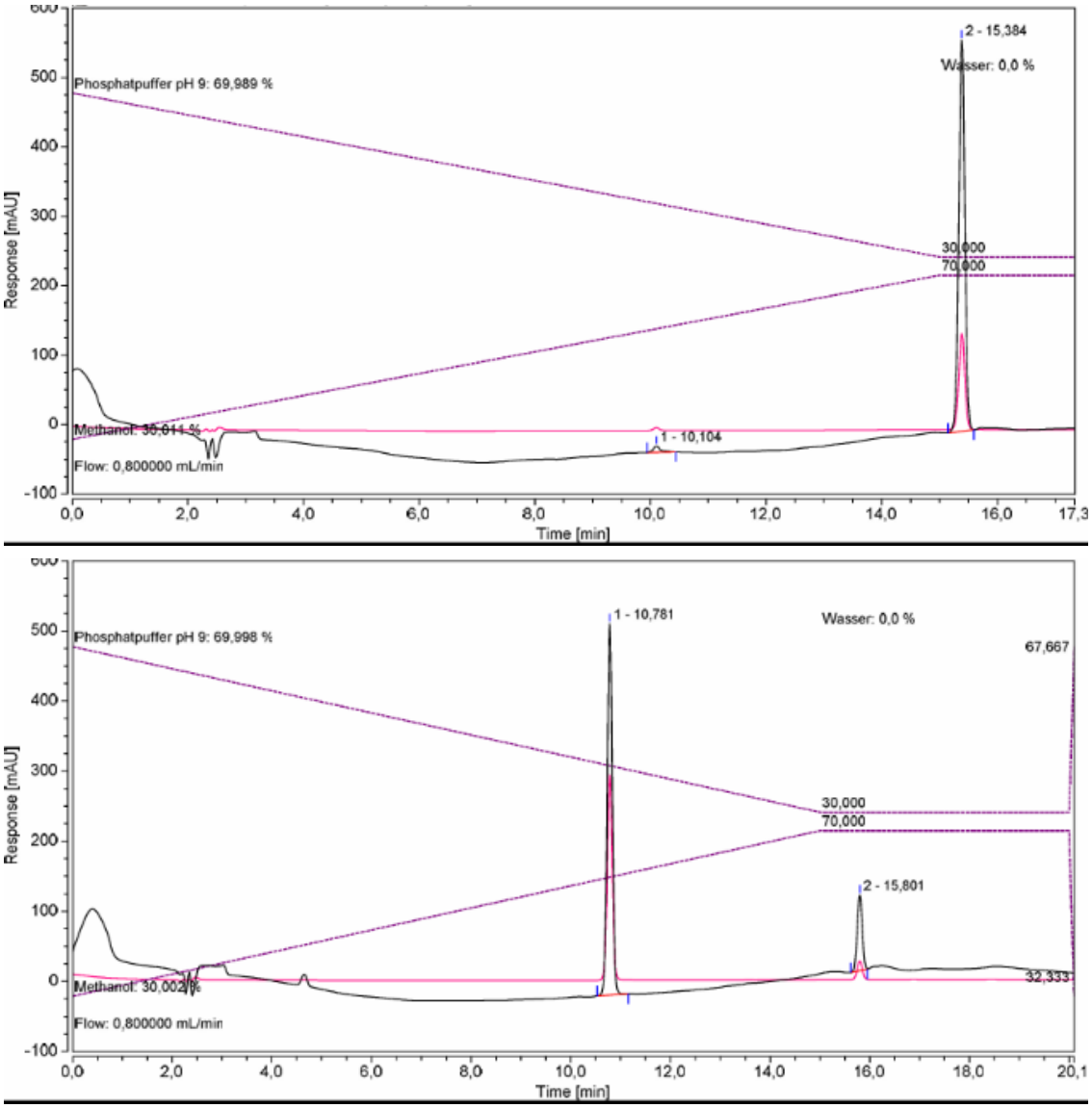


Figure 15: HPLC measurement of compound **98** respectively 10 minutes after probe preparation (above) and 24 h later (below).

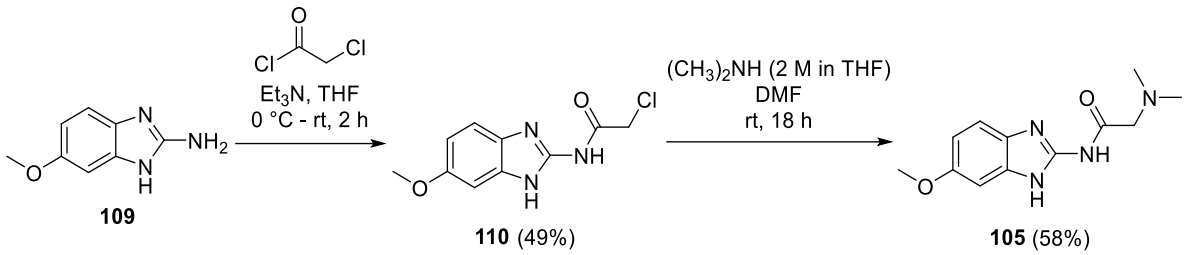
3.2.4 Heteroanalogues of compound 68

Another series of compounds focused on the replacement of the aromatic moiety of **68** with heteroaromatic analogues of benzothiazole. As described in this Chapter, benzimidazole **105**, benzoxazole **106**, thiazolopyridine **107**, and thiazolopyrimidine **108** were planned, synthesised, and tested.

3.2.4.1 Synthesis

Benzimidazole heteroanalogue

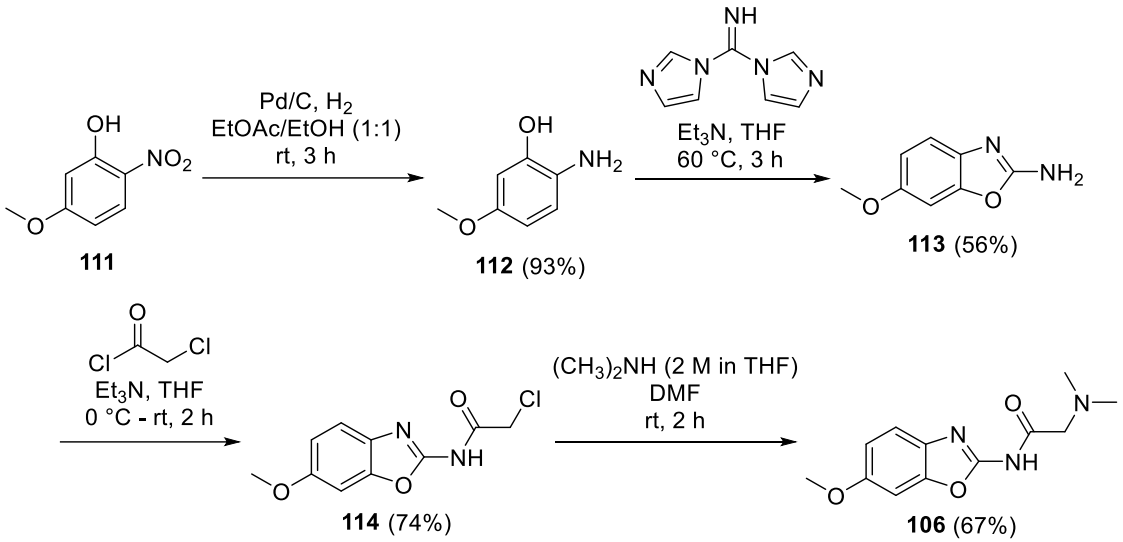
Benzimidazole **105** (Scheme 26) was obtained performing the two standard steps of amide formation starting from commercially available 2-aminobenzimidazole **109** to chloroacetamide **110** and subsequent substitution with *N*,*N*-dimethylamine to obtain the desired product **105**.



Scheme 26: Synthesis of benzimidazole **105**.

Benzoxazole heteroanalogue

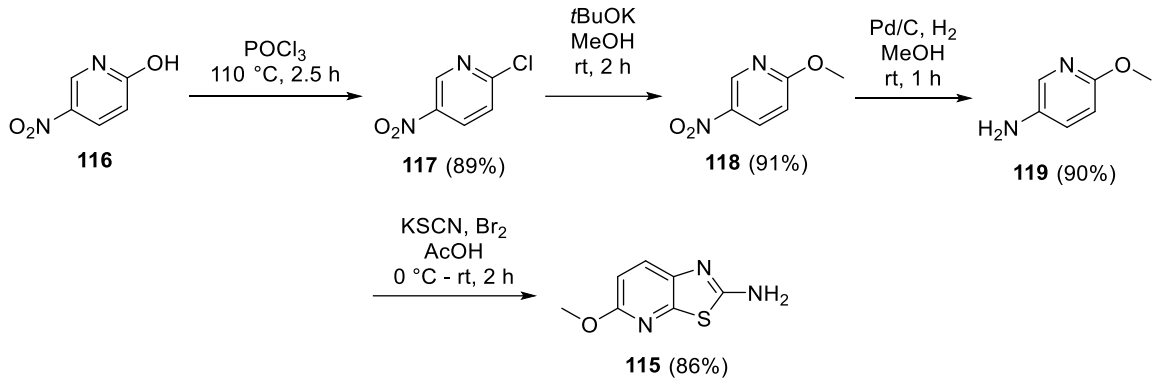
The synthesis of benzoxazole analogue **106** was achieved starting from 5-methoxy-2-nitrophenol (**111**, Scheme 27). After hydrogenation with palladium on charcoal of nitroarene **111**, the attempt of cyclisation of the obtained 2-aminophenol **112** was carried out with cyanogen bromide[118] at reflux for 3 h, without any product forming. Rynearson *et al.*[119], on the other hand, succeeded in cyclisation of variously substituted aminophenols with di(1*H*-imidazol-1-yl)methanimine and this protocol was successfully applied to obtain 2-aminobenzoxazole **113**. The latter was then functionalised with the typical glycinamide chain under the established conditions.



Scheme 27: Synthesis of benzoxazole **106**.

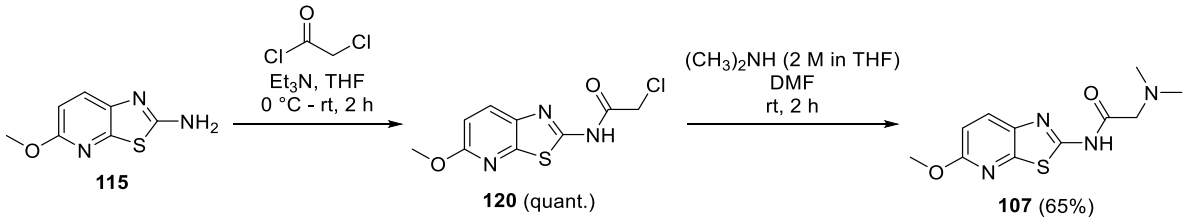
Thiazolopyridine heteroanalogue

Thiazolopyridine **107** was obtained from 2-aminothiazolopyridine **115**. The latter was synthesised in a high-yielding four-step procedure starting from 2-hydroxy-5-nitropyridine (**116**, Scheme 28). This compound was converted following published conditions into a 6-cloro-substituted analogue **117**[120] and then to methoxypyridine **118**[121]. The reduction *via* palladium catalysed hydrogenation led to aminopyridine **119**, which was then cyclised by treatment with bromine and potassium thiocyanate[122].



Scheme 28: Synthesis of 2-aminothiazolopyridine **115**.

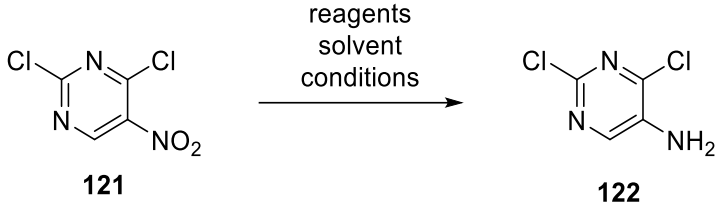
2-Aminothiazolopyridine **115** was then functionalised under standard conditions, as depicted in Scheme 29, to give the final product **107**, bearing the *N*,*N*-dimethylamino acetamide as lateral chain.



Scheme 29: Functionalisation of **115** to obtain final product **107**.

Thiazolopyrimidine heteroanalogue

The synthesis of thiazolopyrimidine **108** started with the reduction of 2,4-dichloro-5-nitropyrimidine (**121**), as depicted in Table 10. Reduction of the nitro group *via* catalytic hydrogenation (Table 10, entry 1), applying the same successful protocol seen for reduction of nitroarene **118** to **119** (see Scheme 28), did not lead to an equally satisfying yield. Wang *et al*.[123] succeeded in this reduction treating nitropyrimidine **121** with iron and ammonium chloride (entry 2). The protocol was reproduced as described in literature, without leading to the expected good result. A third attempt with $SnCl_2$ as reducing agent (entry 3), a standard reducing reagent for nitroarene to aromatic amine, led to amine **122** with a yield of 90% in a fast and efficient way.

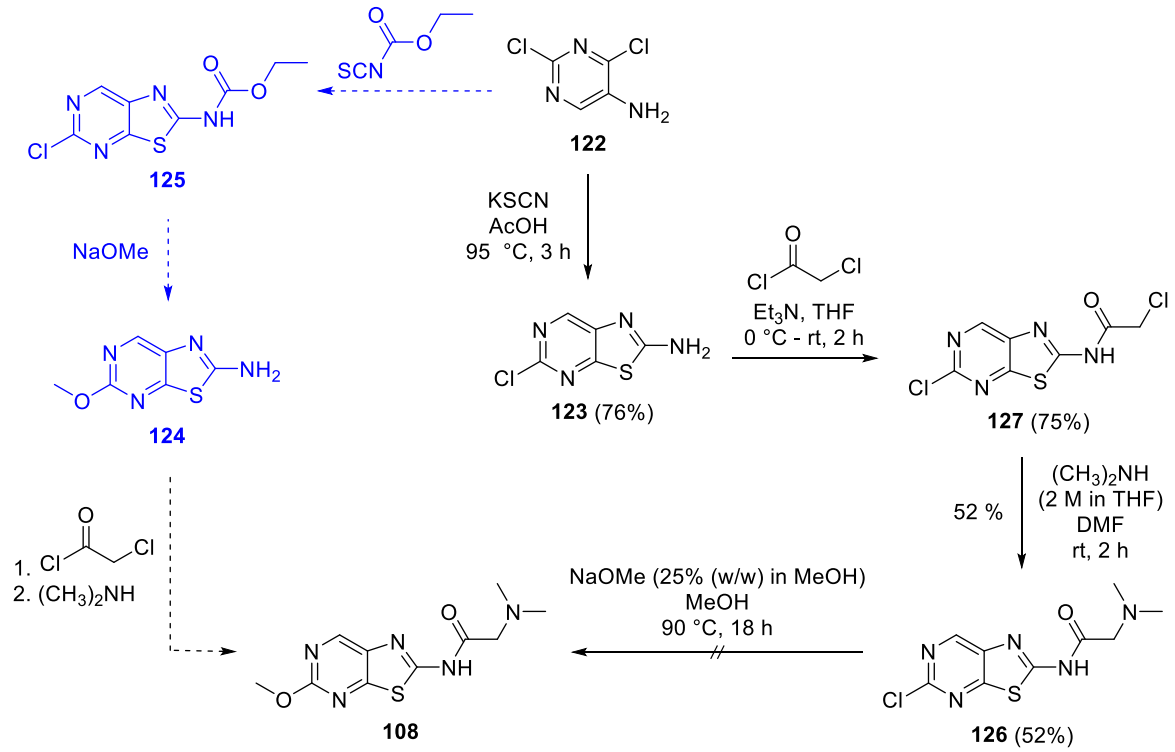


Entry	Reagent	Solvent	Conditions	Yield
1	Pd/C, H_2	MeOH	rt, 1.5 h	20%

2	Fe, NH_4Cl	DCM/MeOH	50 °C, 2.0 h	36%
3	$SnCl_2$	EtOH	80 °C, 1.0 h	90%

Table 10: Reduction of nitropyrimidine **121** to aromatic amine **122**.

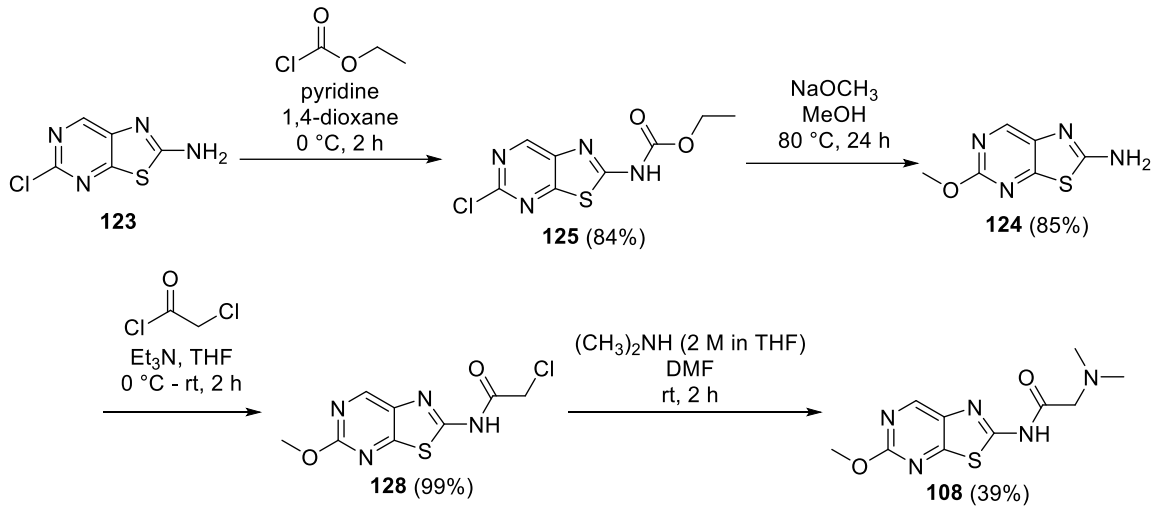
The subsequent ring closure was obtained with potassium thiocyanate in acetic acid (Scheme 30), giving thiazolopyrimidin-2-amine **123**. In literature[124], 6-methoxythiazolopyridine-2-amine **124** was obtained treating ethyl carbamate **125** with a large excess of sodium methoxide, achieving at the same time the methoxy-to-chloride exchange and the deprotection of the amine, as depicted in Scheme 30 in blue. Then, the two additional steps to chloroacetamide and subsequent substitution with *N*,*N*-dimethylamine would bring to the desired product **108**. A pathway functionalising **123** with the lateral chain before the methoxy-to-chloride exchange was attempted. Thiazolopyrimidin-2-amine **123** was converted into *N*,*N*-dimethylacetamide **126** with the two steps extensively applied for the aforementioned analogues. The last step (S_NAr) with sodium methanolate did not lead to any isolable product.



Scheme 30: Attempt to synthesise **108** *via* **126**.

Due to the failure of the last reaction, the quickest way to obtain **108** was to apply the blue synthetic route, depicted in Scheme 30, with some modifications, starting from thiazolopyrimidine **123**, as shown in Scheme 31. Ethyl carbamate **125** was synthesised using ethyl chloroformate, to be then treated with sodium methanolate to give 6-methoxy

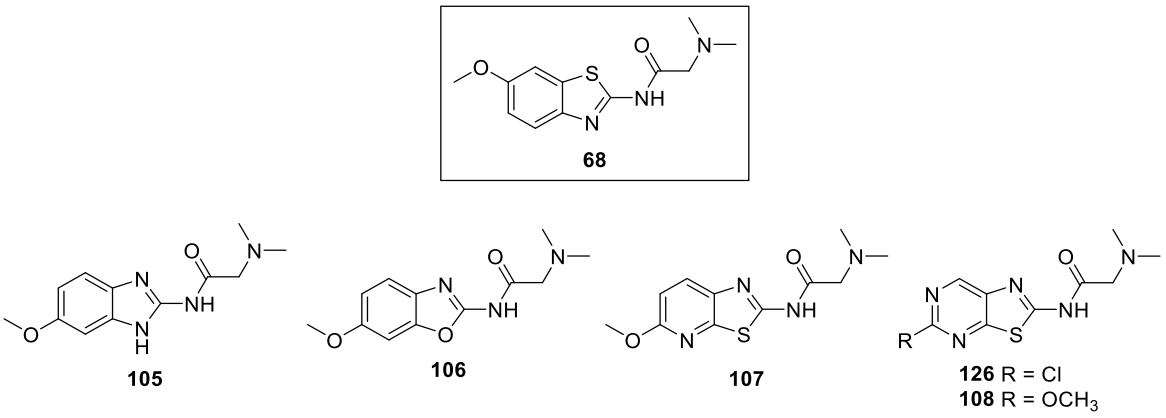
thiazolopyrimidine **124**. The latter was in turn further derivatised to chloroacetamide **128** and to the *N*,*N*-dimethylglycinamide **108**.



Scheme 31: Synthesis of thiazolopyrimidine **108**.

3.2.4.2 NMR-based results

The NMR-based results of heteroanalogues are shown in Table 11. Compound **68** was again taken as reference and higher CSP and T_2 were obtained for benzimidazole **105** (Table 11, entry 2) with only a slightly lower wLOGSY, while higher CSP and wLOGSY were achieved with thiazolopyridine **107** (entry 4). The binding was very weak for benzoxazole **106** (entry 3) and almost completely lost for thiazolopyrimidines **126** and **108** (entries 5 and 6).



Entry	Compound	CSP [Hz]	wLOGSY	T_2 reduction [%]	Highest CSP	Highest wLOGSY	Highest T_2	Sum	Ranking
1	**68**	12.23	0.72	79.26	3	2	2	7	3
2	**105**	25.08	0.62	87.00	1	3	1	5	1
3	**106**	7.32	0.59	21.05	4	4	4	12	4
4	**107**	16.14	1.22	73.62	2	1	3	6	2

5	**126**	1.20	0.11	0.00	6	6	6	18	6
6	**108**	2.28	0.13	7.02	5	5	5	15	5

Table 11: NMR-based results of heteroanalogues of **68**.

Benzothiazole **68** and benzimidazole **105** were chosen as most promising moiety for further modifications. Due to time limitations, the only slightly better thiazolopyridine **107** was left aside.

3.2.5 Second basic site

Further modifications focused on the variation of the lateral *N*,*N*-dimethylamino structure in the glycinamide residue, aiming at the same time at improved solubility in the buffer conditions and improved affinity with 5_SL1.

3.2.5.1 Design

The structure of potential 5_SL1 binders was further modified substituting the *N*,*N*-dimethylamino structure in the glycinamide residue with different lateral chains containing a second basic site. The modifications were applied on mono- and dimethoxybenzothiazole, based respectively on the structural characteristics of **68** and **89** (see Chapters 3.2.1 and 3.2.2). This structure motif was proposed to further improve the solubility in the aq. buffer used for NMR-based measurements, which is a strong limiting factor, and, on the other hand, for promising additional interactions of (under physiological conditions) protonated amines with the phosphate groups of RNA. The planned newly introduced structures consist of piperazine and *N*-methylpiperazine derivatives and 4-amino-2,2,6,6-tetramethylpiperidine derivatives (Figure 16, respectively structures **I** and **II**), whose structure is based on the TEMPO compound (S-391, **129**), which was used for computational calculations and is described in more details in Chapter 3.2.6.1. Moreover, compounds bearing a 3-aminopiperidine moiety were also planned with the attempt of mimicking 2-deoxystreptamine (2-DOS, **130**), known as RNA binding structure[125, 126], depicted in Figure 16. Being the selective derivatisation of 2-DOS (**130**) functional groups particularly time consuming[127-129], 3,5-diaminopiperidine (DAP, **131**) was considered a promising candidate to mimic the cyclic structure of 2-DOS (**130**), with the advantage of containing an additional aliphatic amine for connection to the basic core. Finally, 3-aminopiperidine, still bearing the additional secondary amino group, was chosen as best option, with the added advantage of the commercial availability of the two pure enantiomers (structures **III**).

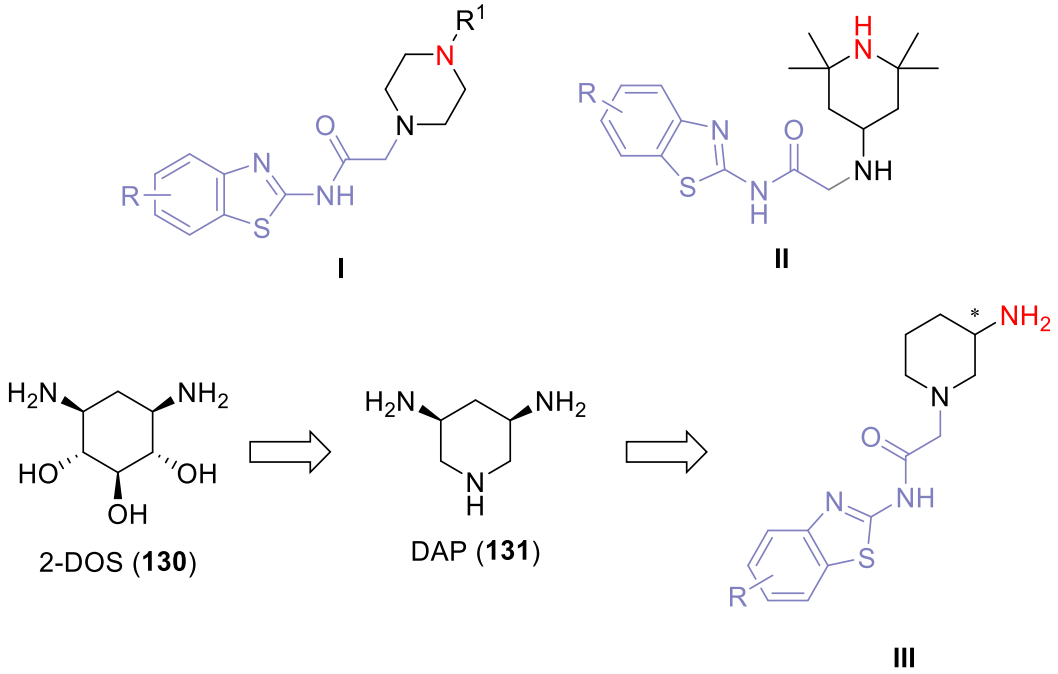
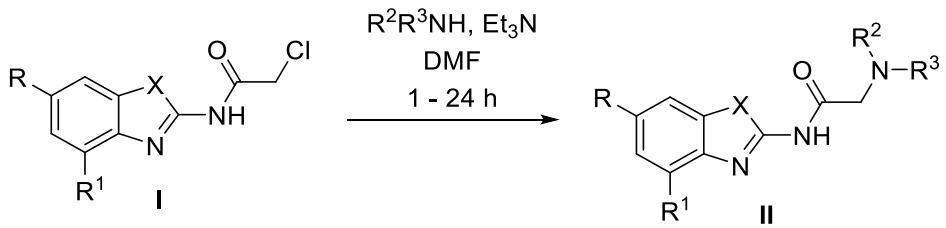
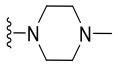
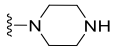
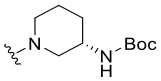
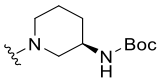
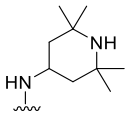
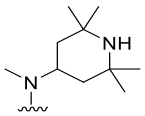
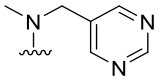
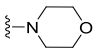
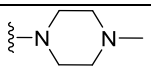
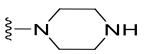
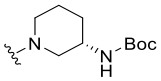
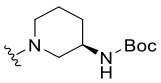
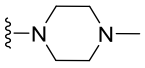


Figure 16: Planned structures with lateral chain containing a second basic site (in red): piperazine and *N*-methylpiperazine (structures **I**); 4-amino-2,2,6,6-tetramethylpiperidine derivatives (structures **II**); 3-aminopiperidine derivatives (structures **III**), as mimic of 2-DOS (**130**). General structure for benzothiazole scaffolds is depicted in light purple.

3.2.5.2 Synthesis

Various substituted aminoacetamides were obtained from chloroacetamides **67** and **82**. As aforementioned, piperazine and *N*-methylpiperazine analogues **132** – **135** were synthesised (Table 12, entries 1, 2, 9, and 10) *via* nucleophilic substitution, using triethylamine as auxiliary base, along with tetramethylpiperidine derivatives **136** and **137** (entries 5 and 6) and *N*-methyl-*N*-pyrimidinemethyl derivative **138** (entry 7). Morpholine substituted analogue **139** (entry 8) was also synthesised, as described in literature[130]. The 3-aminopiperidine derivatives were obtained, utilising the same conditions, as Boc-protected intermediates (entries 3, 4, 11, and 12) in both configurations. One representative with benzimidazole moiety, compound **140** (entry 13), was synthesised. The structure consists of a fusion between **105**, with benzimidazole moiety, based on the promising results obtained for this compound (see Chapter 3.2.4.2, Table 11), and the *N*-methylpiperazine in the lateral chain.

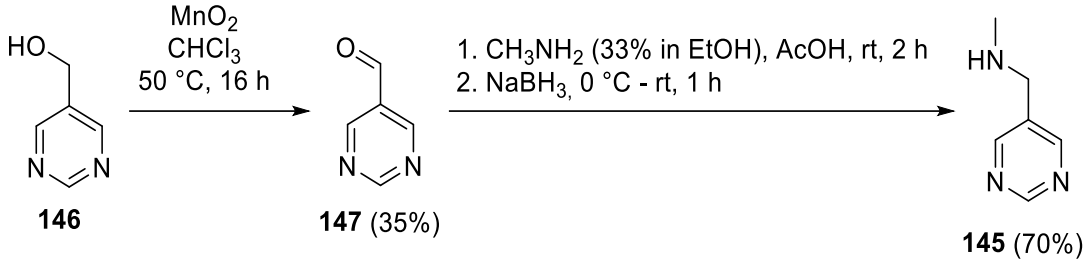


Entry	Starting material (I)	R^2R^3N	II (yield)
1			**132** (79%)
2			**133** (23%)
3			**141** (90%)
4			**142** (95%)
5	**67** (R = OCH_3, R^1 = H, X = S)		**136** (47%)
6			**137** (48%)
7			**138** (36%)
8			**139** (67%)*
9			**134** (91%)
10			**135** (44%)
11	**82** (R = R^1 = OCH_3, X = S)		**143** (85%)
12			**144** (83%)
13	**110** (R = OCH_3, R^1 = H, X = NH)		**140** (48%)

*Conditions for **139**: Morpholine, EtOH, 80 °C, 6 h.

Table 12: Additional basic site analogues.

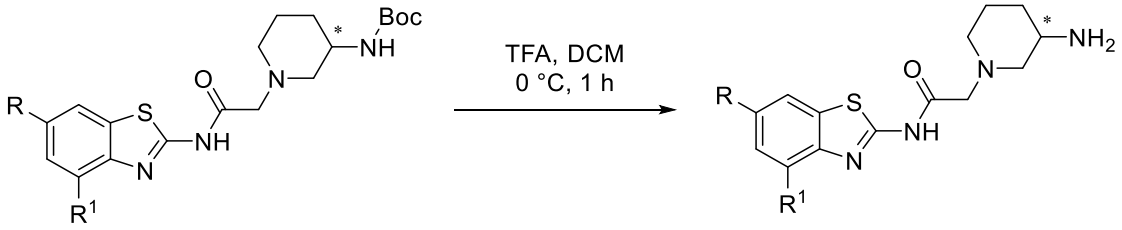
To obtain compound **138**, the secondary amine *N*-methylpyrimidinemethanamine **145** was synthesised, unlike all the other required amines available in the lab, as shown in Scheme 32: pyrimidine-5-methanol (**146**) was oxidised with MnO_2[131] to aldehyde **147**, which was then reductively aminated with methylamine to give secondary amine **145.**



146 **147** (35%) **145** (70%)

Scheme 32: Synthesis of building block **145** used to obtain **138** (see Table 12, entry 7).

The free piperidines **148** – **151** were obtained by Boc deprotection with TFA in DCM, respectively from *N*-Boc-protected amines **141** – **144** (Scheme 33).



141 (*S*), R = OCH_3, R^1 = H
142 (*R*), R = OCH_3, R^1 = H
143 (*S*), R = R^1 = OCH_3
144 (*R*), R = R^1 = OCH_3

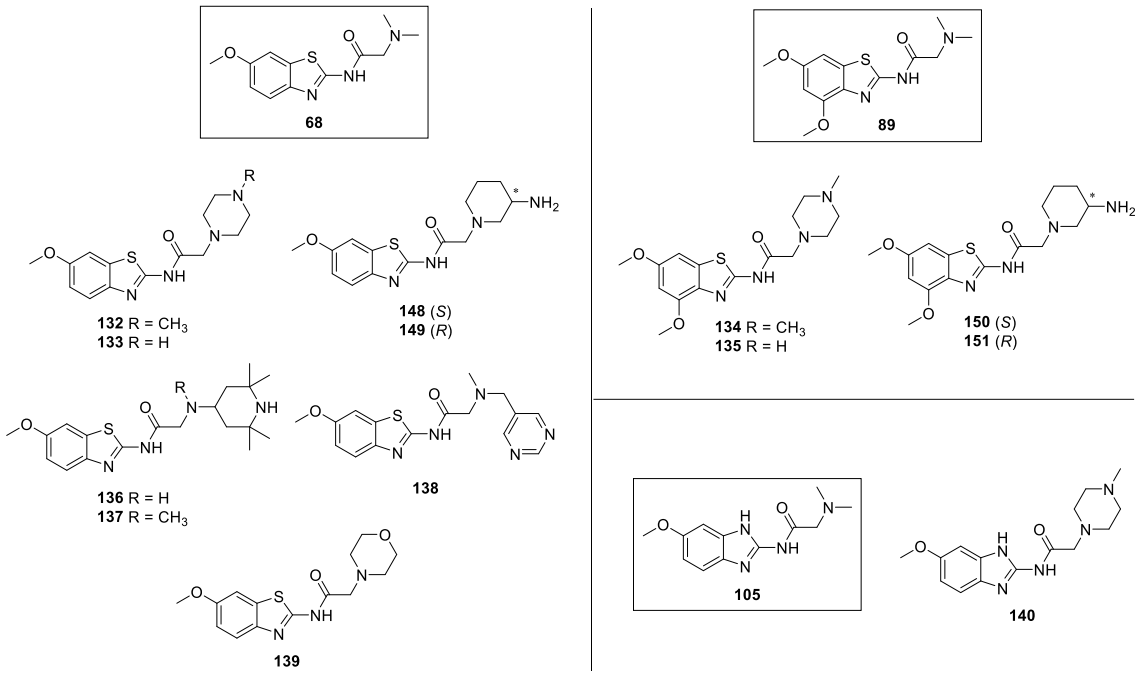
148 (*S*), R = OCH_3, R^1 = H (80%)
149 (*R*), R = OCH_3, R^1 = H (87%)
150 (*S*), R = R^1 = OCH_3 (99%)
151 (*R*), R = R^1 = OCH_3 (99%)

Scheme 33: *N*-Boc deprotection of **141 – 144** to obtain respectively 3-aminopiperidines **148** – **151**.

3.2.5.3 NMR-based results

The NMR-based results of analogues containing an additional basic group in the lateral chain are shown in Table 13. For compounds **134**, **135**, **150**, and **151** the wLOGSY values were not assignable (Table 13, entries 12 – 15) and therefore the values were considered as 0.00. The binding was improved for compounds **132** – **135** (entries 4, 5, 12, and 13), which share the *N*-methylpiperazine or piperazine moiety, compared to **68** and **89**. Compounds **148** – **151** (entries 6, 7, 14, and 15), containing the 3-aminopiperidine residue, displayed the highest overall scores of the series. Having a closer look, the results were higher (CSP and T_2) and comparable (wLOGSY) for **148** and **149** in comparison with **68**, and the impact was very pronounced for **150** and **151** on the T_2 in comparison with **89**. Pyrimidine derivative **138** (entry 10) showed a lower binding effect compared to compounds **132** – **135**, probably related to the weaker basicity of the amine compared to the aliphatic ones. Morpholine derivative **139** (entry 11) displayed an even lower binding effect, lacking the second basic site, and confirming the crucial role of an oligoamine side chain in gaining activity towards the target. On the other hand, compound **140** (entry 16), obtained by the fusion of the promising benzimidazole **105** and *N*-methylpiperazine group, did not lead to the same effect registered for compounds **132** – **135**. Compound **137** displayed better values compared to the almost inactive *N*-demethylated **136** (entries 8 and 9). The lower values, in comparison with 6-methoxy

substituted **132**, **133**, **148**, and **149**, could possibly be explained by the steric hindrance of the four methyl groups of 2,2,6,6-tetramethylpiperidine, which might prevent interaction with the second basic site in the lateral chain. Another noteworthy effect is the fact that almost all compounds with the additional basic site bearing the 6-methoxybenzothiazole (entries 4 – 9), with exception of **136** (entry 8), gave higher overall scores than **68**, along with lowest effects for **138** and **139** (entries 10 and 11), and the same trend was respected for 4,6-dimethoxybenzothiazole derivatives **134**, **135**, **150**, and **151** (entries 12 – 15) in comparison to **89**.



Entry	Compound	CSP [Hz]	wLOGSY	T_2 reduction [%]	Highest CSP	Highest wLOGSY	Highest T_2	Sum	Ranking
1	**68**	12.23	0.72	79.26	12	7	10	29	12
2	**89**	31.92	2.66	36.00	5	1	13	19	7
3	**105**	25.08	0.62	87.00	7	9	8	24	10
4	**132**	22.14	0.80	91.00	10	6	7	23	9
5	**133**	24.81	1.54	86.71	8	2	9	19	7
6	**148**	30.18	0.82	100.00	6	5	1	12	1
7	**149**	24.72	1.01	100.00	9	4	1	14	2
8	**136**	10.09	0.00	0.00	13	12	16	41	15
9	**137**	12.99	1.19	76.80	11	3	11	25	11
10	**138**	2.88	0.32	28.89	15	10	14	39	14
11	**139**	2.06	0.28	21.25	16	11	15	42	16

12	**134**	32.79	-	100.00	3	12	1	16	5
13	**135**	31.95	-	100.00	4	12	1	17	6
14	**150**	34.62	-	100.00	1	12	1	14	2
15	**151**	34.05	-	100.00	2	12	1	15	4
16	**140**	9.48	0.68	41.23	14	8	12	34	13

Table 13: NMR-based results of analogues of **68**, **89**, and **105** containing a second basic group in the lateral chain.

With all the considerations taken together, the most promising compounds were the enantiomeric 3-aminopiperidine derivatives **148** – **151**.

3.2.6 Second site binders

During the course of this work, the promising 6-methoxybenzothiazole moiety was confirmed to bind the internal loop of 5_SL1 by total correlation spectroscopy (TOCSY) experiments, as described in detail in the next Chapter. Additionally, a possible expansion of the structure based on docking experiments was proposed. The promising ligands that were planned on this new information were synthesised, as presented in the following Chapters, and tested by NMR-based screening.

3.2.6.1 Design

5_SL1 of SARS-CoV-2 RNA was recently screened using a poised fragment library as described before[92]. For one of the fragment hits, the paramagnetic analogue S-391 (**129**) was synthesised (Figure 17) by Anna van der Sluis under the supervision of Dennis P. Piet and Peter Maas (Specs, part of eMolecules, Netherlands). It contains a TEMPO (2,2,6,6-tetramethylpiperidin-1-oxyl) group with an unpaired electron at the NO substituent. This electron has paramagnetic properties and induces relaxation in a distance dependent way. TOCSY and docking experiments on S-391 (**129**) were performed by Dr. Marcel J. J. Blommers, Dr. Kaspar Zimmermann, and Dr. Kamal Azzaoui from Saverna Therapeutics (Switzerland) and Dr. Claus Ehrhardt, University of Zurich. The synthesised molecule S-391 (**129**) was tested for binding to 5_SL1 by TOCSY in the following way. First, S-391 (**129**) was reduced by adding ascorbic acid in a 1:1 ratio, to obtain a diamagnetic compound. Subsequently, TOCSY spectra were recorded for 5_SL1 and for 5_SL1 in the presence of 5-fold and 10-fold excess of the diamagnetic compound (Figure 17). 5_SL1 resonance assignments were taken from Richter and Hohmann *et al.*[76]. For some cross peaks, clear chemical shift perturbations were observed, indicating specific binding of the compound. In the TOCSY spectrum, the 5_SL1 RNA aromatic proton resonances of uridine and cytidine that are

coupled are observed (Figure 17): in absence (blue) and in presence of diamagnetic S-391 (**129**), respectively at 1:5 (red) and 1:10 (green) concentration ratio. The largest CSPs were identified in the bulge, nucleotides U11, U13, U25, and C28, and in the apical loop, nucleotide C20.

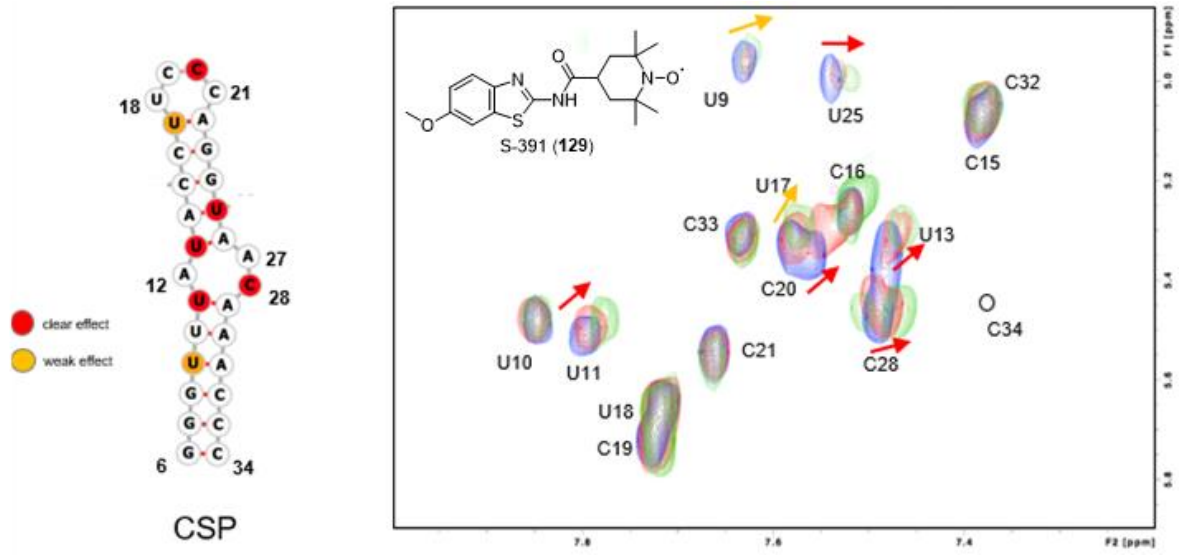


Figure 17: TOCSY spectrum of induced CSP of 5_SL1 RNA upon binding of diamagnetic S-391 (**129**).

In the next experiment, the TOCSY spectrum was recorded of 5_SL1 after addition of paramagnetic S-391 (**129**, Figure 18). This spectrum shows induced relaxation of aromatic protons of nucleotides U10 and U11 and not of other aromatic protons. It can be concluded that S-391 (**129**) binds exclusively in the bulge region and not in other regions where for some nucleotides CSPs were observed, most likely due to a long-range conformational effect, caused by binding.

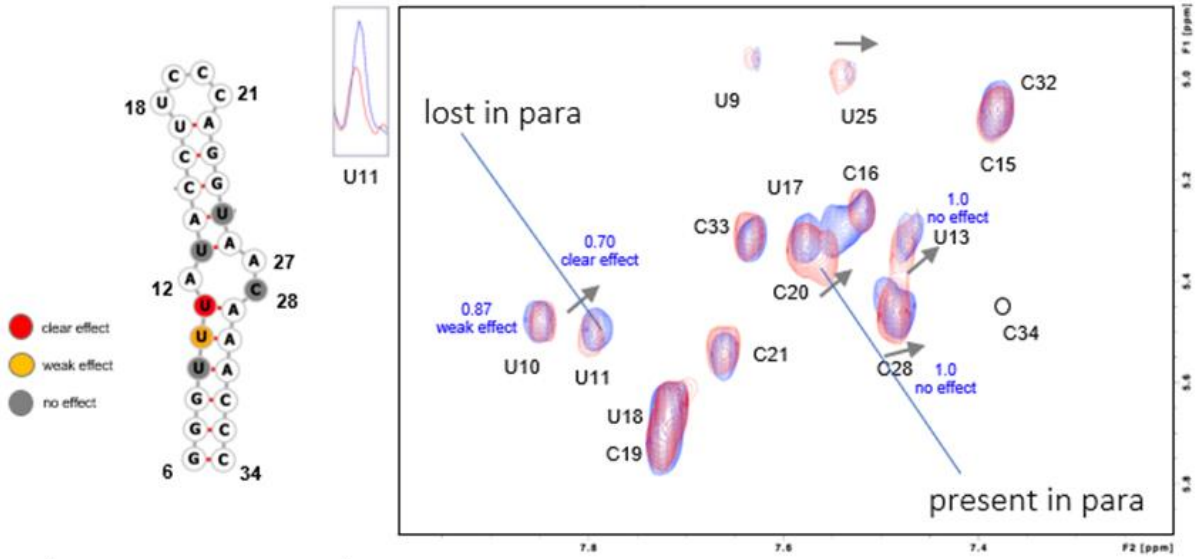


Figure 18: TOCSY spectrum of paramagnetic induced relaxation of 5_SL1 RNA upon binding paramagnetic S-391 (**129**).

The reduction in signal intensity of the cross peaks was measured. As can be derived as followed, the relative distances between aromatic protons and the paramagnetic centre were calculated and used in docking of S-391 (**129**) into a structural model of 5_SL1. Since no structure with an obvious binding pocket of 5_SL1 was available, a homology model was built from the crystal structure of the A-site of the bacterial ribosome in complex with an aminoglycoside. In this model, a binding pose of S-391 (**129**) that fulfils the experimental distance restraints is obvious, and the docking pose was subsequently energy minimised using the AMBER force field. It is noted that the structure was later subjected to molecular dynamics simulations and the ligand docking pose remains, giving confidence to the proposed model. The docking mode is shown in Figure 19. The two distances between aromatic protons and the paramagnetic centre are in agreement with the experimental data and the paramagnetic centre faces the major groove.

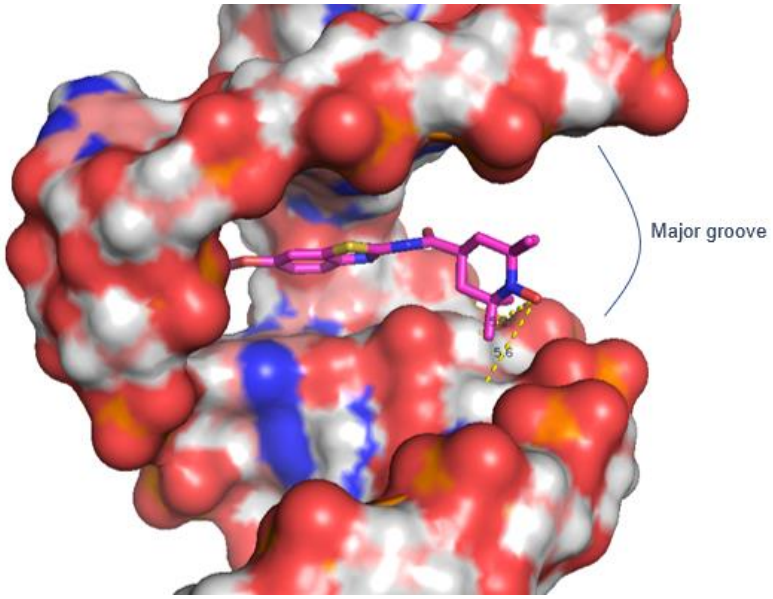


Figure 19: Docking model of the binding of S-391 (**129**) to 5_SL1.The ligand is represented by sticks. The RNA binding pocket is shown with a solid surface. Distances are shown with dashed lines.

After identifying and validating S-391 (**129**) as 1st-site ligand, this compound was further used to find proximate ligands, called 2nd-site ligands, as previously described by Jahnke *et al.*[132]. In the presence of the paramagnetic fragment S-391 (**129**), the same poised fragment library as described before was screened. Several hits were found, and the experiment was repeated to validate them, with the aim to observe an S-391 (**129**) concentration dependent paramagnetic relaxation. This effect was observed for the three validated hits that are shown in Figure 20, and the paramagnetic relaxation was observed in the aliphatic region of these hits (orange dotted line).

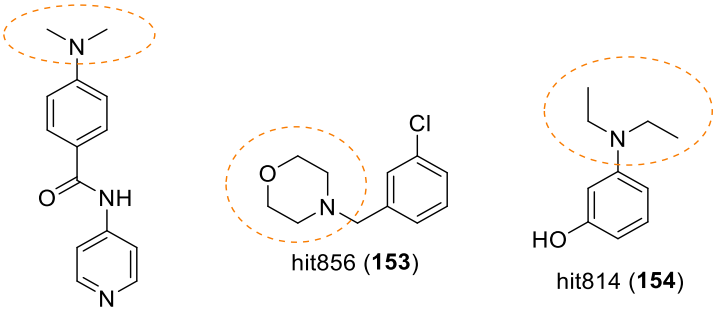


Figure 20: Area of observed paramagnetic relaxation of 2nd-site hits in the presence of the paramagnetic 1st-site binder S-391 (**129**).

Since the paramagnetic centre faces the major groove in the model of the complex with S-391 (**129**), it is expected that 2nd-site ligands bind in the major groove of 5_SL1. The identified 2nd-site binding ligands were docked in the major groove and subjected to MD stimulations using AMBER, suggesting the alignment of the 1st- and 2nd-site binder, as shown in Figure 21. This alignment was derived from the fact that the aliphatic resonances are in close proximity to the paramagnetic centre (relaxation effect), whereas the aromatic resonances are further away (no relaxation effect). Figure 21 shows an example of a docking model of S-391 (**129**) and a 2nd-site ligand, as predicted, binding in the major groove. In order to design a new molecule, that can replace both ligands while keeping its interactions with 5_SL1, the optimal length of the linker and the relative orientation of the 1st-site ligand, bearing the benzothiazole, and 2nd-site ligand, hit814 (**154**), a 3-aminophenol, were studied. It was concluded that probably three atoms between the two moieties were needed.

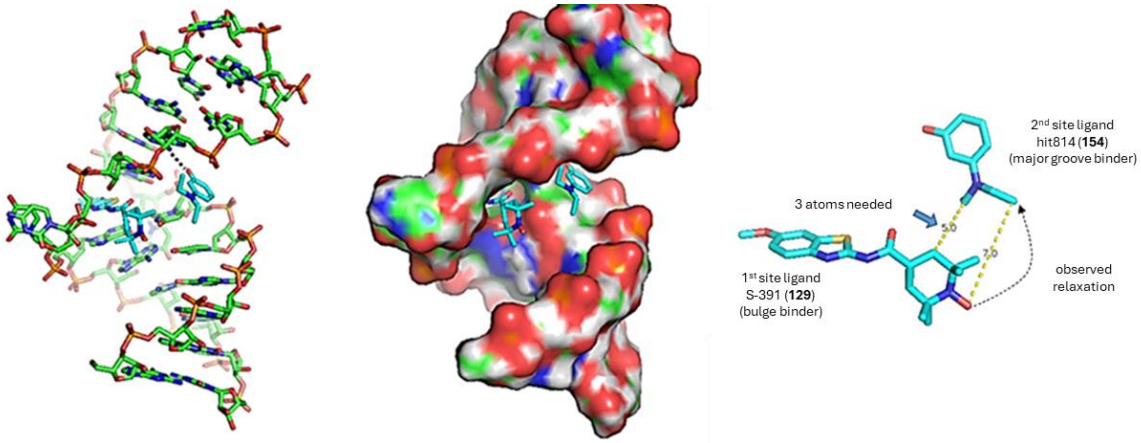


Figure 21: Alignment obtained by docking simulations between the 1st-site ligand S-391 (**129**) and 2nd-site ligand hit814 (**154**).

Based on the results described above, a first structure was proposed (Figure 22, structure **A**), with the benzothiazole portion as 1st-site binder, highlighted in blue, and the hit814 (**154**) portion as 2nd-site binder, in red. Structure **A** was then further implemented into the three structural proposals, containing basic aliphatic amines to increase the polarity: **v1**, on the left,

and **v2**, in the centre, containing 3-aminopiperidine as linker, based on the promising results of **148** and **149** (see Chapter 3.2.5.3, Table 13); structure **v3**, on the right, in which the fragments are connected with a linear linker. The structural proposals **v1**, **v2**, and **v3** were then developed into actual aimed structures, depicted in Figure 22: starting from structure **v1**, (*S*)-configured **155** and **156** and (*R*)-configured **157** and **158**; the two enantiomers **159** and **160**, based on structure **v2**; from structure **v3**, the six analogues with an ethylidene chain (**161** and **162**), a propylidene chain (**163** and **164**), and butylidene chain (**165** and **166**) were planned. For the R chain, methyl and ethyl group were chosen in order to have more than one representative for each class.

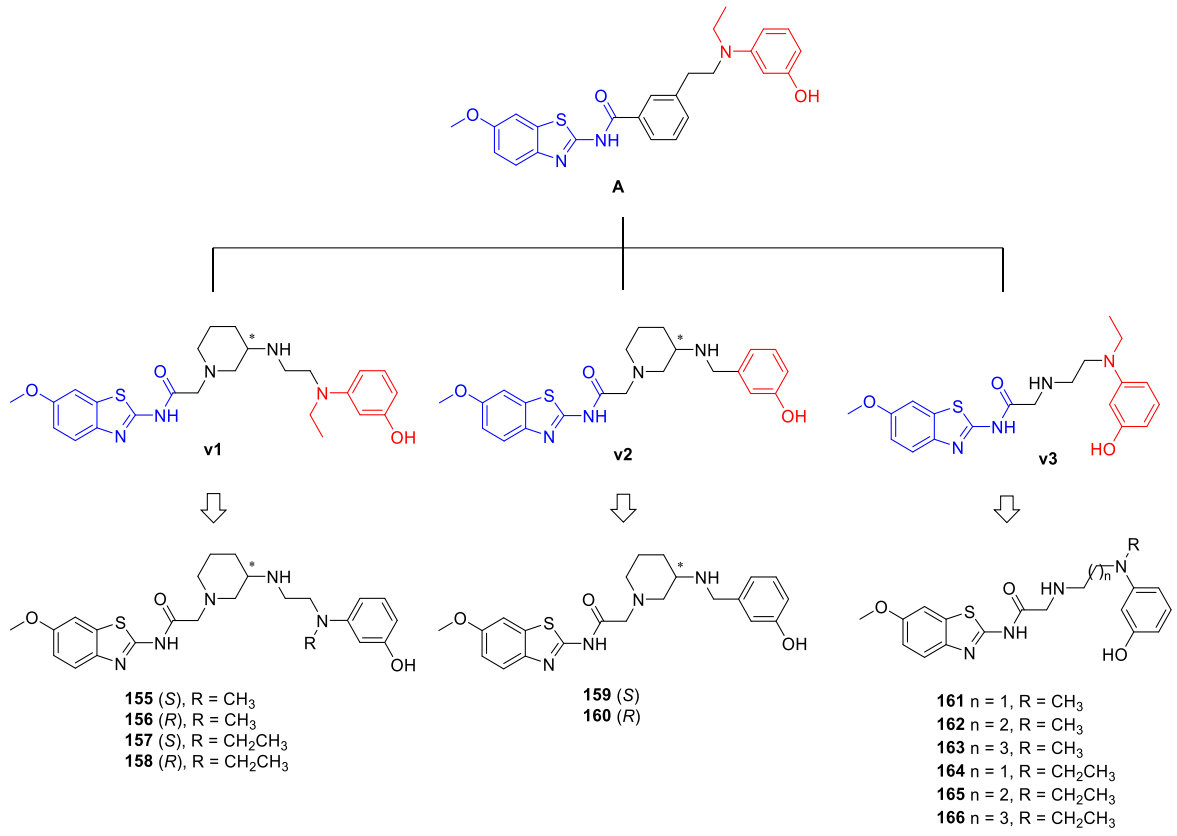


Figure 22: Design of 2nd-site binding analogues, starting from the proposed structures **v1**, **v2**, and **v3**.

The proposed structures **v1**, **v2**, and **v3** were also docked, leading to the conclusion that the introduction of a 3-hydroxypropyl chain as substituent of the aromatic amine could bring additional interactions with the phosphates of RNA, improving the binding with the target, as shown in Figure 23.

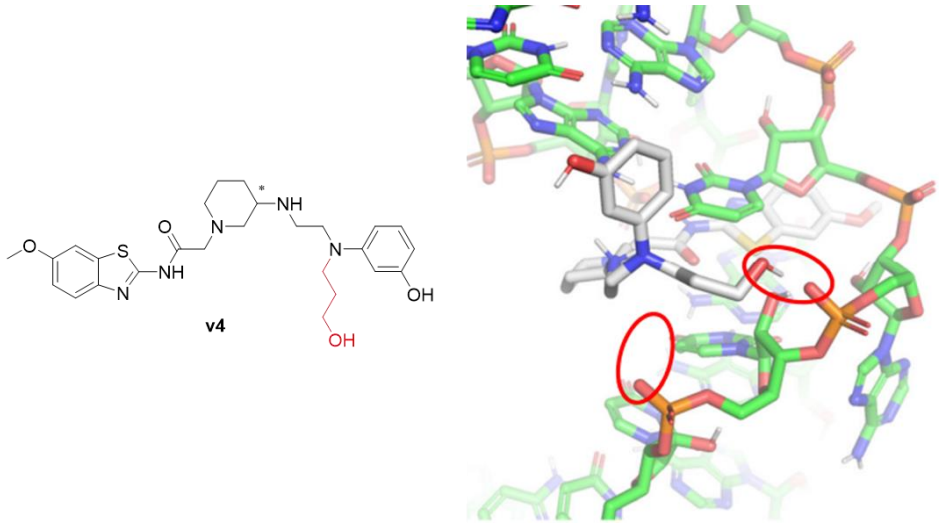
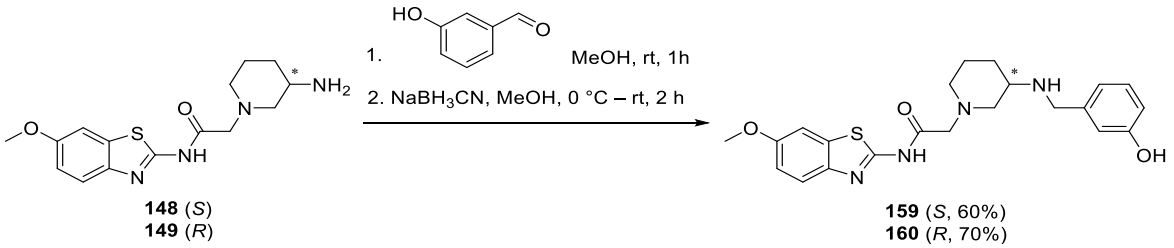


Figure 23: Introduction of 3-hydroxylpropyl residue after docking experiments of the merged structure **v1** (**v4**, in grey). Circled in red the O^- groups of RNA phosphates that could develop additional interactions with the OH of 3-hydroxypropyl chain of **v4**.

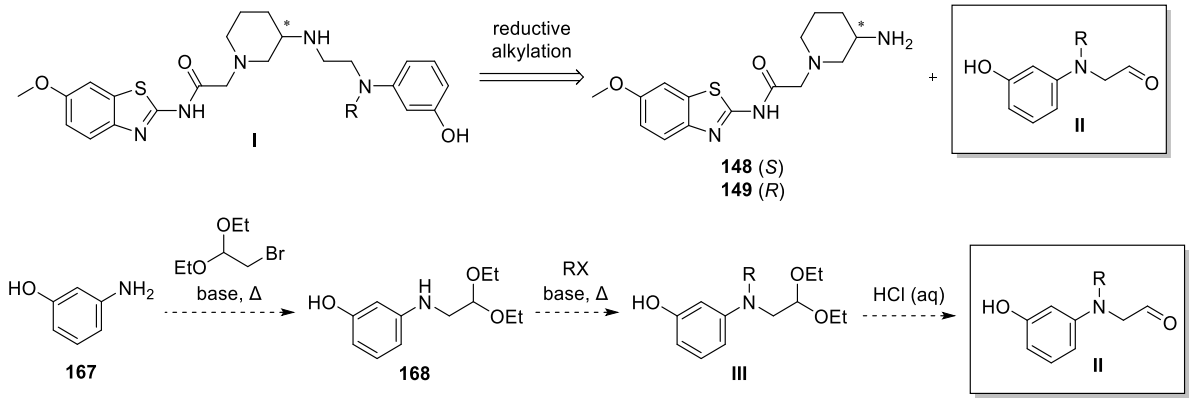
3.2.6.2 Synthesis – 3-aminopiperidine linker (v1 and v2)

The analogues from **v2** were obtained starting from available benzothiazoles **148** and **149** (see Chapter 3.2.5) and performing a reductive *N*-alkylation using 3-hydroxybenzaldehyde. Due to the poor solubility of amines **148** and **149** in most organic solvents, $NaBH_3CN$ was chosen as reducing agent, allowing the reaction to be conducted in methanol (Scheme 34).



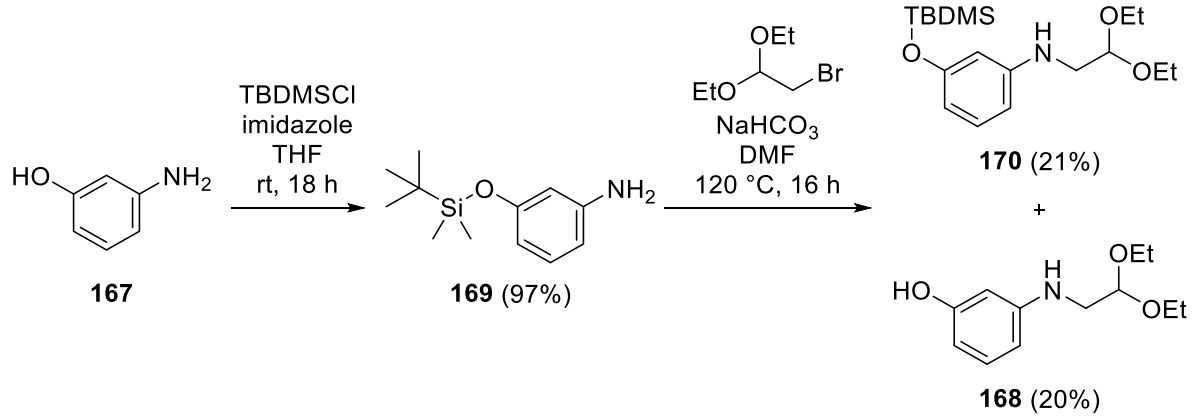
Scheme 34: Reductive alkylation of amines **148** and **149** with 3-hydroxybenzaldehyde to obtain derivatives **159** and **160**.

The idea to construct the analogues based on **v1** (Scheme 35, structures **I**) was to derivatise the free amino group of compounds **148** and **149** with a second building block containing the 3-aminophenol moiety. A lateral chain bearing an aldehyde (structures **II**) was planned to provide the final product *via* reductive alkylation as depicted in the retrosynthesis of Scheme 35. The plan to synthesise structures **II** was the following: two subsequent alkylations, the first one using bromoacetaldehyde, protected as diethyl acetal, and the second with alkyl halides, to further derivatise the aromatic amine to obtain structures **III**, followed by deprotection of the acetal to release the desired aldehyde derivatives **II**.



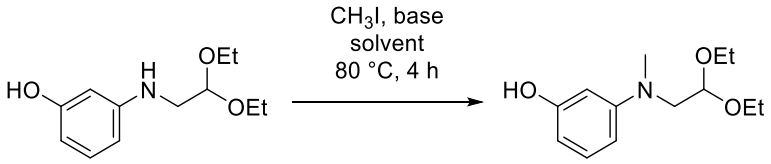
Scheme 35: Retrosynthesis of structure **I** and initial synthetic pathway for the synthesis of structure **II**.

An additional consideration was the introduction of a protective group on the phenol moiety to avoid possible side reactions, such as alkylation of the phenol, that could lead to lower yields, and, on the other hand, to facilitate the purification avoiding additional intermolecular interactions between the functional groups present in the molecules. The choice of a suitable protective group is strictly limited from the synthetic pathway, as it must be stable in both basic (alkylations) and acidic conditions (deprotection) and to reduction (reductive alkylation). Moreover, the cleavage needs to be selective and preferably performed under mild conditions due to the presence of the amide in the final structure. At first, the standard protection as TBDMS ether was tried, since it is possible to selectively protect the phenol in the first step in the presence of the aromatic amine, it's stable to bases, and the cleavage with tetrabutylammonium fluoride (TBAF) is selective and performed under mild conditions. The problem could be the cleavage under the acidic conditions of acetal deprotection to release the aldehyde. This first approach is depicted in Scheme 36. The protection with TBDMSCl was conducted as described in literature[133]. A trial reaction of substitution with bromoacetaldehyde diethyl acetal was conducted in DMF in slightly basic conditions. After treating silyl ether **169** overnight at 120 °C, along with the expected product **170**, the deprotected analogue **168** was isolated.



Scheme 36: 3-Aminphenol (**167**) protection with TBDMS and substitution with bromoacetaldehyde diethyl acetal to obtain compound **170**.

More attempts with reduced temperature (100 °C) were conducted, applying longer reaction times (20 – 36 h) with the aimed product **170** found in traces, confirmed only by atmospheric solids analysis probe (ASAP) and ^{1}H-NMR and, therefore, not characterised, and with no reaction occurring at 80 °C after 24 h. In the meantime, as the amount of secondary aromatic amine **168** obtained with the previous reaction was enough, *N*-methylation with iodomethane was performed (Table 14) and the protection step was postponed. The great reactivity of iodomethane allows milder reaction conditions and shorter reaction times. Two alternative protocols were applied one using potassium carbonate as auxiliary base in acetonitrile (ACN, Table 14, entry 1) and the other using *N*,*N*-diisopropylethylamine (DIPEA) in DMF (entry 2), respectively proposed by Wang *et al.*[134] and Jiao *et al.*[135]. The first protocol was chosen due to the higher yield. Moreover, the lack of obvious formation of the by-product, methylated also on the phenol makes protection in the first two substitution steps unnecessary.

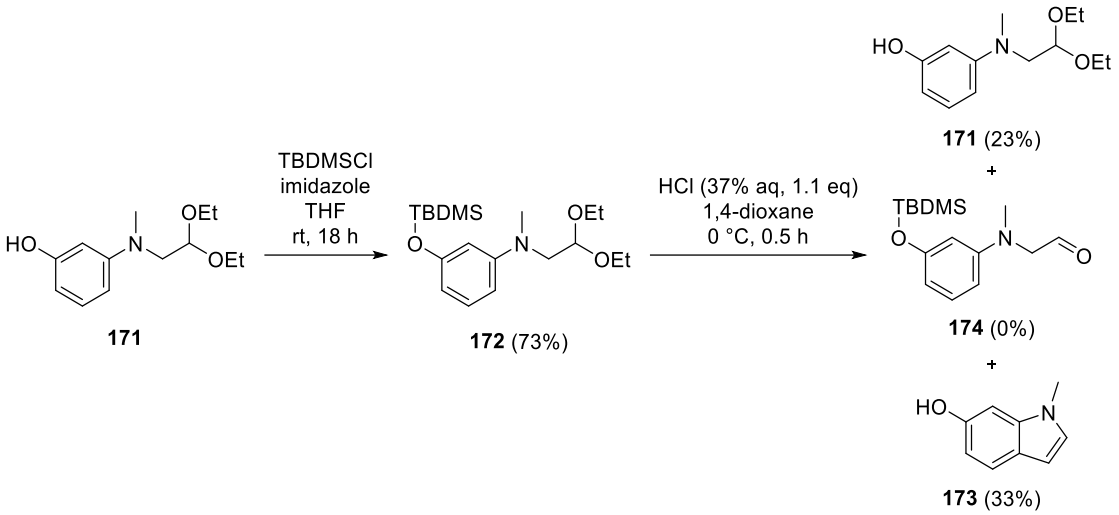


168 **171**

Entry	Base	Solvent	Yield
1	K_2CO_3	ACN	67%
2	DIPEA	DMF	36%

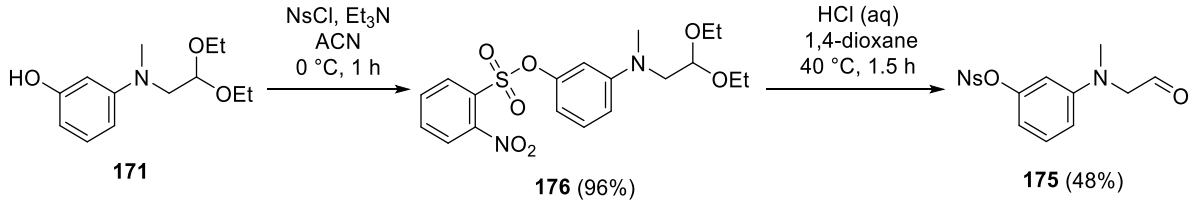
Table 14: Methylation of secondary aromatic amine **168**.

Compound **171** was protected again with TBDMS (Scheme 37) to test for the probable lability in strong acidic conditions. Through acidic treatment of TBDMS-protected **172**, the only two products isolated were either TBDMS-deprotected **171** or 6-hydroxyindole **173**, obtained as main product *via* stereoselective intramolecular cyclisation.



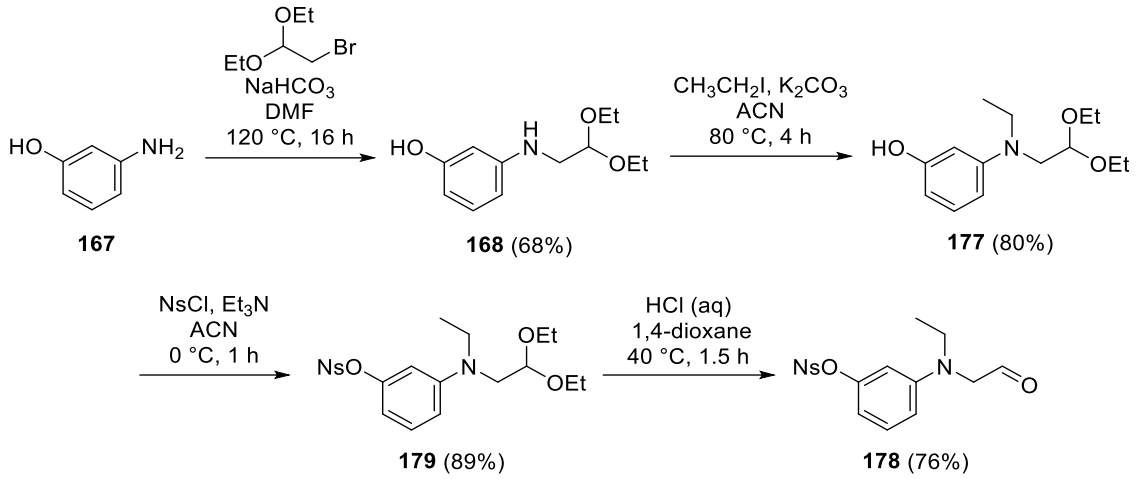
Scheme 37: TBDMS-protection of **171** and treatment in acidic environment.

Based on the latter reaction, TBDMS-protection was abandoned and the need of a protecting group on the phenol stable in acidic condition was confirmed by the cyclisation reaction. In this regard, the conversion of phenol into a sulfonate was considered. Aihara *et al.*[136] published the use of nosylates as protecting group, providing an excellent alternative to the standard tosylate, being it easily and selectively cleaved under mild conditions with soft nucleophiles. This difference in reactivity is attributable to the nitro substituent in *ortho* position, which with strong electron-withdrawing effect increases the electrophilic character of the sulfonate. In Scheme 38 is depicted the nosyl protection of phenol **171** with nosyl chloride (NsCl), quick and efficient, and the subsequent treatment in acid condition to release aldehyde **175**. Compared to the conditions applied for **172**, the temperature was raised to 40 °C to shorten the reaction duration, as there was no risk for side reactions. The rather low yield of acetal deprotection to aldehyde derivative **175** (48%) was probably due to the instability of the product, since a large part was lost during purification by flash column chromatography.



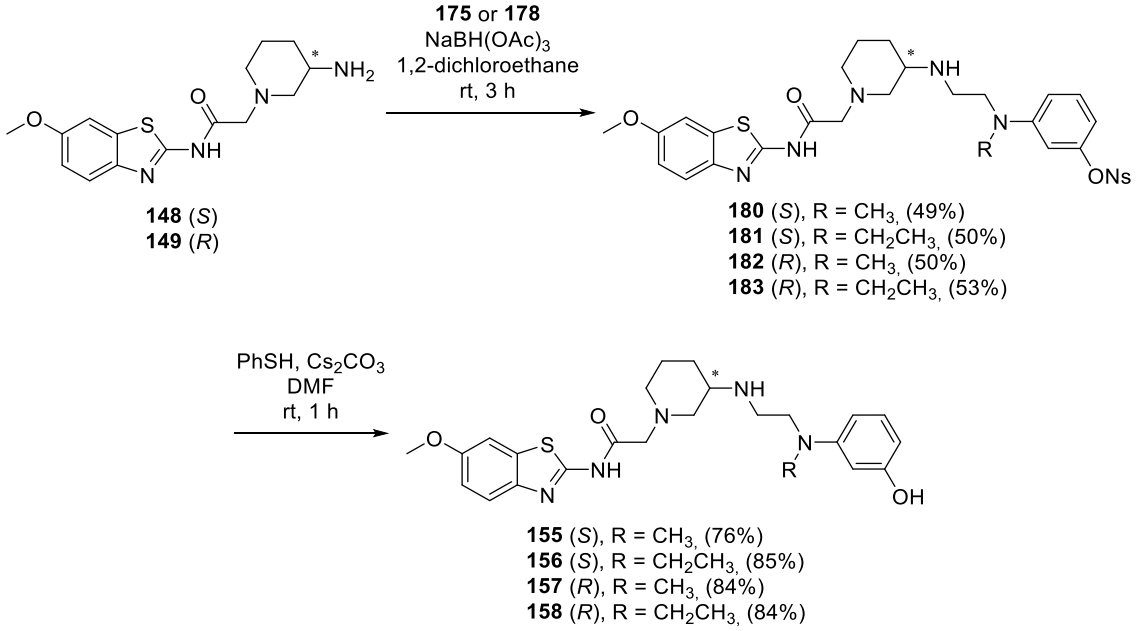
Scheme 38: Nosylation of **171** and treatment in acid condition to release aldehyde **175**.

The same pathway was tested on the *N*-ethyl derivative. As shown in Scheme 39, secondary aromatic amine **168** was synthesised from 3-aminophenol (**167**), performing the two alkylation steps with bromoacetahldeyde diethyl acetal and iodoethane. The free phenol **177** was then nosylated and aldehyde **178** released, following the same reaction conditions as applied for **175** (see Scheme 38). The yield of aldehyde release was raised through a shorter and faster flash column chromatography process, in comparison to aldehyde **175** purification.



Scheme 39: Aldehyde **178** synthesis based on the previous result on *N*-methyl homologue **175**.

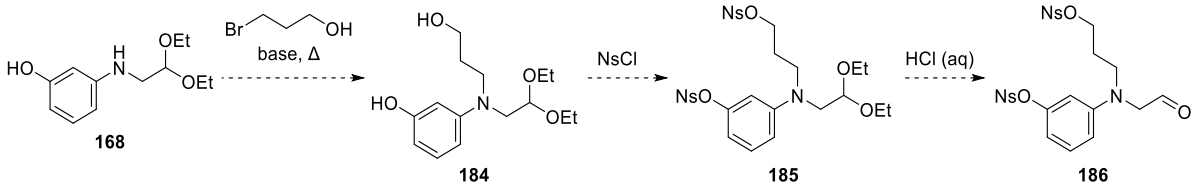
Once aldehyde **175** and **178** were obtained, the fusion with the benzothiazole moiety was achieved by reductive amination respectively with **148** and **149**. The same protocol, described for the synthesis of compounds **159** and **160** (see Scheme 34), was applied, using $NaBH_3CN$ in methanol. At the TLC, it was possible to notice aldehyde consumption but no product forming, suggesting a reduction of the aldehyde to alcohol before the imine intermediate formation. Therefore, the reducing agent was replaced with sodium triacetoxyborohydride, a weaker reducing agent but selective for imines over aldehydes and ketones. One limitation is the rapid hydrolysation that this reagent undergoes with methanol and, therefore, the need to perform the reaction in 1,2-dichloroethane. Despite the very low solubility of amines **148** and **149** in this organic solvent, the nosylated products **180** – **183** (Scheme 40, first step) were obtained with acceptable yield. The nosyl deprotection, performed with thiophenol and caesium carbonate, led to the corresponding final desired products **155** – **158**.



Scheme 40: Synthesis of 2nd-site binders **155** – **158**.

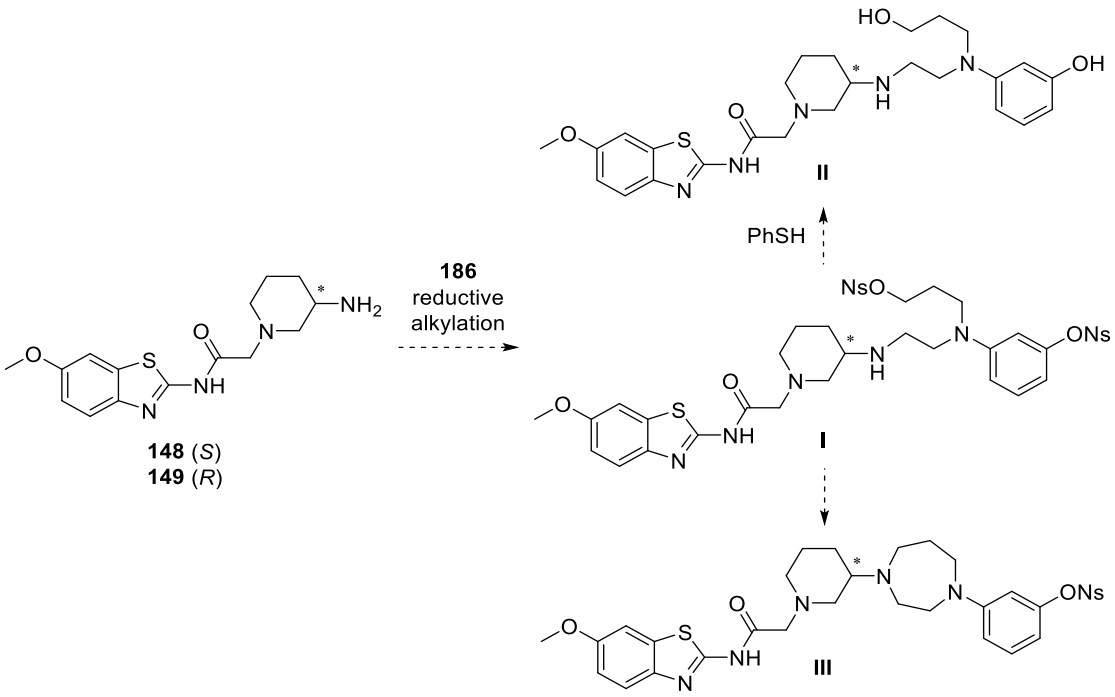
*Hydroxypropyl lateral chain – structure **v4***

In this chapter are presented the attempts to synthesise the analogues based on **v4** with the hydroxypropyl moiety as substituent on the aromatic amine (see Chapter 3.2.6.1, Figure 23). The initial idea was to follow a similar synthetic pathway as the one presented for analogues **155** – **158**, with the additional challenge of the presence of the alcoholic group in the structure (Scheme 41). Starting from **168**, the plan was to convert it into a *N*-(3-hydroxypropyl) derivative **184** with 3-bromo-1-propanol, followed by simultaneous protections of the phenolic and alcoholic functions, and then aldehyde release from the diethyl acetal **185**.



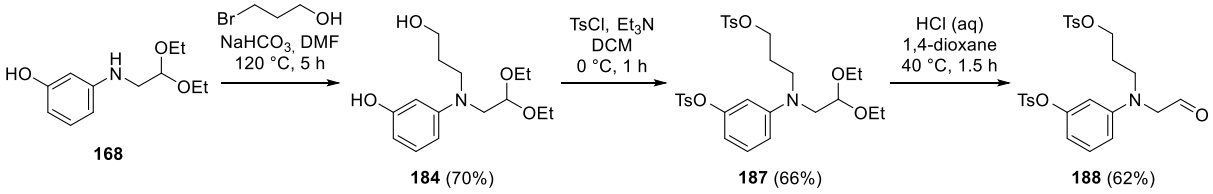
Scheme 41: Synthetic plan of aldehyde **186** with nosylated hydroxypropyl lateral chain.

De Castro *et al.*[137] performed the nosylation on 2-(3-hydroxy-1-phenylpropyl)-4-methylphenol, bearing both phenolic and alcoholic functional groups. The aim of De Castro *et al.*[137] was to simultaneously protect the phenolic group and convert the alcohol into a better leaving group to undergo nucleophilic substitution with diisopropylamine, which was not successfully obtained with tosylation. In the present case, the problem could arise in the following step of reductive alkylation. In Scheme 42 is depicted the possible synthetic pathway with reductive alkylation, followed by denosylation to give the desired structures **II**. A probable side reaction in this synthetic route is the 7-ring formation *via* nucleophilic substitution of secondary amine on the alkyl nosylate group in structures **I** to give by-products **III**.



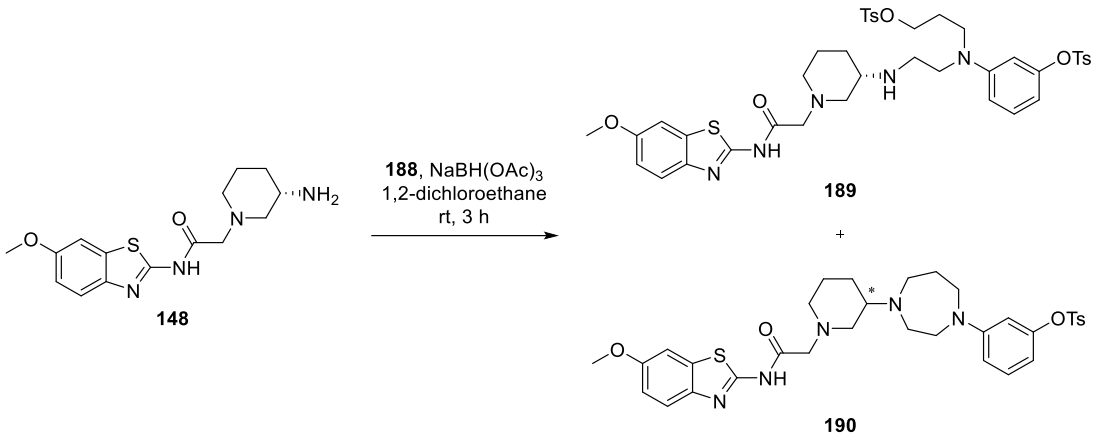
Scheme 42: Reductive alkylation on amines **148** and **149** with aldehyde **186** and deprotection.

With this limitation in mind, the nosylate was replaced by tosylate, due to its lower reactivity. As shown in Scheme 43, secondary amine **168** was substituted with 3-bromo-1-propanol to hydroxypropyl derivative **184**, which was then tosylated on both phenol and alcohol leading to compound **187**. Aldehyde **188** was released upon treatment with aq. HCl as mentioned above.



Scheme 43: Synthesis of aldehyde **188**.

Due to the instability of aldehyde **188**, it was characterised only by ^{1}H NMR and HRMS (see Experimental section, Chapter 5.7.2.2), as the 70 mg available were directly used for the following step of reductive alkylation of **148**, depicted in Scheme 44. Product **189** was formed, according to ASAP. After quenching the reaction, cyclised product **190** was also detected by ASAP. Through purification by flash column chromatography, it was possible to separate a little amount of by-product **190**, while the rest was mixed fractions. The mass to charge ratio of by-product **190** was confirmed by high-resolution mass spectrometry (HRMS) for the clean fractions and both of product **189** and by-product **190** for the mixed fractions.



Scheme 44: Reductive alkylation on amine **148** with aldehyde **188**.

In Figure 24 is shown a ^{1}H NMR spectrum of the first spot, zoomed in the aromatic region. The set of aromatic signals integrated for 11 H showed that one of the two tosylate groups has been lost, confirming, together with the HRMS results, formation of by-product **190**.

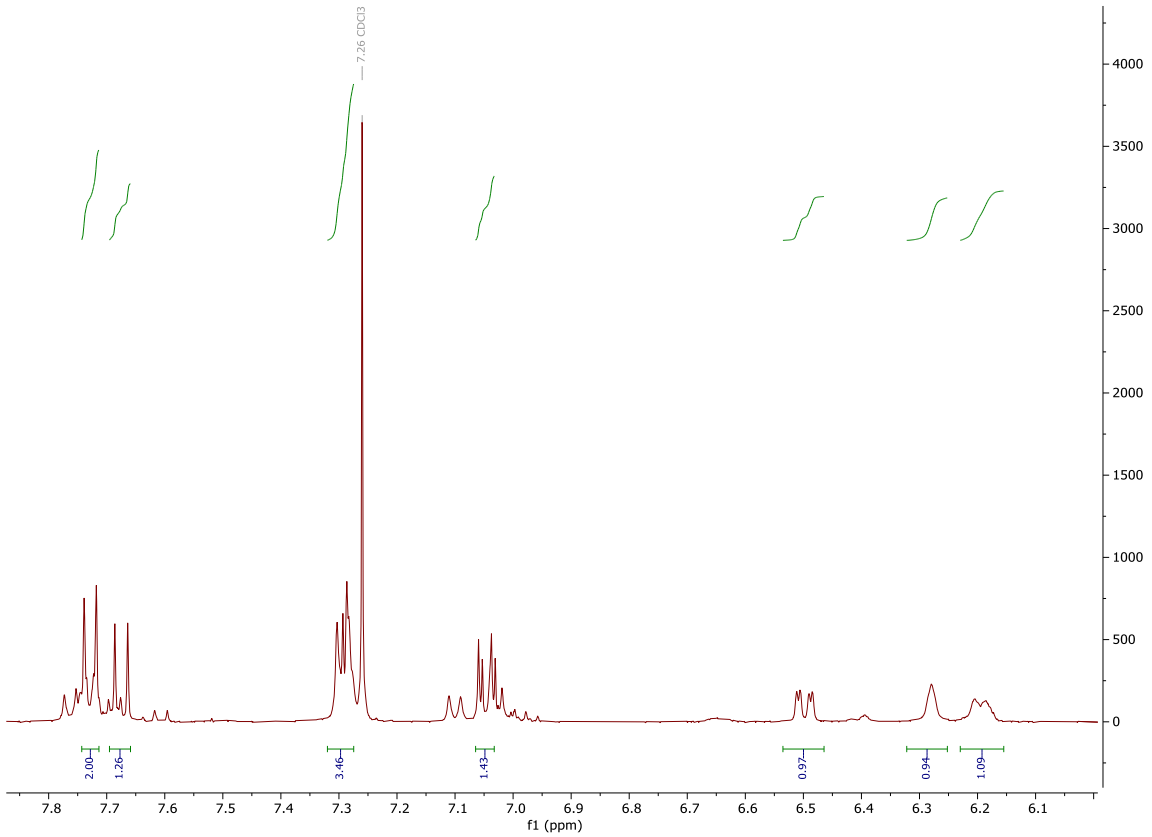
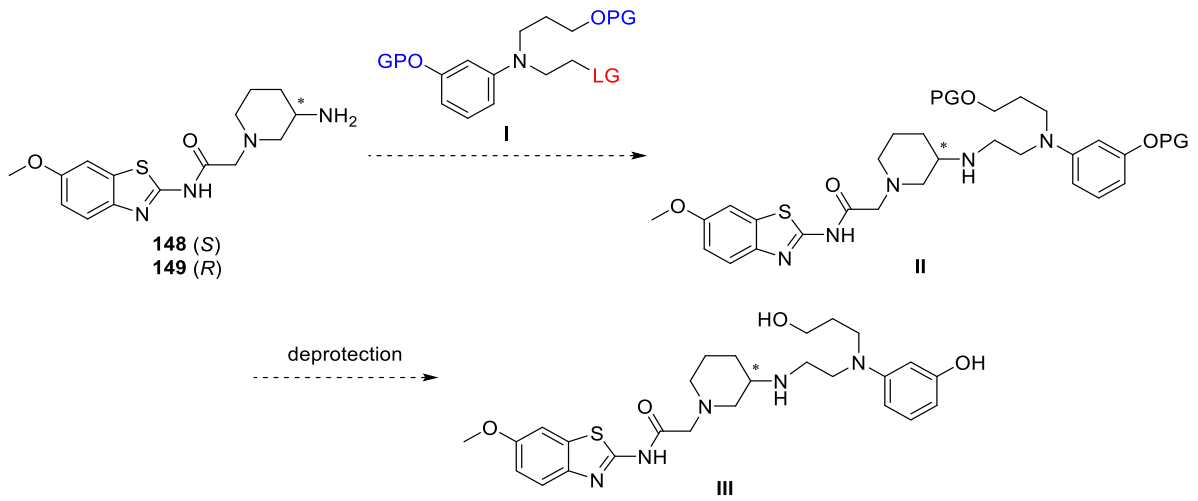


Figure 24: ^{1}H NMR spectra zoomed in the aromatic region of supposed by-product **190**.

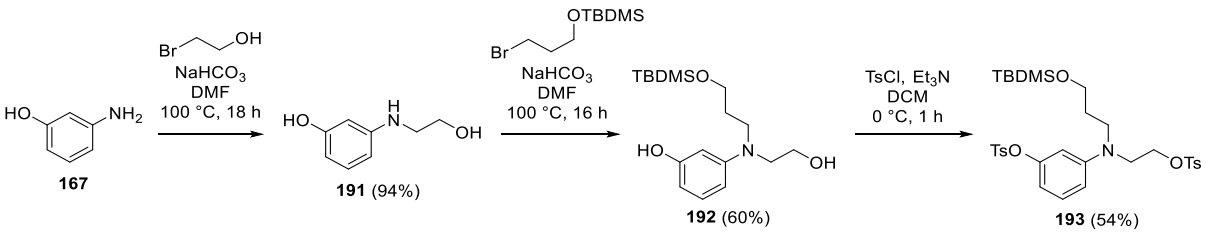
No further analysis was conducted on the sample due to the little amount of substance available. Stating the failure of this last step and the instability of aldehyde **188**, another approach was adopted. The formation of by-product **190** in the latter step was exploited to achieve the desired product. The idea was to design an intermediate (Scheme 45, structures **I**) with the phenol and alcohol groups of the 3-aminophenol protected (in blue) and a good leaving group in 2-position of the ethyl lateral chain (in red). This intermediate could react in a

nucleophilic substitution with amine **148** and **149**. The side reaction of di-substitution of the amine is most likely severely limited by steric factors.



Scheme 45: Alternative route *via* nucleophilic substitution. GL = good leaving group, PG = protecting group.

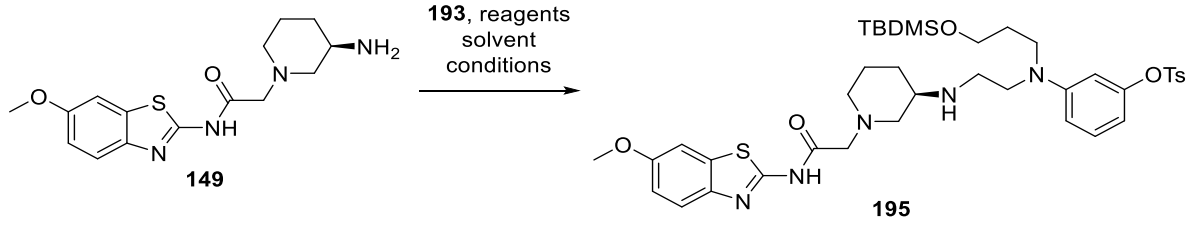
This route *via* nucleophilic substitution, in place of reductive alkylation, allows to avoid the acidic step to release the aldehyde, reopening the possibility of using TBDMS as protecting group, particularly useful in this case being stable to nucleophilic attacks of amines. The first attempt aimed at reproducing a similar mechanism seen for the cyclisation reaction in Scheme 46. With this purpose, 3-aminophenol (**167**) was substituted with 2-bromo-1-ethanol to intermediate **191** and then again with (3-bromopropoxy)(*tert*-butyl)dimethylsilane to **192**. The latter was then di-tosylated to intermediate **193**, exploiting the protection feature on the phenol and activating property on the alcohol, converting it in a better leaving group.



Scheme 46: Synthesis of bis-tosylated derivative **193** based on structures **I** from Scheme 45.

Tosylate **193** was then used to alkylate **149**, as shown in Table 15. A first attempt was performed using triethylamine as auxiliary base and potassium iodide in catalytic amount (Table 15, entries 1 and 2), used in literature to alkylate benzylamine with tosylate esters[138]. In the first case, possibly due to solubility issue of **149** in THF, no reaction occurred after 48 h at 80 °C. Replacing the solvent with MeOH (entry 2), the only reaction occurring, according to ASAP analysis, was silyl ether cleavage of **193**, giving by-product **194**. Following another protocol using primary amine and methanesulfonate in MeOH at 140 °C[139], amine **149** and tosylate **193** were dissolved in MeOH in a pressure tube and different temperature and reaction times were applied (entries 3 – 5): at 80 °C for 24 h (entry 3) there was no reaction occurring;

at 100 °C for 3 h (entry 4) it was only possible to observe the formation of 2-aminobenzothiazole **55**, confirmed by means of ASAP and TLC; at 140 °C for 3 h (entry 5) secondary amine **195** was obtained in low yield (14 mg, 9%), confirmed only by ^{1}H-NMR and HRMS due to the little amount available, along with a 13% of 2-aminobenzothiazole **55**.



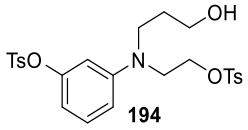
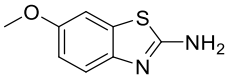
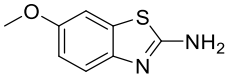
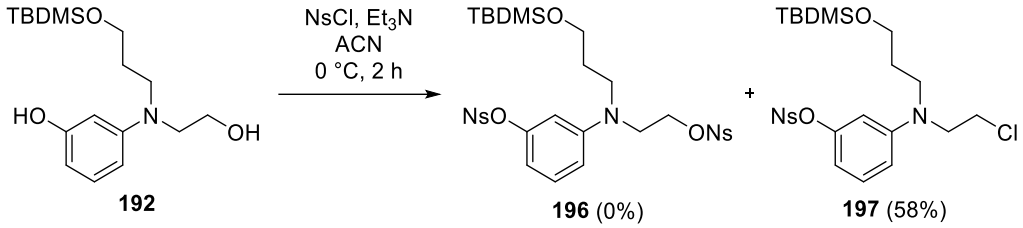
Entry	Reagents	Solvent	Conditions	Yield	By-product
1	Et_3N, KI (cat.)	THF	80 °C, 48 h	-	-
2	Et_3N, KI (cat.)	MeOH	80 °C, 24 h	-	 **194**
3	DIPEA	MeOH	80 °C, 24 h	-	-
4	DIPEA	MeOH	100 °C, 3.0 h	-	 **55** (traces)
5	DIPEA	MeOH	140 °C, 3.0 h	9% (14 mg)	 **55** (13%)

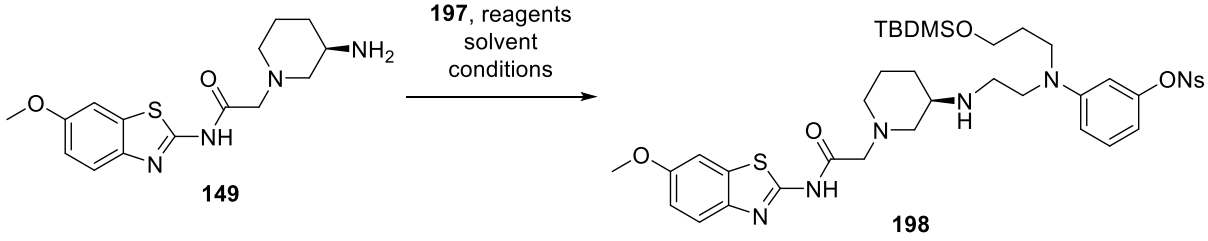
Table 15: Attempt of nucleophilic substitution of amine **149** on tosylate derivative **193**.

Due to the little amount obtained for product **195** and the fact that two additional deprotection steps were needed for preparation of the final desired product (see Scheme 45, structures **III**), this route was abandoned to find a more efficient one. On the other hand, the formation, albeit in small quantities, of the final product **195**, made it worthy to follow this route by replacing the tosylate with more reactive leaving groups, in order to avoid the harsh condition that led to amide bond breaking of **149** (see Table 15, entries 4 and 5, by-product **55**). The idea was to replace the tosylate with the nosylate, more reactive towards nucleophilic substitution. To achieve this, the double nosylation of compound **192** was performed, resulting in nosylation of the phenol moiety and chlorination of the alcohol (Scheme 47), a side reaction due to the formation of anion Cl^- with attacks in turn the formed nosylate.



Scheme 47: Attempt of double nosylation of **192**.

The undesired alkyl chloride **197**, being as well a good electrophile, was used to perform the nucleophilic substitution of **149** (Table 16). Using the same protocol with DIPEA in MeOH (Table 16, entries 1 – 3), applied to **149** in Table 15, the desired reaction occurred only at 140 °C (entry 3), confirmed by ASAP, along with the expected formation of 2-aminobenzothiazole **55**. It was not possible to separate compound **198** from 2-amino-6-benzothiazole **55**, and, therefore, it was not characterised. With triethylamine in DMF, aimed at enhancing the solubility of amine **149**, no reaction occurred even raising the temperature (entries 4 – 7). The only exception was at 140 °C (entry 7) at which the silyl ether deprotection of compound **197** occurred giving by-product **199**, confirmed by ASAP.



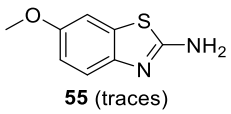
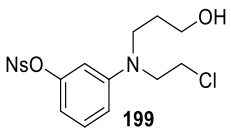
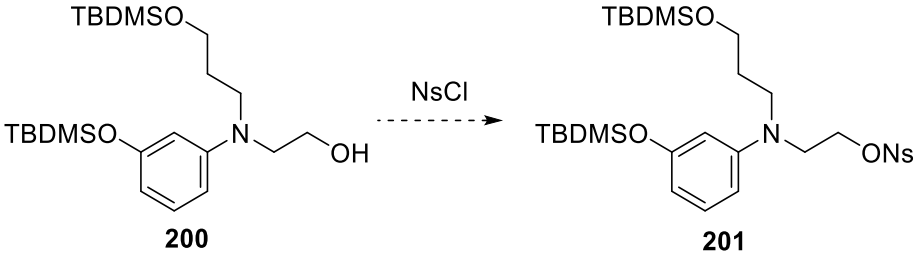
Entry	Base	Solvent	Conditions	Yield	By-product
1	DIPEA	MeOH	80 °C, 18 h	-	-
2	DIPEA	MeOH	100 °C, 8.0 h	-	-
3	DIPEA	MeOH	140 °C, 18 h	<18% (40 mg)	 **55** (traces)
4	Et_3N	DMF	rt, 72 h	-	-
5	Et_3N	DMF	80 °C, 16 h	-	-
6	Et_3N	DMF	100 °C, 16 h	-	-
7	Et_3N	DMF	140 °C, 3.0 h	-	 **199**

Table 16: Attempt of nucleophilic substitution of amine **149** with alkyl chloride **197**.

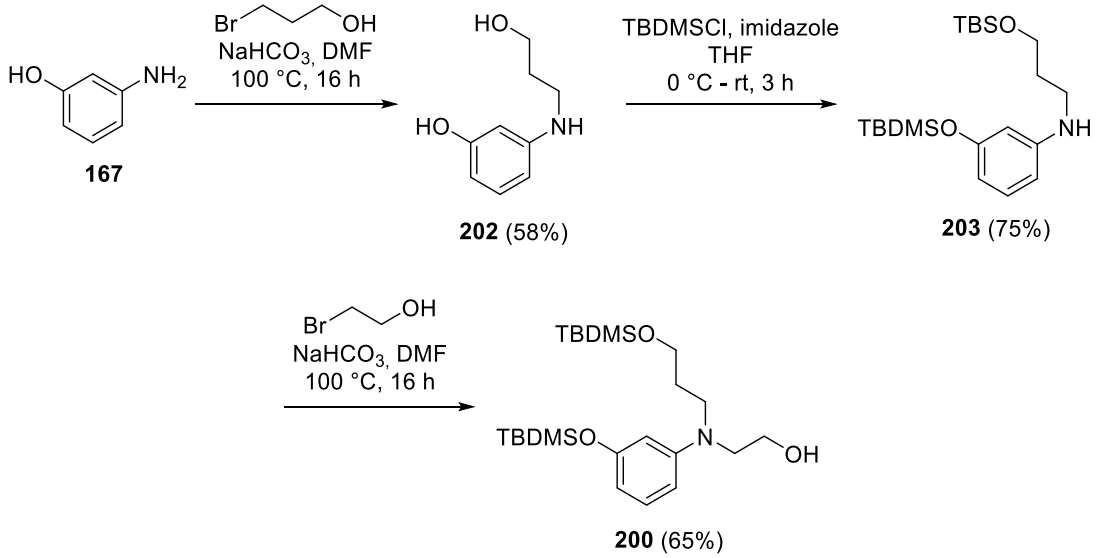
These attempts listed in Table 16, entries 3 and 7 confirmed that also in the case of alkyl chloride **197**, as seen above with tosylate **193** (see Table 15), the conditions for nucleophilic substitution were too harsh, as the two side reactions, amide bond breaking of **149** and silyl ether deprotection of **197**, occurred easier and faster.

One positive takeaway from the failure of double nosylation reaction (see Scheme 47) is the actual great reactivity of nosylate ester of alcohols to nucleophilic substitutions. To reduce the amount of chloride formed during the reaction, the aim was to perform the nosylation on structure **200**, TBDMS-protected on the alcohol as well as on the phenol (Scheme 48), in order to reduce chloride formation. Another great advantage of this pathway is the protection of hydroxypropyl alcohol and phenol with the same group, which translates in a single deprotection step at the end.



Scheme 48: Nosylation of alcohol **200** to synthesise the desired intermediate **201**.

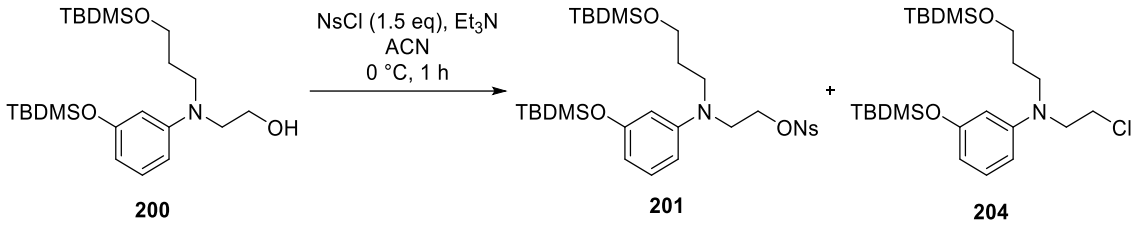
To obtain the free alcohol **200**, 3-aminophenol (**167**) was substituted at first with 3-bromo-1-propanol to obtain alcohol **202**, which was then TBDMS protected, on both alcohol and phenol, to give product **203** (Scheme 49). The latter was then alkylated with 2-bromo-1-ethanol to obtain the desired free alcohol **200**.



Scheme 49: Synthesis of double TBDMS protected intermediate **200**.

The results for the nosylation of alcohol **200** are listed in Table 17. It was possible to control the production of desired nosyl derivative **201** and chloride derivative **204**. The addition of nosyl

chloride before triethylamine (Table 17, entry 1) gave exclusively alkyl chloride **204**. With the caution of adding nosyl chloride after the auxiliary base (entries 2 and 3) and using a greater excess of triethylamine (entry 3), the yield of nosyl derivative **201** was improved to 50%.

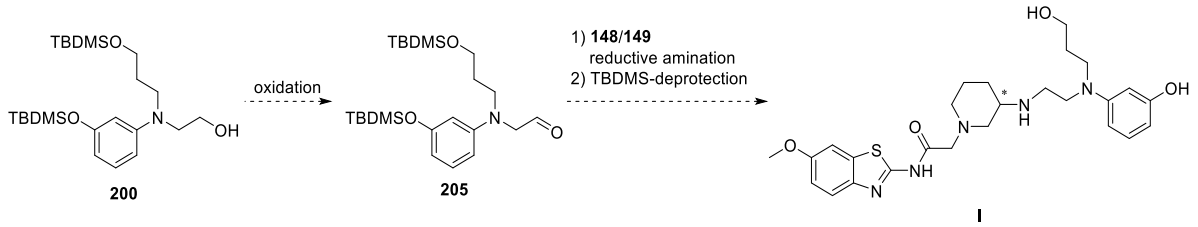


Entry	Eq of Et_3N	Order of addition	Yield (201)	Yield (204)
1	1.5	NsCl before Et_3N	0%	94%
2	1.5	Et_3N before NsCl	34%	6%
3	2.0	Et_3N before NsCl	50%	0%

Table 17: Synthesis of nosyl derivative **201**.

Nosylate **201** was then used for the nucleophilic substitution with **149** under the same conditions as described in Table 15, with no reaction occurring. Further substitution of chloride **204** was not attempted due to the disappointing results aforementioned in Table 16.

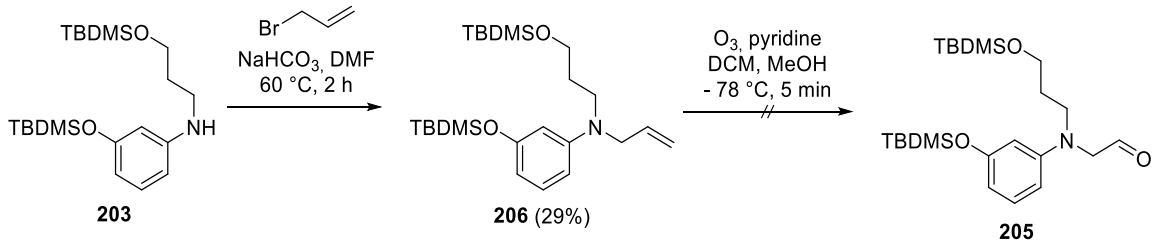
At this point, the route *via* nucleophilic substitution was also abandoned and an attempt of oxidation of the available alcohol **200** to aldehyde **205** was attempted (Scheme 50), in order to perform a reductive alkylation with **148** and **149**.



Scheme 50: Synthetic plan of structures **I** *via* reductive alkylation with aldehyde **205**.

The first step of alcohol oxidation to aldehyde was attempted with Dess-Martin periodinane, DMSO and oxalyl chloride, and PCC, leading in all three cases to several spots on the TLC not isolable and with no product mass detected.

Another and last attempt consisted in obtaining the aldehyde by ozonolysis of alkene **206** (Scheme 51), which was in turn synthesised from secondary amine **203** and allyl bromide. Ozonolysis was performed on alkene **206** leading to a series of not identified by-products.



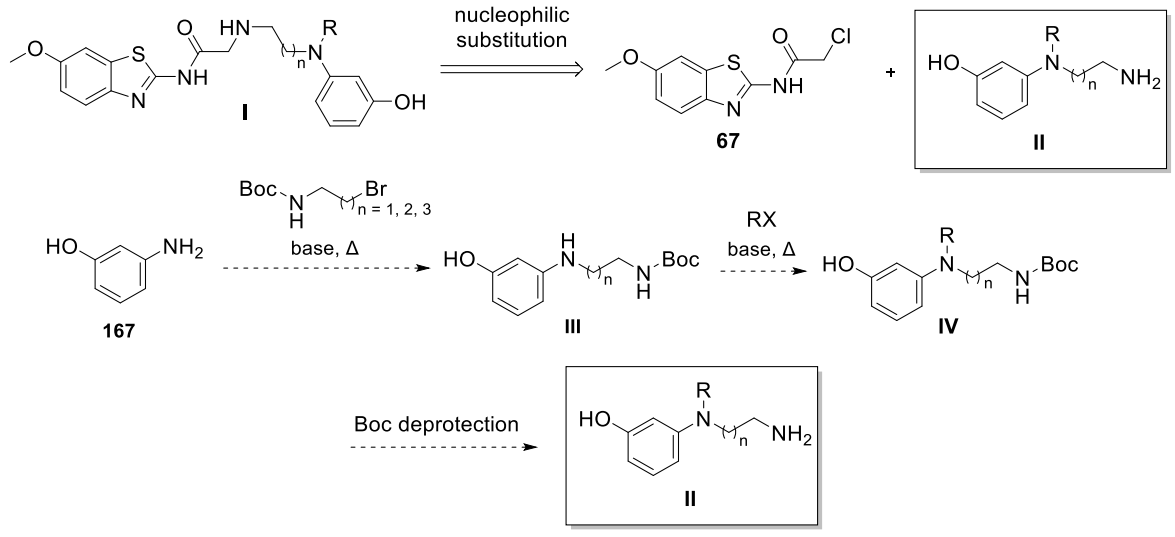
Scheme 51: Last attempt of synthesis of aldehyde **205**.

Due to time constrains, no further attempts were undertaken to synthesise the hydroxypropyl analogue based on structure **v4** (see Chapter 3.2.6.1, Figure 23).

3.2.6.3 Synthesis – linear linker (v3)

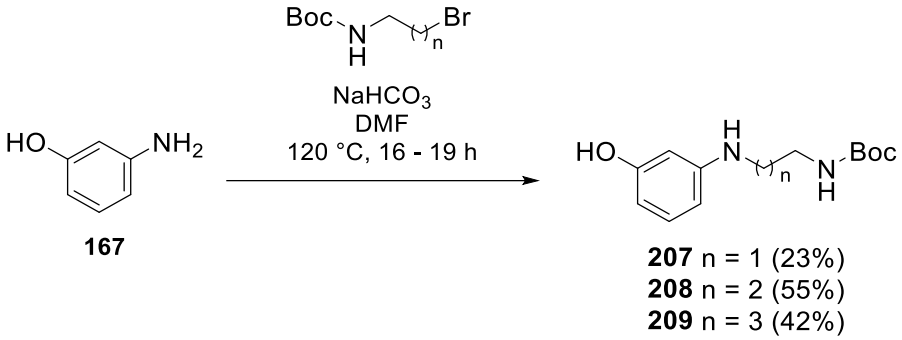
The present Chapter describes the syntheses of linear analogues of 2nd-site binders (see Chapter 3.2.6.1, Figure 22, structure **v3**) performed by Ludovica Bellino, during her master thesis as Erasmus student from Sapienza University of Rome, under my supervision. Here, the main findings are presented.

The synthetic plan to construct the analogues based on **v3** (Scheme 52, structures **I**) consisted of performing a nucleophilic substitution between chloroacetamide **67**, previously synthesised (see Chapter 3.2.1.1, Scheme 18), and the respective amines (structures **II**). The strategy to obtain building blocks of type **II** was to derivatise 3-aminophenol (**167**) with alkyl bromides of different length - the future ethylidene, propylidene, or butylidene linker chains - to synthesise compounds of type **III**. Second *N*-alkylation at the aromatic amine to structures **IV** and a subsequent *N*-Boc deprotection step should lead eventually to the desired amines (structures **II**).



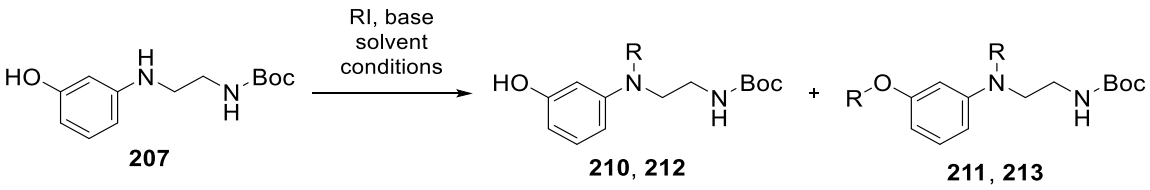
Scheme 52: Synthetic plan for the synthesis of linear analogues based on structure **v3** (see Chapter 3.2.6.1, Figure 22).

The first step was performed following the same procedure aforementioned for substitution of 3-aminophenol (**167**) with alkyl bromides (see Chapter 3.2.6.2, Scheme 39), using $NaHCO_3$ as auxiliary base in DMF. The reaction was performed using *N*-Boc-protected 2-bromoethyl amine, 3-bromopropyl amine, and 4-bromobutylamine, obtaining respectively secondary aromatic amines **207** – **209** in moderate yields (Scheme 53).



Scheme 53: *N*-Alkylation of 3-aminophenol (**167**).

Amine **207** was then methylated (Table 18, entries 1 – 3) following the same procedure applied for *N*-methyl derivative **171** (see Chapter 3.2.6.2, Table 14, entry 1) and the one described above with $NaHCO_3$ in DMF (see Scheme 53). The reaction was initially performed in ACN at 80 °C (Table 18, entry 1) with 2.0 eq of both iodomethane and base. As the reaction was not complete after 24 h, 2.0 eq of both iodomethane and base, for a total of 4.0 eq, were added and the reaction was left stirring for additional 24 h, giving *N*-methylated derivative **210** with a 50% yield. On the other hand, performing the reaction in DMF with 3.0 eq of iodomethane at 120 °C, by-product **211**, methylated also on the phenol, was isolated as main product (entry 2). The protocol was adjusted reducing the temperature to 100 °C, the reaction time to 4 h, and the equivalents of CH_3I to 2.0 eq (entry 3): product **210** was obtained with a yield of 70% with no by-product **211** forming. In the meantime, amine **207** was ethylated with iodoethane and $NaHCO_3$ in DMF (entry 4) to give *N*-ethyl derivative **212** with a satisfactory yield of 75% at 120 °C and no by-product **213**, ethylated on the phenol, forming.

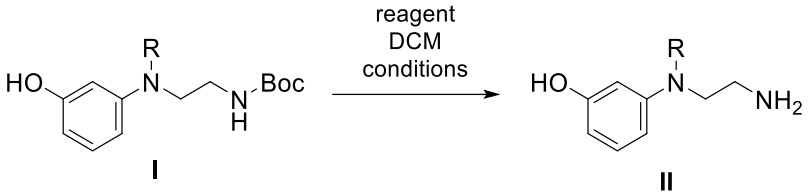


Entry	Reagents	Solvent	Conditions	R	Yield	By-product
1	CH_3I (4.0 eq), K_2CO_3 (4.0 eq)	ACN	80 °C, 48 h	CH_3	**210** (50%)	-

2	CH_3I (3.0 eq), $NaHCO_3$ (3.0 eq)	DMF	120 °C, 16 h	CH_3	**210** (32%)	**211** (37%)
3	CH_3I (2.0 eq), $NaHCO_3$ (2.0 eq)	DMF	100 °C, 4.0 h	CH_3	**210** (70%)	-
4	CH_3CH_2I (2.0 eq), $NaHCO_3$ (2.0 eq)	DMF	120 °C, 19 h	CH_2CH_3	**212** (75%)	-

Table 18: *N*-Methylation and *N*-ethylation of secondary aromatic amine **207**.

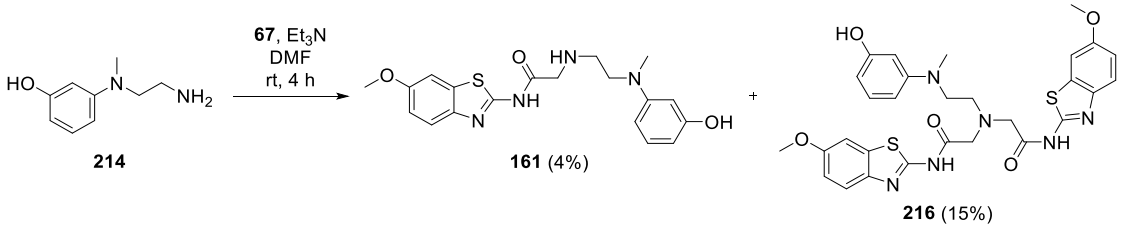
As depicted in Scheme 52, the following step was the Boc deprotection under acidic conditions. This reaction was carried out on the previously obtained tertiary amines **210** and **212**, with TFA in DCM (Table 19, entries 1 and 3) and HCl in dioxane (entries 2 and 4). In both cases, the latter protocol gave the best yield.



Entry	Starting material (I)	Reagent	Conditions	II (yield)
1	**210**	TFA	0 °C, 4 h	**214** (34%)
2	(R = CH_3)	HCl in dioxane	0 °C, 1 h	**214** (75%)
3	**212**	TFA	0 °C, 4 h	**215** (21%)
4	(R = CH_2CH_3)	HCl in dioxane	0 °C, 1 h	**215** (68%)

Table 19: Boc deprotection of derivatives **210** and **212**.

As already introduced in the synthetic strategy (see Scheme 52), the nucleophilic substitution of **214** with chloroacetamide **67** was attempted in DMF with triethylamine as auxiliary base (Scheme 54). The desired product **161** was obtained only in traces, along with the expected double substituted product **216**, also obtained with a low 15% yield.



Scheme 54: Nucleophilic substitution of primary amine **214** with chloroacetamide **67**.

To avoid this side reaction, the target structure was slightly modified introducing a methyl group on the aliphatic amine (Figure 25, structures **II**, methyl group in red). This *N*-methylation should not affect the binding with the target, since all the compounds previously synthesised and tested bear a tertiary amine at that position and have shown to effectively bind the target (see Chapters 3.2.2.2, Table 9, Chapter 3.2.4.2, Table 11, and 3.2.5.3, Table 13).

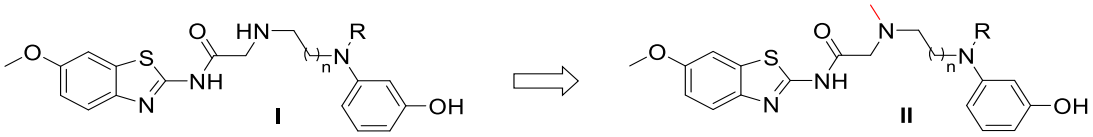
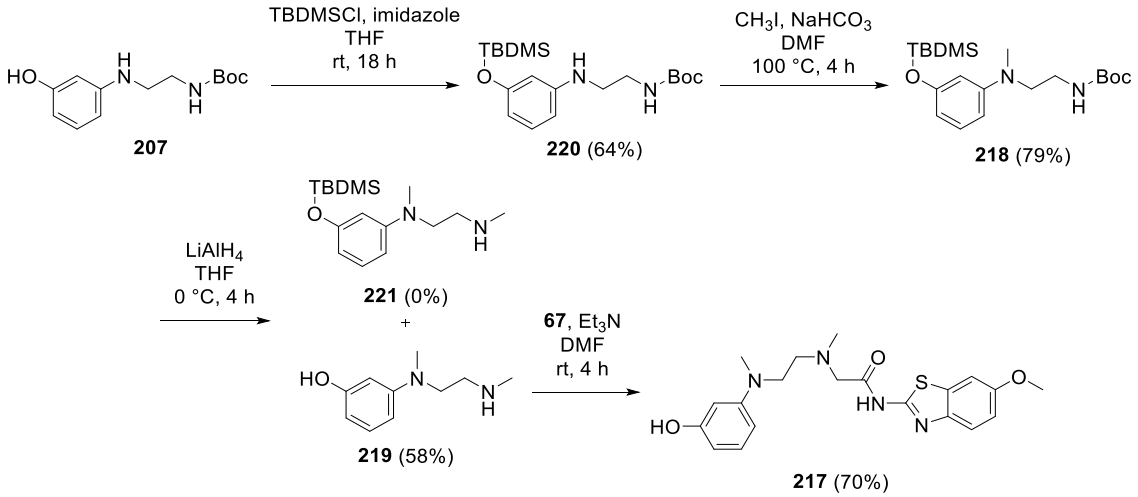


Figure 25: Additional methyl group (in red) on the new target structures **II**.

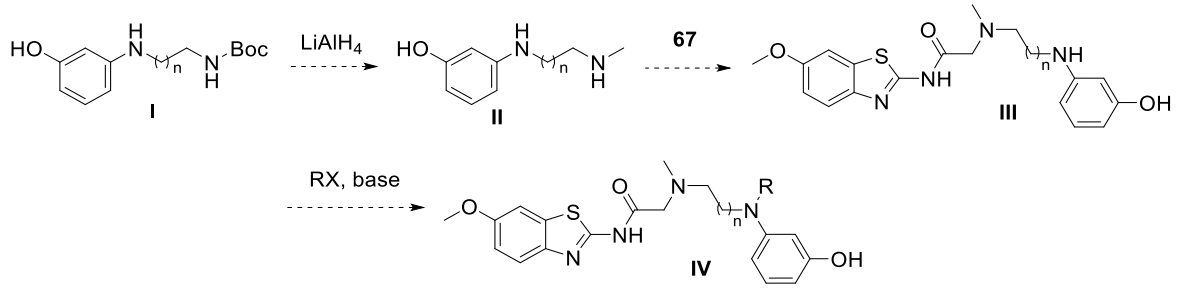
In our case, the fastest strategy to obtain the methylation was to reduce the carbamate group of intermediates **210** and **212** to an *N*-methyl group. As the purification of amines **214** and **215** (see Table 19) was very time consuming due to their high polarity, resulting in poor solubility in most organic solvents, a protection step of the phenol precursor with TBDMSCl was attempted, since silyl ethers showed stability under reductive conditions, unless there is adjacent amine or hydroxyl[140, 141]. This synthetic strategy was applied on the *N*-methyl derivative **217**, as depicted in Scheme 55. The first step of protection of phenol **210** as TBDMS ether was followed by *N*-methylation to compound **218**. The reduction of carbamate **218** was performed with $LiAlH_4$ as reducing agent, leading exclusively, and unexpectedly, to *N*-methylated free phenol **219**. As the purification of amine **219** resulted smother that of the primary amines **214** and **215**, the protection step on the phenol was removed from the synthetic plan. Aminophenol **219** underwent the nucleophilic substitution with chloroacetamide **67**, giving the desired final amide **217**.



Scheme 55: Synthesis of *N*-methylated analogue **217**, bearing the tertiary aliphatic amine of the new target structure (see Figure 25, structures **II**).

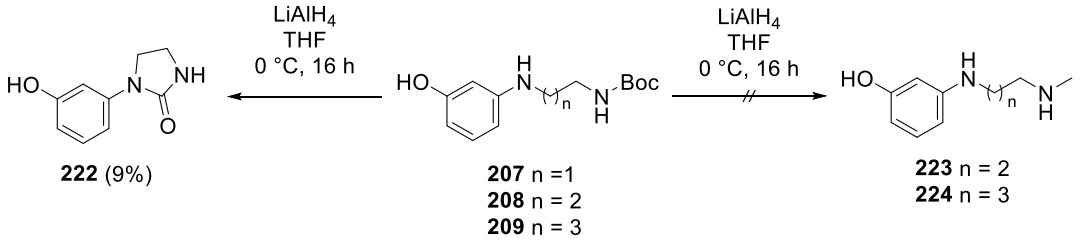
To improve the efficiency of the synthetic strategy, it was thought to change the order of the reactions (Scheme 56), performing the reduction step before the alkylation to synthesise

structures **II**, then the substitution with chloroacetamide **67**, and, as last step, the introduction of the alkyl chains R (methyl, ethyl, and hydroxypropyl), in order to differentiate the route of the analogues at the last step.



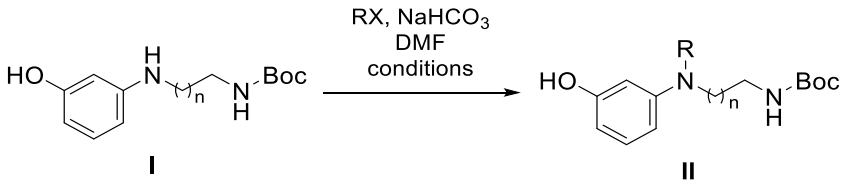
Scheme 56: More efficient synthetic strategy to structures **IV**.

The reduction with $LiAlH_4$ was then attempted on the three carbamates **207** – **209** (Scheme 57). Strating from **207** the only product isolated was the cyclic urea **222**. Most likely the hydride from $LiAlH_4$ acts as a strong base deprotonating the secondary aromatic amine, which results in an amide anion, a strong nucleophile that attacks in an intramolecular manner giving elimination of the *tert*-butanolate and consequent cyclisation to urea **222**. In the case of carbamates **208** and **209**, neither product nor by-products were isolated, even thought there was complete consumption of the starting materials.



Scheme 57: Attempted reduction of carbamates **207** – **209** to *N*-methyl amines.

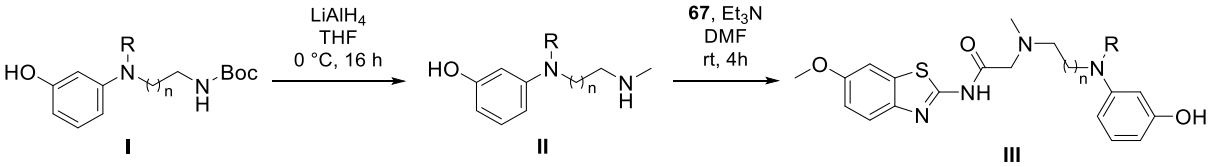
Based on the disappointing results shown in Scheme 57, the *N*-alkylation step was maintained as second step and the results are described in Table 20. Analogues bearing the hydoxpropyl chain based on structure **v4** (see Chapter 3.2.6.1, Figure 23) were also synthesised. The conditions, listed in Table 18, entries 3 and 4, were therefore applied to **210** with 3-bromo-1-propanol (Table 20, entry 1) and to secondary amine **208** and **209** respectively substituted with iodomethane (entries 2 and 5), iodoethane (entries 3 and 6), and 3-bromo-1-propanol (entries 4 and 7). The lower temperature (100 °C) and shorter reaction time (4.0 h) were kept only for methylation due to the higher reactivity of iodomethane as alkylation reagent.



Entry	Starting material (I)	RX	Conditions	R	II (yield)
1	**207** (n = 1)	$HO(CH_2)_3Br$	120 °C, 16 h	$(CH_2)_3OH$	**225** (23%)
2		CH_3I	100 °C, 4.0 h	CH_3	**226** (37%)
3	**208** (n = 2)	CH_3CH_2I	120 °C, 16 h	CH_2CH_3	**227** (62%)
4		$HO(CH_2)_3Br$	120 °C, 16 h	$(CH_2)_3OH$	**228** (31%)
5		CH_3I	100 °C, 4.0 h	CH_3	**229** (57%)
6	**209** (n= 3)	CH_3CH_2I	120 °C, 19 h	CH_2CH_3	**230** (62%)
7		$HO(CH_2)_3Br$	120 °C, 16 h	$(CH_2)_3OH$	**231** (27%)

Table 20: *N*-Alkylations of secondary amines **207** – **209** with iodomethane, iodoethane, and 3-bromo-1-propanol.

The alkylated analogues **212** and **225** – **231** underwent reduction of the carbamates to *N*-methyl derivatives **232** – **239** in acceptable to very good yields, and subsequent substitution of the generated secondary amines with chloroacetamide **67** yielded the desired final products **240** – **247** in poor to moderate yields, as depicted in Table 21. Due to time restrictions, it was not possible to synthesise the target structure **241**.



I II III

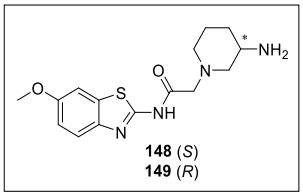
Entry	n	R	Starting material (I)	Products (yield) II	Products (yield) III
1	1	CH_2CH_3	**212**	**232** (61%)	**240** (29%)
2		$(CH_2)_3OH$	**225**	**233** (89%)	**241***
3	2	CH_3	**226**	**234** (39%)	**242** (56%)
4		CH_2CH_3	**227**	**235** (67%)	**243** (49%)
5		$(CH_2)_3OH$	**228**	**236** (66%)	**244** (32%)
6	3	CH_3	**229**	**237** (52%)	**245** (51%)
7		CH_2CH_3	**230**	**238** (49%)	**246** (30%)
8		$(CH_2)_3OH$	**231**	**239** (93%)	**247** (9%)

*Not synthesised.

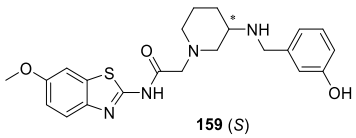
Table 21: Two last synthetic steps to the desired linear 2nd-site binders.

3.2.6.4 NMR-based results

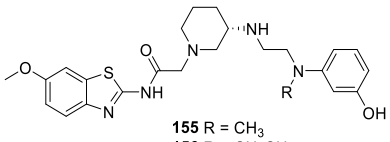
The NMR-based results of the potential 2nd-site binders are shown in Table 22 and include compounds **155** – **160**. Among the 2nd-site binders with the linear linker (see Chapter 3.2.6.3), only compounds **217**, **240**, **243**, and **246** were soluble enough in the buffer condition to be tested. Even for these four cases, the solubility was so poor that the measurements and interpretations of the parameters were unreliable, and these compounds were therefore excluded. For the potential 2nd-site binders with 3-aminopiperidine linker, the wLOGSY values were not assignable, therefore the factor was excluded from the combined rank score (value assigned: 0.00). For all the compounds in this series, lower values (CSP and T_2) were registered. Compounds **159** and **160** (Table 22, entries 3 and 4) showed slightly higher values compared to **155** – **158** (entries 5 – 8), characterised by a longer distance between the two aromatic moieties. The structural constrains could explain the lower the binding affinities with 5_SL1 in comparison to the truncated **148** and **149**. The effect was also considered independent of the configuration of the enantiomers ((*S*)-configured **159**, **155**, and **156** vs **148** and *(R*)-configured **160**, **157**, and **158** vs **149**). From these results, it was not possible to establish a gained effect with the introduction of the 3-aminophenol moiety, as stated by computational calculations (see Chapter 3.2.6.1).



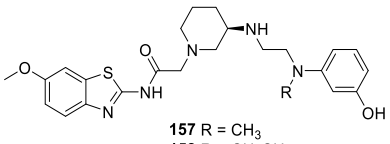
148 (*S*)
149 (*R*)



159 (*S*)
160 (*R*)



155 R = CH_3
156 R = CH_2CH_3



157 R = CH_3
158 R = CH_2CH_3

Entry	Compound	CSP [Hz]	wLOGSY	T_2 reduction [%]	Highest CSP	Highest wLOGSY	Highest T_2	Sum	Ranking
1	**148**	30.18	0.00	100.00	1	1	1	3	1
2	**149**	24.72	0.00	100.00	2	1	1	4	2
3	**159**	20.01	0.00	83.78	4	1	5	10	5
4	**160**	21.03	0.00	85.86	3	1	4	8	3
5	**155**	17.28	0.00	74.30	6	1	6	13	6
6	**156**	15.15	0.00	72.85	8	1	7	16	8
7	**157**	15.17	0.00	72.85	7	1	7	15	7

8	**158**	19.56	0.00	88.75	5	1	3	9	4

Table 22: NMR-based results of the 2nd-site binders.

Although it was not possible to confirm the expectations of the computational calculations, stipulating additional interactions of the 2nd-site ligand, the NMR-based results of the 2nd-site binders could adjust and improve the computational model. Due to time limitations, no attempts to improve the solubility in the buffer conditions of linear linkers **217**, **240**, **242** – **247** were undertaken during the course of this work.

3.2.7 Further biological characterisation

The compounds tested by NMR-based screenings were further characterised. Additional experiments performed by Sabrina Toews, Schwalbe group, are briefly presented (Chapters 3.2.7.1 and 3.2.7.2). MTT and agar diffusion assays (Chapters 3.2.7.3 and 3.2.7.4), routine analysis in Bracher group, were performed for all the final compounds by Martina Stadler.

3.2.7.1 Binding site mapping *via* 2D-^{1}H,^{1}H-TOCSY measurements

The RNA-ligand interactions were monitored by TOCSY by Sabrina Toews, Schwalbe group. This technique allows to detect interactions between pyrimidine protons H5 and H6 of uridines and cytosines and the ligand *via* spin-spin coupling, as introduced in Chapter 3.2.6.1. Figure 26 presents the results for **52** and **68**, the first two promising binding fragments: in black are depicted the cross-peaks of 5_SL1 in its apo-state; in purple the cross-peaks of holo-state 5_SL1 after addition of compound **52** (on the left) and compound **68** (on the right). With **68**, the strongest CSPs were identified in the asymmetrical internal loop, nucleotides U11 and C28, while for **52** clear CSP were registered in the same region, nucleotides U13 and C28, and in the apical loop, nucleotides C20 and C21, with a smaller impact for U18 and C19. The smaller CSPs observed in the apical loop, compared to the ones registered for the internal loop, might suggest that **52** potentially interacts directly with the internal loop, causing structural changes that propagate through 5_SL1.

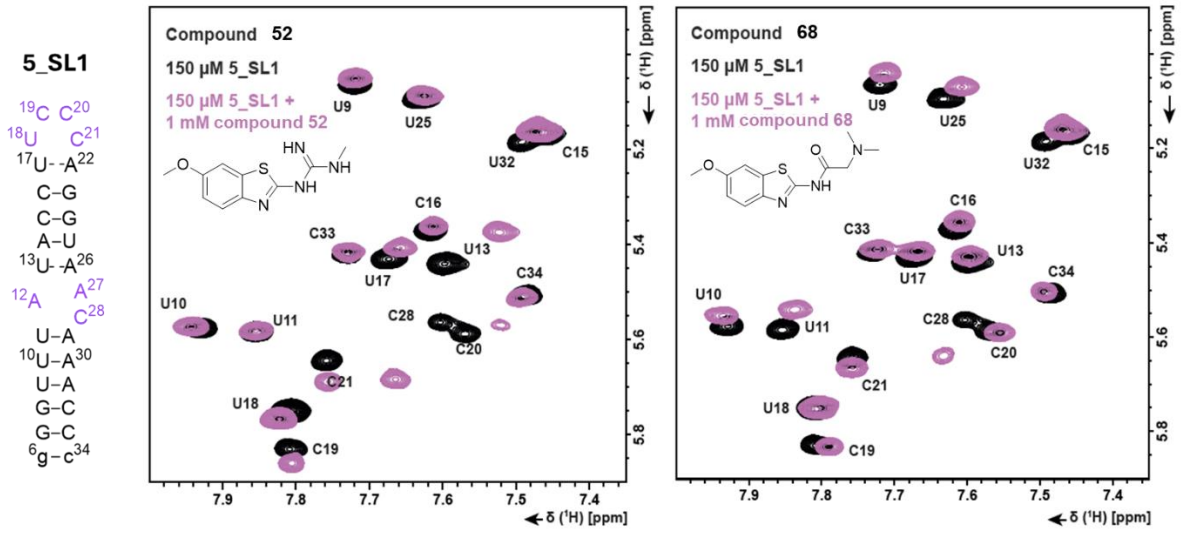


Figure 26: 2D-^{1}H,^{1}H-TOCSY experiments of 5_SL1, before (in black) and after (in purple) addition of **52** (on the left) and **68** (on the right).

Some of the compounds further synthesised were tested with TOCSY, while other measurements are still on-going. Compounds were tested at a concentration ratio of 1:5 RNA/ligand, slightly different from the condition used for **52** and **68**, due to limited RNA availability at the time. Therefore, the results have been only qualitatively compared in this Chapter. Among the compounds bearing a different pattern on the aromatic ring, nitroarene **87**, 6-bromo substituted **88**, and 4,6-dimethoxy substituted **89** were tested, along with benzimidazole **105** and *N*-methylpiperazine analogue **132** (Figure 27). For compounds **87** and **88** were registered clear shifts for nucleotides U11, U13, and C28, and smaller for U25, all belonging to the asymmetrical internal loop or right next to it. Compound **89** showed additionally shifts for U9 and U10. Similar results were obtained for **132**, suggesting that these compounds might improve the binding due to the additional shift of U13, which was not registered in the case of **68**. Benzimidazole **105** did not showed improvement, as a clear shift was registered only for nucleotide C28.

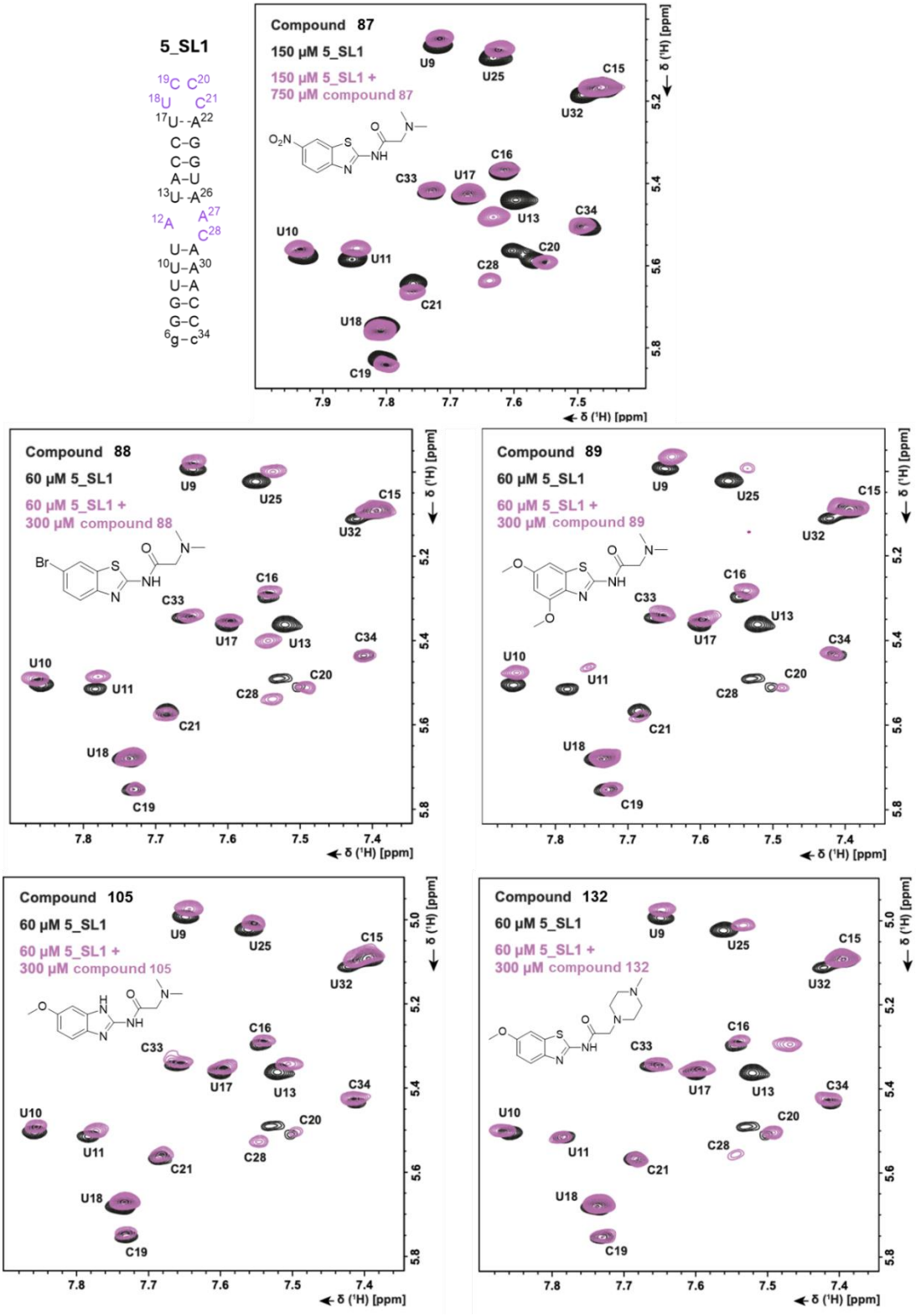


Figure 27: 2D-^{1}H,^{1}H-TOCSY experiments of 5_SL1, before (in black) and after (in purple) addition of **87**, **88**, **89**, **105**, and **132**.

These preliminary results are of great importance, as they confirm the asymmetrical internal loop for 5_SL1 as the primary binding site of these benzothiazoles. Moreover, the result obtained for *N*-methylpiperazine analogue **132** validate the strategy of adding second basic sites in the structure to increase the binding (see Chapters 3.2.5).

Further TOCSY experiments on the best compounds, in particular the 3-aminopiperidine derivatives **148** – **151**, which showed the best results overall (see Chapter 3.2.5.3, Table 13), are still on-going.

3.2.7.2 Mutant counterscreen

One of the main issues in drug development is the selectivity of binding compounds towards the target. The compounds that were considered as hits (see Chapter 2.2, compounds that respected at least two of the statements: CSP ≥3 Hz, wLOGSY ≥0.7, and T_2 ~40%) were tested. These selected compounds for each generation were screened against a modified construct of 5_SL1 by Sabrina Toews, Schwalbe group. Since most of the compounds tested here lead to shifts in the H5H6 resonances of uridines and cytosines residues located within and surrounding the internal loop of 5_SL1, the internal loop was classified as the major binding epitope of this viral RNA element. A modified version of 5_SL1, lacking this internal loop, was therefore designed as a counterscreen RNA. The modified construct lacks nucleotides A27 and C28 and an additional U has been inserted at position 27 for Watson-Crick base pairing, resulting in an overall paired stem (Figure 28).

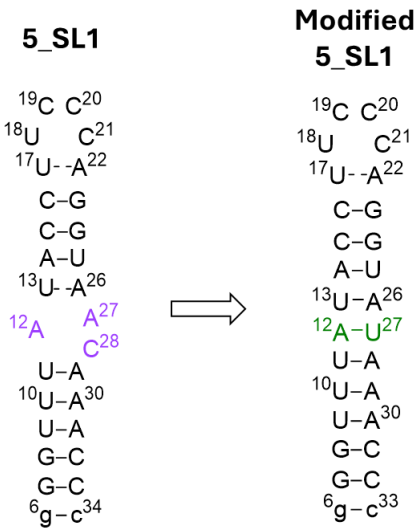


Figure 28: 5_SL1 construct on the left; modified 5_SL1 construct on the right, missing the internal loop for the counterscreen.

The results of NMR-based screenings with the mutated 5_SL1 are presented in Figure 29. If binding was detected, CSPs were measured. Most of the selected compounds did not show binding to the mutant of 5_SL1, implying a selectivity for wild-type 5_SL1 of 74% (25 out of 34 selected compounds, depicted in grey). In case of detected binding, the CSPs were significantly lower compared with the ones measured with 5_SL1 (shown in parenthesis). The highest rate of binding to the mutant 5_SL1 was detected for compounds containing the additional basic site in the lateral chain (compounds **133**, **136**, **134**, and **150**) suggesting possible additional interactions with 5_SL1. Moreover, the interaction between 2nd-site binders

155 and **158** and mutant 5_SL1, not detected for **148** and **149**, could indicate the existence of the postulated second binding site (see Chapter 3.2.6.1).

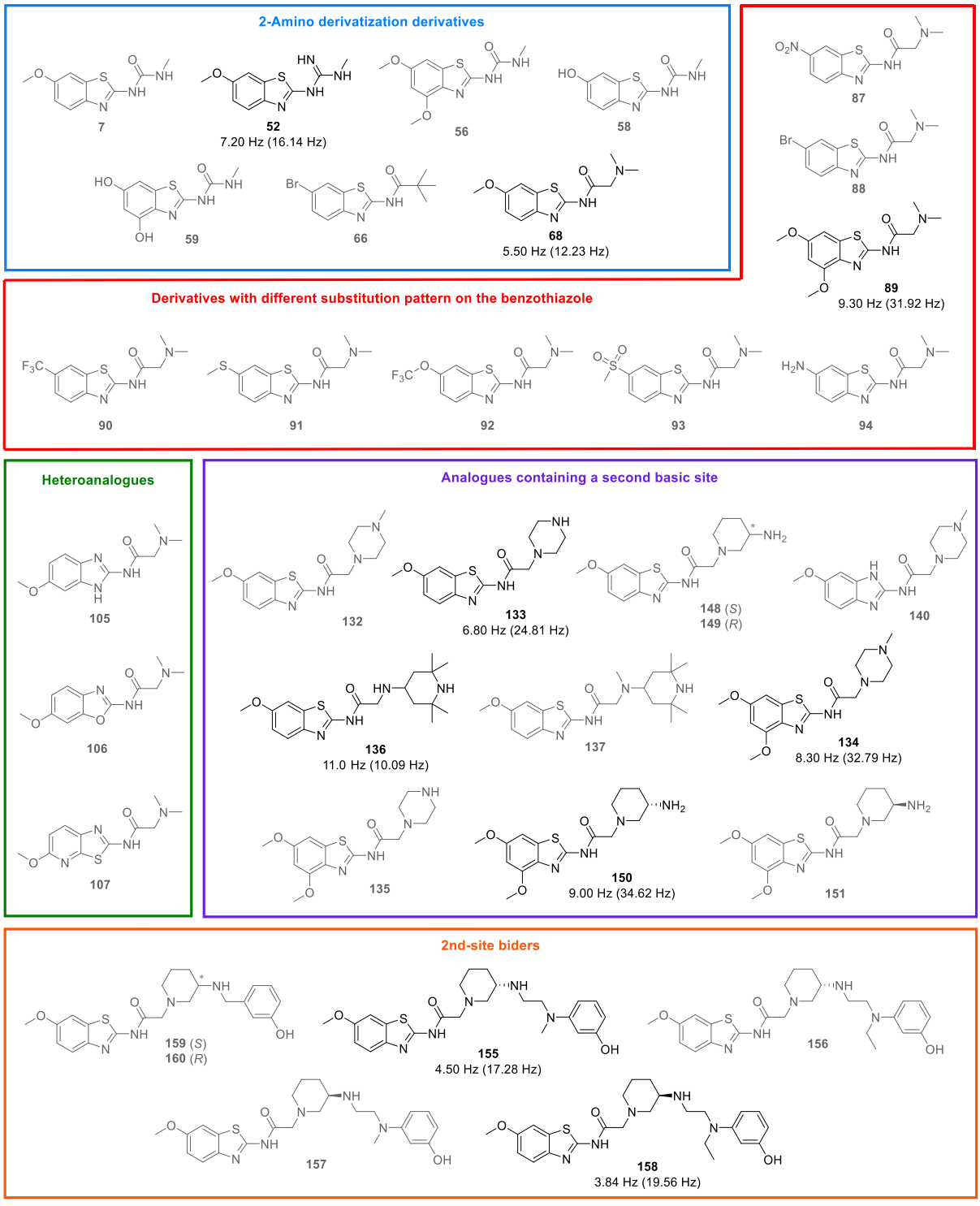


Figure 29: Counterscreen analysis of selected compounds on a modified 5_SL1. Compounds belonging to the same chemical group are highlighted by colours: 2-amino derivatisation (see Chapter 3.2.1) in light blue; different substitution pattern on the benzothiazole (see Chapter 3.2.2) in red; heteroanalogues (see Chapter 3.2.4) in green; second additional basic site (see Chapter 3.2.5) in purple; 2nd-site binders (see Chapter 3.2.6) in orange. Compounds with negative counterscreen (no binding) are depicted in grey. In case of a positive counterscreen, CSPs were measured. CSP values in parenthesis are reported from the previous measurements on 5_SL1 (see

Chapter 3.2.1.2, Table 7, Chapter 3.2.2.2, Table 9, Chapter 3.2.4.2, Table 11, Chapter 3.2.5.3, Table 13, and Chapter 3.2.6.4, Table 22).

Further screening analysis involves tests within a cellular environment to measure translation efficiency. The cell-free translation assay involves a cell-free system derived from rabbit reticulocyte lysates aiming for luminescence detection based on the translation efficiency of the here applied Nano-Luciferase. Luminescence is detected during incubation at 30 °C in the presence of the needed substrate Furimazine. In case of an inhibitor, a decrease in translation and, therefore, in the luciferase output, is to be detected. These experiments are still on-going and therefore not described in this work.

3.2.7.3 MTT assay

The cytotoxicity of all final compounds was assessed using the standard MTT assay on HL-60 cells, as already introduced in Chapter 3.1.3.1. Most of the 58 final compounds showed no cytotoxic effect (IC_{50} >50 µM), except for a few ones with slight toxicity: **66** (IC_{50} = 48 µM), **156** (IC_{50} = 33 µM), **157** (IC_{50} = 42 µM), **158** (IC_{50} = 23 µM), and **247** (IC_{50} = 30 µM). These results might be very promising if supported by further biological assays that can prove *in vivo* activity on SARS-CoV-2, as they could be evaluated as a first selectivity towards the viral RNA genome.

3.2.7.4 Agar diffusion assay

As already described for the TPC2 inhibitors (see Chapter 3.1.3.2), the antimicrobial activity was assessed for all the final compounds (**7**, **52**, **53**, **56**, **58** – **61**, **65**, **66**, **68**, **70**, **71**, **87** – **96**, **105** – **108**, **126**, **132** – **140**, **148** – **151**, **155** – **160**, **217**, **240**, and **242** – **247**) presented in this thesis as SARS-CoV-2 ligands against various model microorganisms. In this assay, none of the compounds tested showed an inhibition zone, and, therefore, no antimicrobial effects were registered against gram-negative bacteria *Escherichia coli* and *Pseudomonas marginalis*, gram-positive bacteria *Staphylococcus equorum* and *Streptococcus entericus*, and yeasts *Saccharomyces cerevisiae* and *Yarrowia lipolytica*.

4 Summary

This thesis dealt with two independent topics and focused on the optimisation of small molecules as TPC2 inhibitors and as ligands of the RNA of SARS-CoV-2.

The lead compound for TPC2 inhibitors was the dimeric chloroquinoline Lys05 (**1**), identified by a HTS campaign performed by Nicole Urban (Schaefer group, Leipzig) in collaboration with Prof. Christian Grimm (LMU). Lys05 (**1**) was particularly promising due to its modality specific inhibition towards one of the two synthetic activators of TPC2, showing a stronger blockage towards TPC2-A1-P (**3**) mediated activation compared to TPC2-A1-N (**2**) mediated activation. Systematic structure modifications of the free base of Lys05 (**1**), Lys01 (**4**), were performed to determine the structure-activity relationships of Lys01 (**4**) as TPC2 inhibitor. All the analogues presented in this work were crucial to underline and confirm the great and unpredictable effects on the activity of small structural variations of the structure, which were proved by calcium imaging experiments. Along with Lys01 (**4**), *N*-nor-Lys01 (**8**) was selected as best inhibitor, and their activity is currently being confirmed by electrophysiological patch-clamp experiments.

The starting point of the SARS-CoV-2 project was the benzothiazole **7**, identified by Sreeramulu and Richter *et al.*[92], binding the first loop (5_SL1) of the viral RNA. The structure optimisation of **7** was achieved by synthesis of a large variety of analogues regularly tested by NMR-based screening by Sabrina Toews (Schwalbe group, Frankfurt). Each group of modifications, categorised according to chemical characteristics, were tested separately, and further compounds, originated by the fusion of the best modifications, led to important structure improvements and insights on the binding pocket of 5_SL1. The results obtained in this project highlighted the crucial interdependence between NMR-based screening and molecular docking, which consistently influenced the strategic direction regarding which compounds to synthesise next. Additionally, it underscored the necessity of continuous adjustments to rapidly and effectively optimise the development of compounds with improved binding affinity.

4.1 TPC2 inhibitors

In this study, the dimeric chloroquinoline derivative Lys05 (**1**) was identified as a potent inhibitor of TPC2 and subsequently used as lead compound for a structure-activity relationships study. In the last years, the increasing interest for TPC2 inhibitors has emerged due to the proven crucial role of this cation channel in cancer[30-37], viral infections[27-29], and neurodegenerative disorders[22, 23]. The starting point of this project was a HTS campaign with about 7,000 compounds (drugs, natural substances, and toxins) *via* a fluorometric calcium assay using a HEK293 cell line stably expressing plasma membrane TPC2$^{L11A/L12A}$-RFP. The two known synthetic low-molecular activators TPC2-A1-N (**2**) and TPC2-A1-P (**3**)[15] were used to assess the inhibitory effects of Lys05 (**1**), which resulted in blocking TPC2-A1-P (**3**) mediated

activation with 9-fold difference inhibitory effect over TPC2-A1-N (**2**) mediated activation (IC_{50}(TPC2-A1-N (**2**)) = 6.1 µM, IC_{50}(TPC2-A1-P (**3**)) = 0.66 µM). In order to exploit the potential of lead structure Lys05 (**1**), systematic modifications of this compound were performed. An overview of all derivatives synthesised is shown in Figure 30. As first derivative of Lys01 (**4**), which serves as precursor to its trihydrochloride salt form Lys05 (**1**), *N*-nor-Lys01 (**8**) was synthesised. The following set of analogues focused on methylation on the two aromatic amines: the *N*,*N*',*N*''-trimethylated analogue **11** and secondary aliphatic amine **12**, exclusively methylated on both aromatic amino groups. To explore the chemical space around Lys01 (**4**), various analogues substituted on the central aliphatic amine were synthesised: the *N*-alkylated compounds **16** and **17**, *N*-benzyl compound **15**, the polar functional group substituted derivatives, such as *N*-cyanomethyl derivative **19**, *N*-acetyl derivative **20**, ethyl ester **18**, *N*-hydroxyethyl derivative **21**, *N*-aminoethyl derivative **23**, and the two hydrazine derivatives **25** and **26**. To assess the need of the symmetric structure, three variations of the monomeric subunit of Lys01 (**4**) were obtained: primary amine **28**, *N*-monomethylated derivative **29**, and the *N*,*N*-dimethyl compound **30**. Moreover, homologue **35** (containing two C_3 spacers instead of C_2), the respective *N*-monomethylated homologue **38**, the bis-dechlorinated analogue **36**, and the respective *N*-methylated **39** were synthesised. For bis-desaza analogue **40** the oligoamine chain was substituted with two propylidene chains bound to the central aliphatic amine.

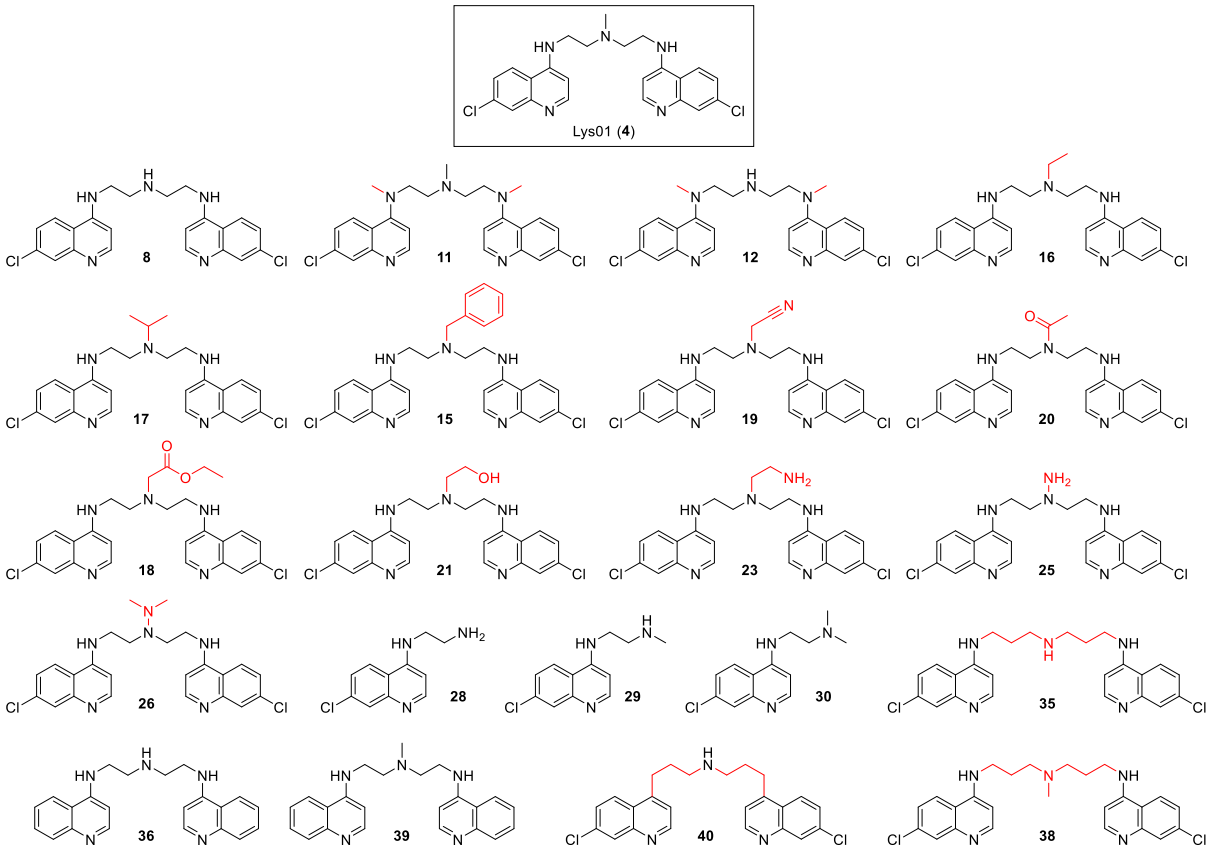
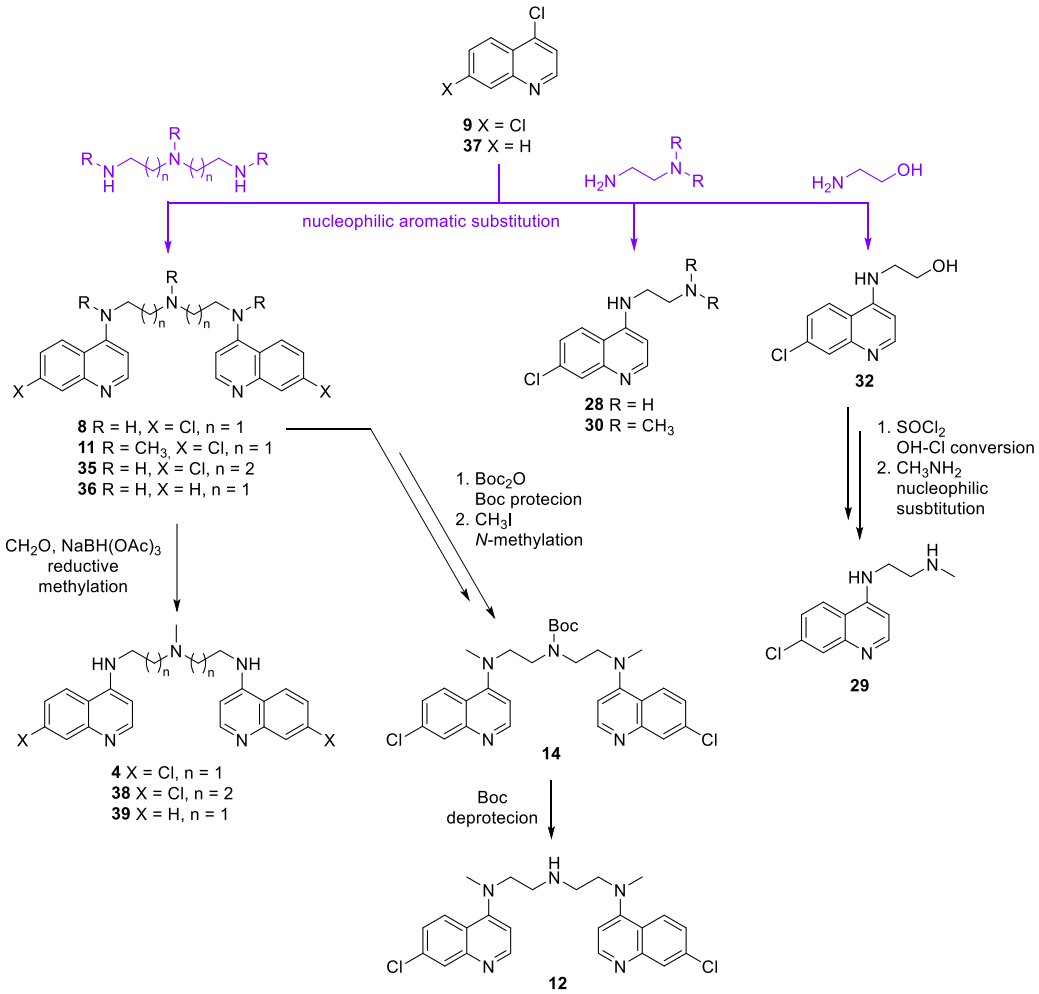


Figure 30: Overview on the Lys01 (**4**) analogues synthesised and tested.

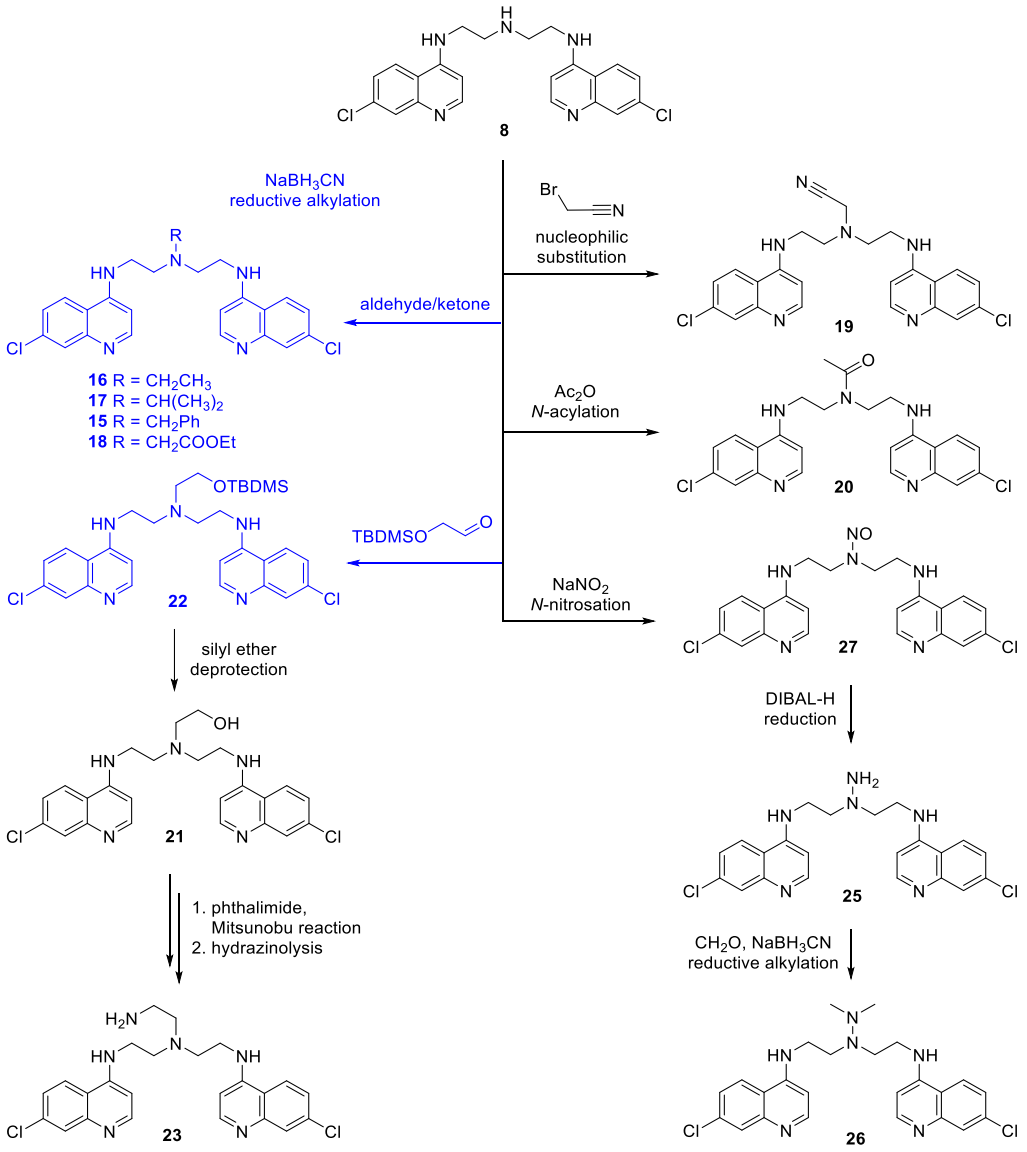
Starting from 4-chloroquinolines **9** or **37** *via* nucleophilic aromatic substitution (Scheme 58, in purple) were synthesised the dimeric quinolines **8**, **11**, **35**, and **36**, using the respective oligoamines, and the monomers **28** and **30**, using a large excess of the corresponding oligoamine as reagent, and *N*-hydroxyethyl intermediate **32**, which, upon conversion into the alkyl chloride, was substituted with methylamine to give the monomethylated monomer **29**. Dimeric quinolines **8**, **35**, and **36** were methylated *via* reductive methylation using formaldehyde and sodium triacetoxyborohydride to *N*-methyl analogues **4**, **38**, and **39**. Selective methylation on the two aromatic amino residues of compound **8** was achieved by Boc protection, *N*,*N”*-methylation with iodomethane, and final Boc deprotection to *N*,*N”*-dimethylated analogue **12**.



Scheme 58: Synthesis of dimeric quinolines **8**, **11**, **35**, and **36** from 4-chloroquinolines **9** and **37**, and monomers **28** and **30** from 4,7-dichloroquinoline (**9**) *via* nucleophilic aromatic substitution with the respective oligoamine. Synthesis of *N*,*N”*-dimethylated **12**. Synthesis of *N*-methyl monomer **29**.

An overview of the syntheses of compounds variously substituted on the central amino group of the linker starting from **8** is depicted in Scheme 59. *N*-Alkylated compounds **15** – **18** and **22** were obtained *via* reductive alkylation (in blue) with the corresponding aldehyde or ketone and sodium cyanoborohydride as reducing agent, along with TBDMS-protected *N*-hydroxyethyl

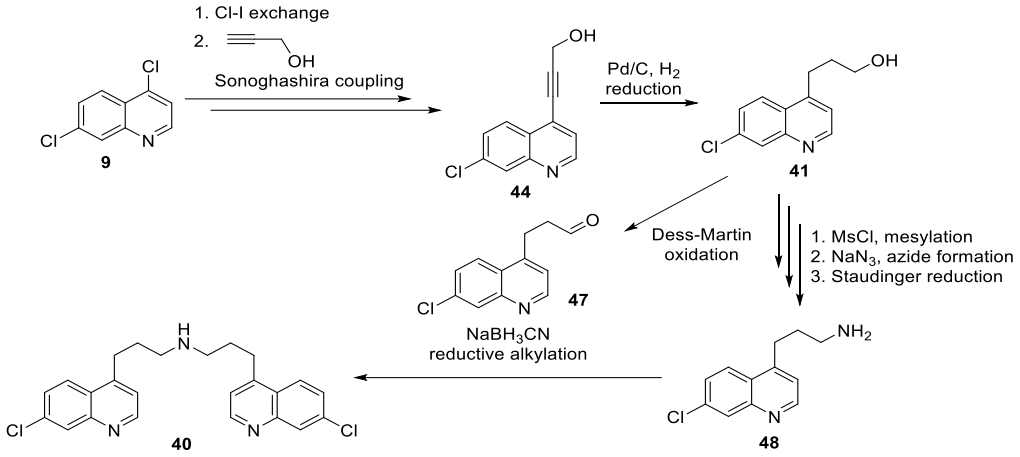
derivative **22**. The latter was then deprotected to *N*-hydroxyethyl derivative **14** to be then converted into *N*-aminoethyl derivative **23** performing a Mitsunobu reaction with phthalimide and DEAD, followed by hydrazinolysis. *N*-Cyanomethyl derivative **19** was obtained by nucleophilic substitution on 2-bromoacetonitrile, and *N*-acylation gave of **8** gave amide derivative **20**. Hydrazine derivative **25** was obtained by *N*-nitrosation of **8** followed by reduction with DIBAL-H, and its methylation *via* reductive alkylation with formaldehyde/sodium cyanoborohydride gave *N*,*N*-dimethylated hydrazine derivative **26**.



Scheme 59: Overview of the synthesis of derivatives **15** – **20**, **21**, **23**, **25**, and **26** variously substituted on the central amine.

The synthesis of the bis-desaza derivative **40** is summarised in Scheme 60. Starting from 4,7-dichloroquinoline (**9**), 4-chloro was exchanged with 4-iodo to efficiently perform in the next step the Sonogashira coupling with prop-2-yn-1-ol. Alkyne **44** was then reduced *via* palladium-catalysed hydrogenation to hydroxypropyl derivative **41**. The latter was in turn converted into two complementary reactive groups: the hydroxy group of **41** was converted into the activated

mesylate to undergo substitution with sodium azide to the azide derivative, which was then reduced to amine **48**; on the other hand, oxidation of **41** with Dess-Martin periodinane gave aldehyde **47**. Reductive alkylation of amine **48** with aldehyde **47** gave the bis-desaza analogue **40**.



Scheme 60: Synthesis of the bis-desaza derivative **40**.

The first biological evaluation of these analogues was performed by means of Fura-2 single cell calcium imaging experiments (see Chapters 3.1.1.3 and 3.1.2.2). First, Lys01 (**4**) was tested and significant inhibition on both activation mechanisms (TPC2-A1-N (**2**) and TPC2-A1-P (**3**)) was registered, confirming the results obtained with the HTS campaign for its trihydrochloride salt Lys05 (**1**). The most promising result was obtained with *N*-demethylated analogue **8**, displaying a similar effect seen for **4**. Modifications regarding the two aromatic amines led to a strong decrease of inhibitory activity towards both activation mechanisms with *N*,*N"*-dimethylated **11** and **12** and bis-desaza analogues **40** displaying no significant (ns) inhibition. Only compound **11** showed inhibition (**) with TPC2-A1-N (**2**) activation, still lower compared to Lys01 (**4**, *** with both TPC2-A1-N (**2**) and TPC2-A1-P (**3**)). Among the substitutions on the central amine, the apolar substituents of *N*-ethyl analogue **16** and *N*-benzyl analogue **15** were better tolerated compared to the variously substituted more polar analogues **18** – **21**, **23**, **25**, and **26** which showed a loss of inhibitory effect especially after TPC2-A1-P (**3**) activation. A crucial role is also played by the dimeric structure, since the monomers lost completely the inhibitory effect on TPC2-A1-P (**3**), with **28** showing a rather opposite modality selectivity trend maintaining a comparable inhibition (***) on TPC2-A1-N (**2**) activation. The dechlorination also led to disappointing results with complete loss of activity, while the elongated homologues **35** and **38** kept a significant inhibitory effect only on TPC2-A1-N (**2**) activation.

Compounds not showing inhibitory activity were discarded and dose-response relationships were determined by Nicole Urban (Schaefer group, Leipzig) using Fluo-4 calcium imaging experiments. Therefore, compounds Lys01 (**4**), **8**, **11**, **15** – **21**, **23**, **25**, **26**, **35**, and **38** were

tested at different concentrations followed by addition of TPC2-A1-N (**2**) or TPC2-A1-P (**3**), and IC_{50} values were determined. The best results were obtained for Lys01 (**4**, IC_{50}(TPC2-A1-N (**2**)) = 9.3 μM, IC_{50}(TPC2-A1-P (**3**)) = 0.84 μM), comparable to the ones obtained for the trihydrochloride salt Lys05 (**1**, IC_{50}(TPC2-A1-N (**2**)) = 6.1 μM, IC_{50}(TPC2-A1-P (**3**)) = 0.66 μM) and *N*-demethylated Lys01 (**4**) analogue **8** (IC_{50}(TPC2-A1-N (**2**)) = 49 μM, IC_{50}(TPC2-A1-P (**3**)) = 0.69 μM), which displayed a 72-fold difference inhibition between the two activation mechanisms. Among the set of analogues substituted on the central amine, *N*-ethyl analogue **16** (IC_{50}(TPC2-A1-N (**2**)) = 13 μM, IC_{50}(TPC2-A1-P (**3**)) = 1.3 μM) and *N*-benzyl analogue **15** (IC_{50}(TPC2-A1-N (**2**)) = 17 μM, IC_{50}(TPC2-A1-P (**3**)) = 1.6 μM) gave comparable results to Lys01 (**4**) in terms of modality selectivity, although displaying a weaker inhibition on both activation mechanisms. The rest of the analogue showed significantly lower inhibition and modality selectivity.

The results obtained by Fluo-4-based calcium imaging were compared with those of the two inhibitors recently described by our group, SG-005 and SG-094[30], structurally related to the bisbenzylisoquinoline alkaloid tetrandrine. They have been tested by Fluo-4-based calcium imaging both in racemic form (*rac*-SG-005: IC_{50}(TPC2-A1-N (**2**)) = 2.5 μM, IC_{50}(TPC2-A1-P (**3**)) = 7.6 μM; *rac*-SG-094: IC_{50}(TPC2-A1-N (**2**)) = 24 μM, IC_{50}(TPC2-A1-P (**3**)) = 8.3 μM) and as pure enantiomers ((*S*)-SG-005: IC_{50}(TPC2-A1-N (**2**)) = 7.8 μM, IC_{50}(TPC2-A1-P(**3**)) = 10 μM; (*R*)-SG-005: IC_{50}(TPC2-A1-N (**2**)) = 2.4 μM, IC_{50}(TPC2-A1-P (**3**)) = 9.1 μM; (*S*)-SG-094: IC_{50}(TPC2-A1-N (**2**)) = 14 μM, IC_{50}(TPC2-A1-P (**3**)) = 15 μM; (*R*)-SG-094: IC_{50}(TPC2-A1-N (**2**)) = 31 μM, IC_{50}(TPC2-A1-P (**3**)) = 15 μM)[30]. Both Lys01 (**4**) and *N*-demethylated derivative **8** displayed an IC_{50} after TPC2-A1-P (**3**) activation 10-fold lower compared to SG-005 and SG-094, confirming the great potential of this new scaffold as inhibitors of TPC2. As final confirmation, patch-clamp electrophysiological experiments were planned but the results are still on-going and preliminary. Therefore, they were not discussed in the present work.

4.2 SARS-CoV-2 RNA ligands

The starting point of this project was compound **7**, whose interactions with the 5_SL1 region of SARS-CoV-2 RNA were identified by Sreeramulu and Richter *et al.*[92] by NMR spectroscopy. 5_SL1 has been proved to be a promising target for blocking viral RNA translation, being largely involved in Nsp1 evasion mechanisms, which results in selective viral translation and impaired cellular host translation[78-81, 83, 84]. Systematic modifications of the structure were performed and the binding to the 5_SL1 was regularly tested by NMR-based screening by Sabrina Toews (Schwalbe group, Frankfurt). The binding affinities were determined analysing three parameters (CSP, wLOGSY, and T_2) and the results for each generation influenced the

following design of ligands. An overview of the compounds synthesised, grouped by chemical characteristics, is presented in Figure 31.

Starting from the variation on the 2-amino group of **7** (Figure 31, **A**, functional groups in blue), amides **61**, **60**, **65**, and **66**, ureas **58**, **56**, **59**, and **70**, thiourea **71**, guanidines **52** and **53**, and glycinamide **68** were synthesised. The latter was selected as most promising compound, being one of the best in terms of combined rank score (see Chapter 3.2.1.2, Table 7), and due to the promising *N*,*N*-dimethylglycinamide moiety, which was crucial in gaining binding efficiency if compared to the other amines (compounds **60**, **61**, **65**, and **66**) and in improving the solubility in comparison to the neutral amides and ureas (structures **7**, **58**, **56**, **59**, and **70**). Starting from **68**, three sets of structural modifications were planned, aiming at analysing each structural characteristic of this compound. Compounds **87** – **96**, bearing different substitution patterns of the benzothiazole (**B**, substituents in red) and the homologous **96** were synthesised. Out of this series, 4,6-dimethoxy substituted **89** was selected for further development (see Chapter 3.2.2.2, Table 9). Heteroanalogues **105** – **108** and **126** of the benzothiazole (**C**, heterocycles in green) were tested with benzimidazole **105** being the most promising (see Chapter 3.2.4.2, Table 11). The *N*,*N*-dimethylamino moiety of **68** was further substituted by structures containing a second basic group (**D**, in purple), aiming at improving solubility in buffer and physiological conditions further and at gaining possible additional interactions with the phosphate groups of the RNA. A few analogues (**E**) fusing the structures with the best results were synthesised, such as the 4,6-dimethoxy substituted **134** and **135**, respectively bearing *N*-methylpiperazine and piperazine, and the two enantiomers **150** and **151** bearing the 3-aminopiperidine moiety, along with benzimidazole **140** bearing the *N*-methylpiperazine. Among these series (**D** and **E**), 3-aminopiperidine derivatives **148** – **151** stood out, directly followed by piperazine derivative **132** – **135** (see Chapter 3.2.5.3, Table 13). Moreover, compounds **148** and **149** were further developed by expanding the structure with a 2nd-site ligand, which was identified by means of TOCSY experiments and computational calculations performed by Dr. Marcel Blommers, Dr. Kaspar Zimmermann, Dr. Kamal Azzaoui (Saverna Therapeutics, Switzerland), and Dr. Claus Ehrhardt (University of Zurich), as described in detail in Chapter 3.2.6.1. These techniques allowed to confirm the position of the paramagnetic S-391 (**129**), containing the 6-methoxybenzothiazole residue (as in **148** and **149**), in the internal loop of 5_SL1, and this property was further exploited to analyse structures potentially binding next to it. The 2nd-site ligand was identified in the 3-aminobenzothiazole hit814 (**154**), which led to the 2nd-site binders as fusion between 3-aminopiperines **148** and **149** and hit814 (**154**, **F**, 2nd-site ligand structures inspired by hit814 (**154**) in orange). The supposed additional interactions couldn't be confirmed by the NMR-based screening (see Chapter 3.2.6.4, Table 22), since all the compounds in this series did not show superior binding affinity to the truncated **148** and **149**, results that were considered independent of the configuration of the enantiomers. Another set of 2nd-site binders

with a linear linker (**217**, **240**, **242** – **247**) was also synthesised but, due to solubility issues in buffer conditions, had to be excluded from the NMR-based screening (in light grey/orange).

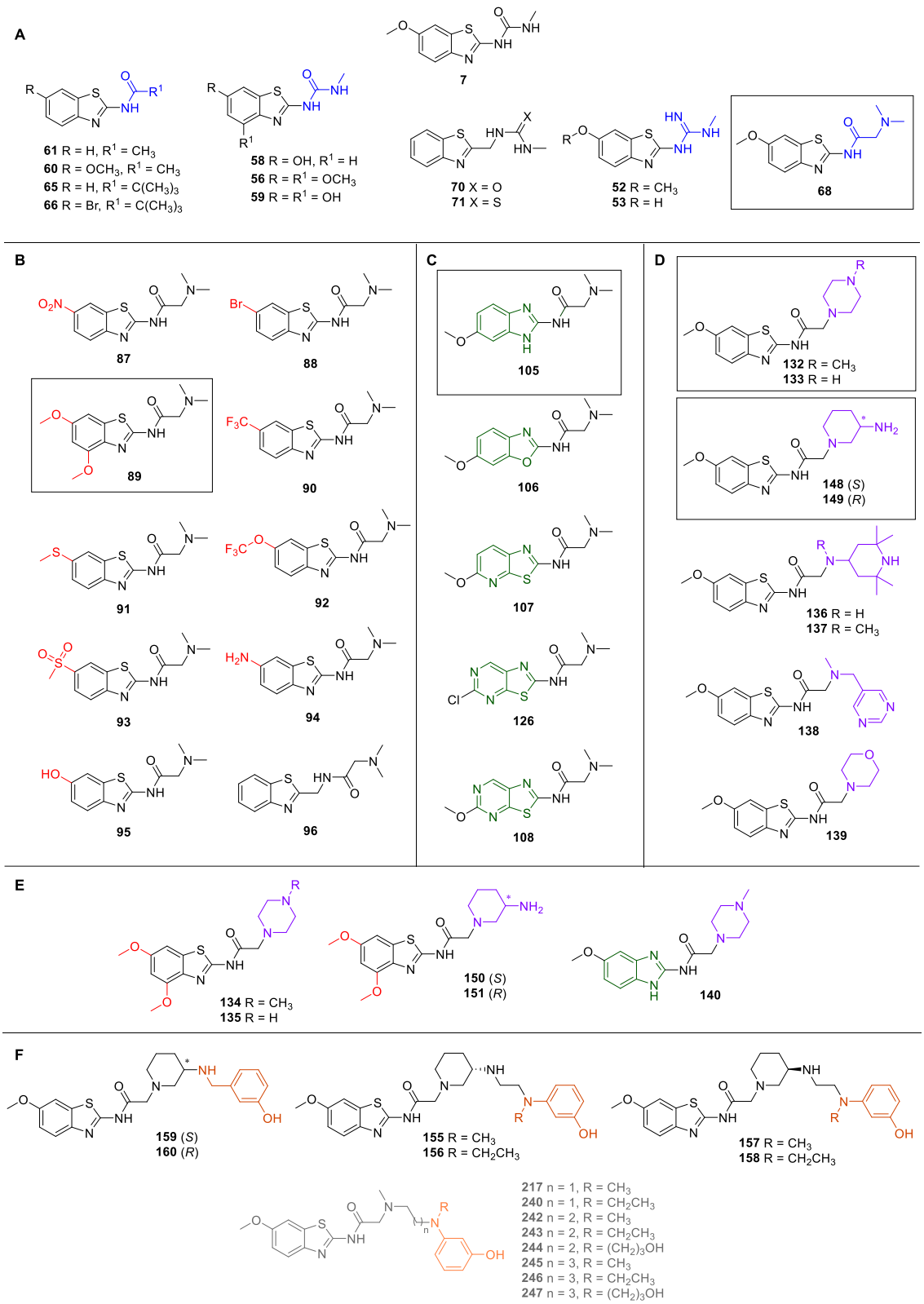
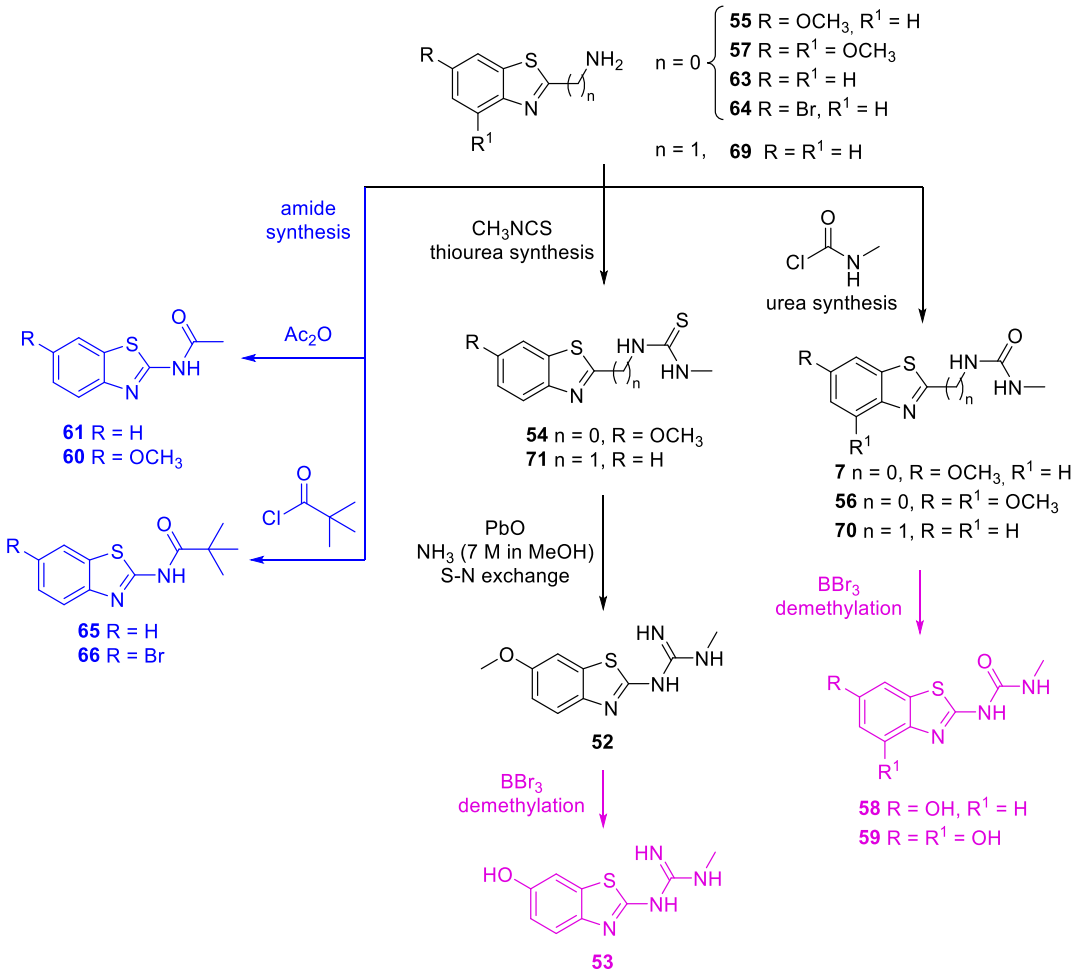


Figure 31: Overview of the structural development of **7**.

Starting from variously substituted 2-aminobenzothiazoles **55**, **57**, **63**, and **64** and 2-aminobenzothiazolemethanamine **69** (Scheme 61), amides **60** and **61** were obtained *via*

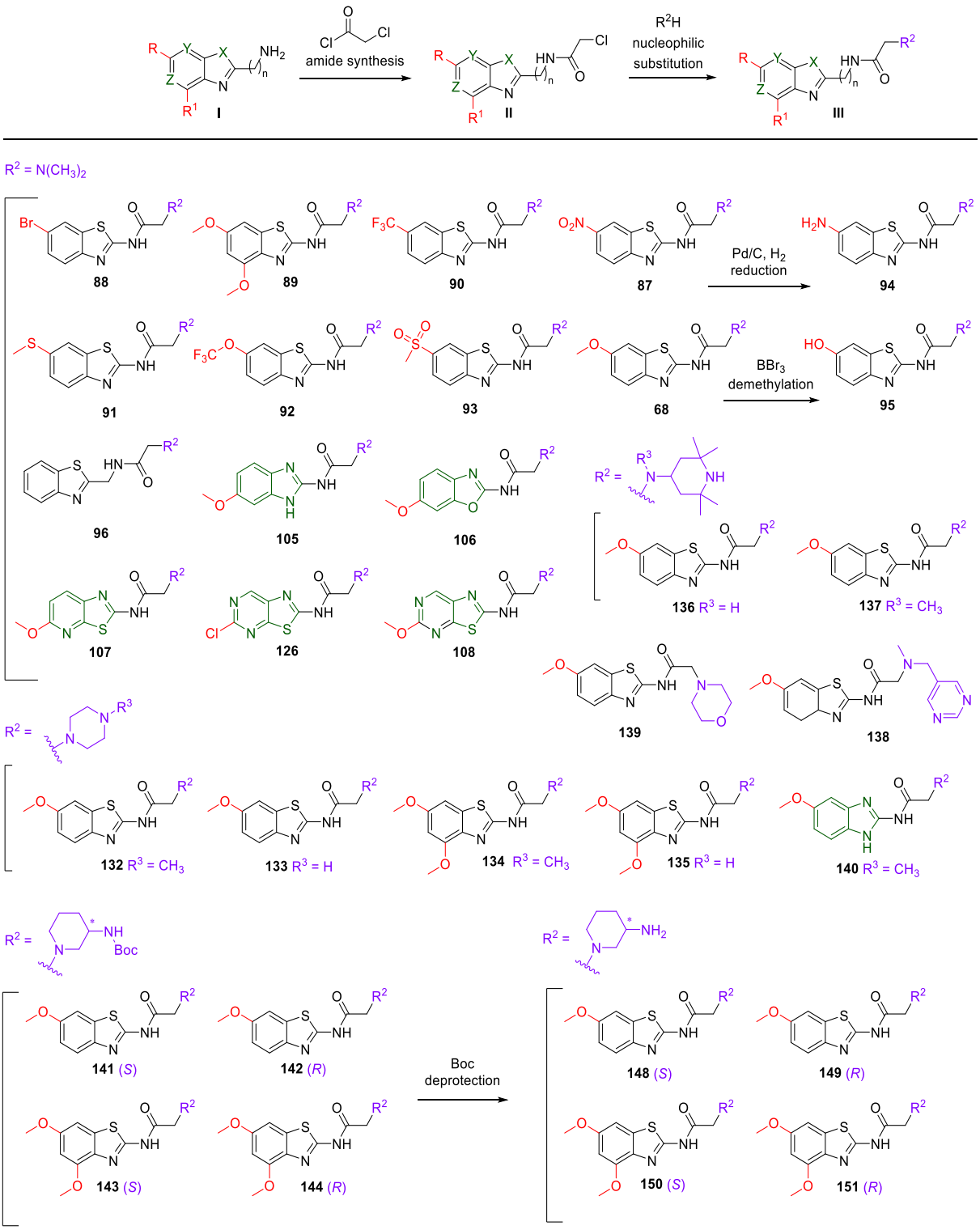
acylation with acetic anhydride, and amides **65** and **66** with pivaloyl chloride. Thioureas **54** and **71** were synthesised starting respectively from **55** and **69** with methyl isothiocyanate. The treatment of **54** with PbO and conc. NH_3 in methanol gave guanidine **52**. Ureas **7**, **56**, and **70** were synthesised *via* urea formation with methylaminoformyl chloride from the respective free amines **55**, **57**, and **63**. BBr_3-mediated *O*-demethylation (in pink) of **52**, **7**, and **56** gave the corresponding hydroxybenzothiazoles **53**, **58**, and **59**.



Scheme 61: Synthesis overview of the first batch of compounds **7**, **52** – **54**, **56**, **58** – **61**, **65**, **66**, **70**, and **71**.

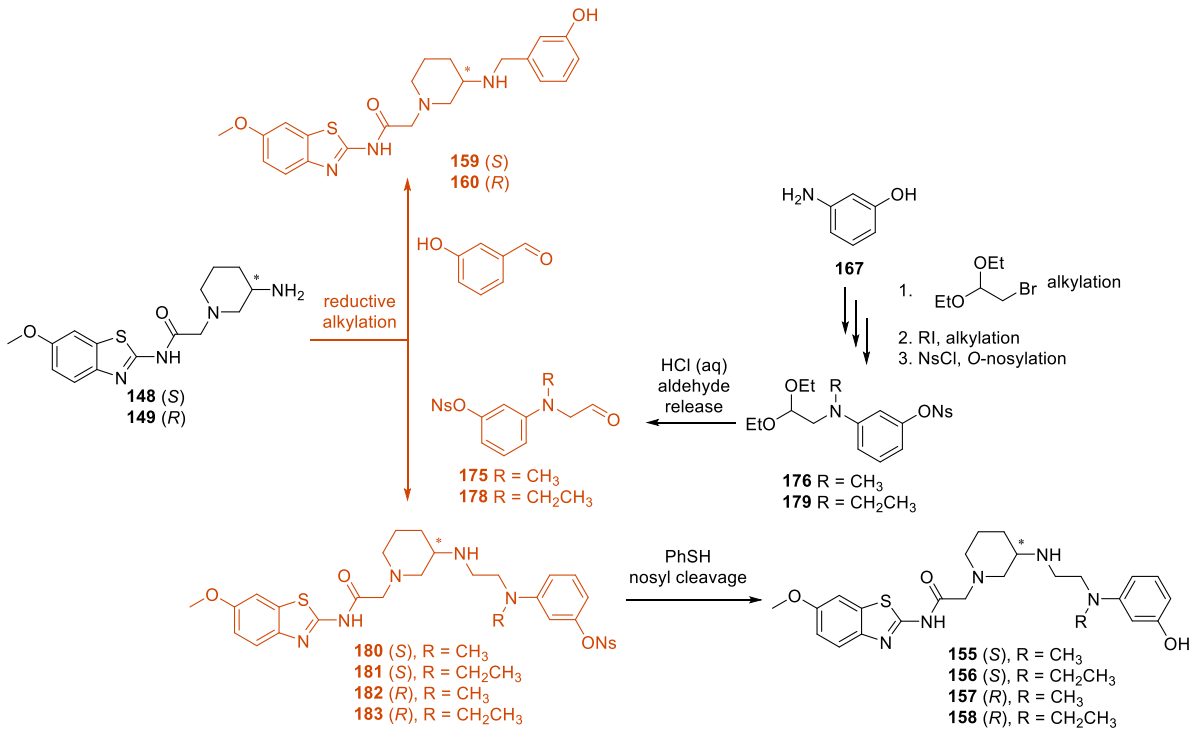
Most of the rest of the compounds shown in Figure 31 were synthesised following the same synthetic pathway. As starting materials (Scheme 62, structures **I**), variously substituted 2-aminobenzothiazoles (**55**, **57**, **63**, **64**, **72** – **79**), 2-aminobenzothiazolemethanamine **69**, 2-aminobenzimidazole **109**, 2-aminobenzoxazole **113**, 2-aminothiazolopyridine **115**, and 2-aminothiazolopyrimidines **123** and **124** were utilised. Among them, 2-aminobenzothiazoles **57**, **72**, and **73** (see Chapter 3.2.2.1, Scheme 20 for the syntheses) and the heteroanalogues of the benzothiazole **113**, **115**, **123**, and **124** (see Chapter 3.2.4.1, Schemes 27, 28, 30, and 31 respectively for the syntheses) were synthesised. Upon alkylation with 2-chloroacetyl chloride, a large variety of compounds was easily accessed *via* nucleophilic substitution using the respective primary and secondary amine. A few compounds were further derivatised: 6-

nitrobenzothiazole **87** was reduced to 6-aminobenzothiazole **94**; 6-hydroxybenzothiazole **95** was synthesised *via* BBr_3-mediated *O*-demethylation of **68**; from *N*-Boc-protected 3-aminopiperidine derivatives **141** – **144**, free amines **148** – **151** were respectively obtained *via* TFA-mediated deprotection.



Scheme 62: Synthetic pathway for most of the analogues synthesised.

To synthesise the 2nd site binders, 3-aminopiperidine derivatives **148** and **149** were *N*-alkylated *via* reductive alkylation (Scheme 63, in orange) with 3-hydroxybenzoaldehyde to derivatives **159** and **160** and with aldehydes **175** and **178** to intermediates **180** – **183**, which were converted into the free phenol derivatives **155** – **158** upon nosyl cleavage. Aldehydes **175** and **178** were in turn synthesised by alkylation of 3-aminophenol (**167**) with bromoacetaldehyde diethyl acetal and, subsequently, with alkyl iodide (iodomethane or iodoethane). The following *O*-protection by nosylation allowed the final release of aldehydes under acidic conditions, avoiding side reaction of intramolecular cyclisation.



Scheme 63: Synthesis overview of 2nd-site binders.

To prove the validity of this system of structure adjustments based on regular NMR screening, further experiments were conducted. 2D-^{1}H,^{1}H-TOCSY measurements detect interactions between the ligand and the pyrimidine protons H5 and H6 of uridines and cytosines, allowing a binding site mapping (see Chapter 3.2.7.1). At first, the most promising structures were tested, such as **52** and **68** (see Figure 31, **A**), **87** – **89** (see Figure 31, **B**), **105** (see Figure 31, **C**), and **132** (see Figure 31, **D**). The main site of binding was confirmed to be the internal loop (U11, U13, and C28) for all the compounds listed. Additionally, small CSPs right next to the internal loop (U9, U10, U11, and U25) were registered for **68**, **88**, and **89**, and in the apical loop (U18, C19, C20, and C21) for **52**, probably due to the propagation of the structural changes upon binding with the internal loop. It was also possible to detect a clear improvement in the CSP of **132**, containing the second basic site in the lateral chain, compared to **68**. Due to limited RNA availability, some of experiments, especially on 2nd-site binders, are still on-going and not included in this work.

To get insights into the selectivity of towards 5_SL1, a mutant counterscreen was performed. Compounds selected from each generation were tested on a mutated 5_SL1 construct missing the internal loop (see Chapter 3.2.7.2). Out of 34 compounds, only 9 showed binding to the mutated 5_SL1 with CSPs that were significantly lower than the ones with wild-type 5_SL1. Interestingly, 6 out of these 9 compounds present the *N*,*N*-dimethylamino moiety (**68** and **89**) or the additional second basic site (**133**, **136**, **134**, and **150**), suggesting possible additional interactions with 5_SL1. Similarly, the small CSPs registered for 2nd-site binders **155** and **158**, which were not detected for **148** and **149** could support the existence of a second binding site in the major groove of the internal loop, as postulated by docking experiments. Further analysis to get a deeper understanding of these interactions with binding site mapping might be crucial to improve the computational model described in Chapter 3.2.5.1, in this interdisciplinary work based on constant updating and adjustment.

The cell-free translation assay to determine the translation efficiency are still on-going and therefore not described in this work.

5 Experimental section

5.1 High-throughput screening (HTS)

Inhibitors of TPC2 were identified by a fluorometric calcium assay-based HTS of the Spectrum Collection library (2000 drugs, natural substances, and toxins) and the Selleck Chemicals library (5000 bioactive compounds) using a HEK293 cell line stably expressing plasma membrane $TPC2^{L11A/L12A}$-RFP. The cells were cultured with Dulbecco's modified Eagle medium (Gibco), supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 0.100 mg/mL streptomycin, and 0.5 μg/mL geneticin (G418, Sigma) at 37 °C in a 5% CO_2-aerated humidified atmosphere. After trypsinisation, cells were suspended in cell culture medium containing 4 μM of the calcium dye Fluo-4/AM (**51**, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and loaded for 30 min at 37 °C. Then cells were centrifuged, resuspended in a HEPES-buffered solution 1 (HBS1: 132 mM NaCl, 6 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5.5 mM D-glucose, 10 mM HEPES, pH 7.4) and dispensed into black pigmented, clear-bottom 384-well microwell plates (Greiner μClear, Frickenhausen, Germany). Plates were placed into a custom-made fluorescence imaging plate reader built into a robotic liquid handling station (Fluent, Tecan, Männedorf, Switzerland) and fluorescence signals (excitation 470 nm, emission 515 nm) were recorded with a Zyla 5.5 camera (Andor, Belfast, UK) and the μManager software like previously described[30]. Compounds of libraries 200 μM-prediluted in 150 mM NaCl, 20 mM HEPES, pH 7.4 and stored in 384-well polystyrene plates were added to the cells with the Tecan 384-tip multichannel arm each at a final concentration of 20 μM. Subsequently, the two known activators TPC2-A1-N (**2**) or TPC2-A1-P (**3**)[15] prediluted in HBS1 were pipetted in each well, and fluorescence signals were recorded for 10 min. After measurement, ImageJ (National Institutes of Health, Bethesda, MD, USA) was used to calculate fluorescence intensities for each well and respective background areas during time responses. Finally, background fluorescences were subtracted, and fluorescence intensities of each time were normalised to initial intensities (F/F_0). The same procedure was applied for generation of concentration response curves of primary screening hits or Lys05 (**1**) derivatives but here compounds were serially prediluted in HBS1 supplemented with 0.1% bovine serum albumin, and obtained data was fitted to a four-parameter Hill equation. The HTS was performed by Nicole Urban, Schaefer Group.

5.2 Single cell calcium imaging

Single cell calcium imaging experiments, as previously described[15, 142], were conducted on HEK293 cells stably expressing $TPC2^{L11A/L12A}$-RFP, cultured at 37 °C with 5% of CO_2 in Dulbecco's modified Eagle medium (Gibco), supplemented with 10% fetal bovine serum,

100 U/mL penicillin, 0.100 mg/mL streptomycin, and 0.5 μg/mL geneticin (G418, Sigma). Cells were plated onto poly-L-lysine (sigma)-coated glass coverslips, grown over two days and then loaded with Fura-2/AM (**34**, 4.0 μM) and 0.005% (v/v) Pluronic® F-127 (both from Thermo Fisher) in HEPES-buffered solution 2 (HBS2). HBS2 was prepared as follows: 138 mM NaCl, 6.00 mM KCl, 1.00 mM $MgCl_2$, 2.00 mM $CaCl_2$, 10.0 mM HEPES, and 5.50 mM D-glucose, using 1M NaOH aq. solution to adjust the pH to 7.4. After the loading with Fura-2/AM (**34**), the cells were incubated 45 min at 37 °C with 10% of CO_2, to be then washed twice with HSB2 and mounted in an imaging chamber (25 mm, RC-40LP, Warner Instrument). Compound stock solutions (10 mM in DMSO) were diluted with HBS2 to obtain a working concentration of 100 μM, 1% DMSO. TPC2-A1-N (**2**) and TPC2-A1-P (**3**) were used to test inhibitors. Activator stock solutions (10 mM in DMSO for TPC2-A1-N (**2**) and 30 mM in DMSO for TPC2-A1-P (**3**)) were diluted with HBS2 to respectively 110 μM and 330 μM, 1% DMSO working concentration. Mere DMSO (1% in HBS2) was used as negative control. Imaging chambers were loaded with HBS2 (450 μL). After recording the baseline for 30 s, the compound solution (50 μL), to reach a final concentration of 10 μM, or the control solution (50 μL) was added. After additional 60 s, the activator solution (50 μL) was added to reach a final concentration of 10 μM for TPC2-A1-N (**2**) and 30 μM for TPC2-A1-P (**3**). A monochromator-based imaging system (Polychrome IV monochromator, TILL photonics or a Leica DMi8 live cell microscope) was used to acquire images every 2 s at 40x magnification. Fura-2 (**5**) was excited at 340 nm/380 nm and emitted fluorescence was captured using 515 nm long-pass filters. All graphs of biological data were plotted using GraphPad Prism 5.

5.3 NMR-based screening

The RNA samples were obtained in-house following the methodology described by Wacker and Weigand *et al.*[143]. Double-stranded template DNA, amplified and linearised, bacteriophage-derived RNA polymerase T7, cofactors, and substrates (nucleoside triphosphates, Mg^{2+}, dithiothreitol (DTT), spermidine) were incubated in a buffer (0.2 M Tris-HCl, pH 8.0) at 37 °C[144, 145]. Transcribed RNA was purified *via* polyacrylamide gel electrophoresis under denaturing conditions and reverse-phase (RP) high-performance liquid chromatography (HPLC). The final RNA samples were buffer-exchanged to 25 mM potassium phosphate (KPi), 50 mM KCl, and pH 6.2 with 3-kDa molecular weight cut-off (MWCO) VivaSpin filtration units (Sartorius). Storage stocks were obtained by dissolving the compounds in a mixture of DMSO-d_6/H_2O (9:1) to a concentration of 25 mM or 50 mM. Working stocks were diluted to 5 mM. Quality control (QC) was performed for each compound recording ^1H spectra with 1 mM of the compounds in NMR screening buffer (25 mM KPi, 50 mM KCl, pH 6.2), 5% DMSO-d_6, and 10 μM sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS). The following solutions were prepared for NMR-based experiments: samples of 40 μL (for 1.7 mm

tubes) or 180 µL (for 3.0 mm tubes) containing 10 µM of unlabeled RNA, 10 µM DSS as a reference, and 200 µM of compound, with a final concentration of 5% (v/v) DMSO-d_6 and 1:20 RNA/ligand ratio. Negative controls were prepared following the same conditions but lacking RNA. For the binding site mapping, the same buffer conditions were applied but using different RNA and ligand concentrations (as specified in Chapter 3.2.7.1, Figure 26 and 27). For 1D-^{1}H experiments, excitation sculpting with gradients or jump return echo water suppression pulse sequences were used. In wLOGSY experiments, the solvent-optimised double gradient spectroscopy (SOGGY) sequence was utilised to suppress water signals[146]. To determine the T_2 relaxation time, Carr-Purcell-Meiboom-Gill (CPMG) experiments a spin-echo pulse sequence were applied[95], utilising SOGGY sequence to suppress water signals. Homonuclear Hartmann-Hahn transfer using the DIPSI2 sequence was applied for total correlation spectroscopy (TOCSY) experiments[147, 148]. An excitation sculpting pulse sequence in the direct dimension was utilised for water suppression, with pulse sequence "dipsi2esfbgpph" from the Bruker library and TOCSY mixing time of 30 ms. The NMR-based screenings were performed by Sabrina Toews, Schwalbe group.

5.4 Docking

All spectra were recorded on a Bruker 600 MHz Advance spectrometer equipped with a 5 mm QNP cryoprobe and a SampleCase sample changer. TOCSY spectra were measured with a 50 ms DIPSI spin-lock pulse and acquired with 400 × 2k data points in T1 and T2. Spectra were measured from 50 µM RNA dissolved in 50 mM phosphate buffer, pH 6.0, and 100% D_2O at 25 °C[149]. Samples for ^{1}H NMR 2nd-site screening contained 50 µM fragments in cocktails of 12, 50 µM S-391 (**129**), and 5 µM RNA dissolved in 50 mM phosphate buffer, pH 6.0, and 10% D_2O. 1D spectra were acquired with excitation sculpting water suppression and an additional perfect echo pulse to correct artifacts caused by *J* modulation of the resonances of the small molecules[150]. The observed line broadening (T2) was used to identify hits. Hits were validated by preparing samples of 50 µM of 2nd-site hit, 50 or 100 µM S-391 (**129**), with or without 50 or 100 µM ascorbic acid, and 5 µM RNA dissolved in 50 mM phosphate buffer, pH 6.0, and 10% D_2O.

Integrated peak volumes, sum of signals using TopSpin:

$$r_{U11} = \sqrt[6]{ln(\frac{I_{U10}^{(1)}}{I_{U10}^{(2)}}\frac{I_{U11}^{(2)}}{I_{U11}^{(1)}})}r_{U10}$$

Integrated peak line width in F2, line fitting using Sparky:

$$r_{U11} = \sqrt[6]{ln(\frac{LW_{U10}^{(1)}}{LW_{U10}^{(2)}}\frac{LW_{U11}^{(2)}}{LW_{U11}^{(1)}})}r_{U10}$$

TOCSY experiments were performed by Dr. Marcel Blommers, Dr. Kaspar Zimmermann, and Dr. Kamal Azzaoui (Saverna Therapeutics, Switzerland).

For the docking experiments, a model of NMR-hit S391 bound to miR155 was minimised with Amber 16 (RNA.OL3/GAFF2, GB with igb=5). The RNA in this model was mutated from miR155 to 5_SL1 and 2nd-site hits were added manually based on RNA-distances. S391 was manually linked with Z814 in complex with 5_SL1 and minimised, leading to the proposals. All manipulations like molecular modeling and docking were done using the modeling package Witnotp, all minimisations and MD with Amber 16. Display of the RNA and ligands was done using PyMOL. The docking was performed by Dr. Claus Ehrhardt (University of Zurich),

5.5 MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed according to a method of Mosmann[151] using HL-60 cells seeded in 96-well plates at 9 x 104 cells/well and incubated for 24 h at 37 °C in a 5% CO_2 atmosphere under standard cell culture conditions in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) without antibiotics. Compounds were dissolved in DMSO (10 mM stock solutions) and tested at different concentrations (7.50 mM, 5.00 mM, 2.50 mM, 1.25 mM, 0.625 mM, and 0.312 mM) with a final well volume of 100 µL, 1% DMSO. Triton® X-100 solution, with a final concentration of 1 µg/mL, was used as positive control and 1% mere DMSO as negative control. Compounds solutions (1 µL) were added to the cell suspensions, and the cells were incubated again for 24 h. After addition of 10 µL MTT solution (5.0 mg MTT in 1.0 mL PBS), the cells were incubated for additional 2 h, followed by addition of 190 µL DMSO. Photometric quantification was conducted after 1 h measuring the absorbance of MTT metabolite formazan at 570 nm with an MRX Microplate Reader (Dynex Technologies, Chantilly, USA). The MTT assay was carried out by Martina Stadler, Bracher group.

5.6 Agar diffusion assay

Agar diffusion assay was performed on microorganisms obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig. Microorganisms were cultivated according to the DSMZ recommendations in liquid culture, using the following autoclaved agars: all-culture agar (AC-agar, Sigma Aldrich), consisting in 35.2 g AC-agar and 20 g agar suspended in 1.0 L water, was used for *Saccharomyces cerevisiae* (DSM number: 1333), *Yarrowia lipolytica* (DSM number: 1345), *Escherichia coli* (DSM number: 426), and *Pseudomonas marginalis* (DSM number: 7527); an agar, 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, was used for *Staphylococcus equorum* (DSM number: 20675) and *Streptococcus entericus* (DSM number: 14446). Warm, liquid agar (15 mL each) was autoclaved and filled onto petri dishes under aseptic conditions. Then, petri dishes were cooled to 8 °C for 1 h. Using cotton swabs, the

germs were brought onto the respective agar. On a test platelet (6 mm diameter, Macherey-Nagel) were given 3.0 µL of 1% (m/V) compound in DMSO, equivalent to 30 µg substance, that was then dried for 24 h at room temperature. As positive controls, clotrimazole (antifungal) and tetracycline (antibacterial) were used, while blind control was conducted with mere DMSO. The platelets containing substances and controls were put onto the petri dishes, which were then incubated for 36 h at 32 °C (bacteria) or 28 °C (yeasts). The measurement of growth inhibition was performed manually. Agar diffusion assay was carried out by Martina Stadler, Bracher group.

5.7 Chemical methods

Solvents and reagents

All chemicals were of analytical grade and were purchased from Sigma-Aldrich (now Merck, Darmstadt, Germany), abcr (Karlsruhe, Germany), Thermo Fisher Scientific (Schwerte, Germany), TCI (Eschborn, Germany), or Th. Geyer (Renningen, Germany). Anhydrous solvents of HPLC grade were purchased from VWR (Darmstadt, Germany) or Sigma-Aldrich. All other solvents were purified by distillation.

Reactions and purifications

Thin layer chromatography (TLC) using polyester sheets POLYGRAM® SIL G/UV 254, coated with 0.2 mm silica gel, from Macherey-Nagel (Düren, Germany), was used to monitor the progress of conversion and the compounds were visualised using UV light (254 nm/365 nm).

Flash column chromatography (FCC) for determination of purities was performed using silica gel 60 (0.040 - 0.063 mm) from Macherey-Nagel. The fractions containing the final compound were unified, and the volatiles were removed under reduced pressure, using Laborota 4001-efficient from Heildolph Instrument (Schwabach, Germany) rotary evaporators and membrane vacuum pumps MD 4C 2006 from VACUUBRAND GMBH (Wertheim, Germany). HPLC analytical measurements for determination of the purities of the products were carried out detecting at 210 nm and 254 nm, utilising the Methods listed in Chapter 5.7.1 and 5.7.2.

Analytical data

Melting points (mp) were measured on Büchi melting point B-540 apparatus. The values are reported in °C and not corrected.

NMR spectra (^{1}H, ^{13}C, DEPT, H-H-COSY, HMQC/HSQC, HMBC) were recorded at 23 °C on Avance III HD 400 MHz Bruker BioSpin. All ^{1}H NMR-spectra and ^{13}C NMR-spectra were recorded respectively at 400 MHz and 101 MHz or 500 MHz and 126 MHz. The δ value of deuterated solvents ($CDCl_3$, DMSO-d_6, or CD_3OD) peaks were taken as internal reference for chemical shifts, which are reported in parts per million (ppm) relative to tetramethylsilane.

^{1}H NMR data are reported as follows: chemical shift *δ* (multiplicity, coupling constants *J*, integral, corresponding H-atom). For the signal multiplicity the following abbreviations were used: s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), dt (double triplet), t (triplet), q (quartet), m (multiplet), br (broad). The coupling constants *J* are reported in Hertz (Hz). ^{13}C NMR-data are reported as follows: chemical shift *δ* (corresponding C-Atom). NMR spectra were analysed by the Software MestReNova 15.0.0-34764 (Mestrelab Research S.L.).

Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR Paragon 1000 instrument. Absorption bands are reported in wave numbers (cm^{-1}). IR spectra were analysed by the Software Spectrum v5.3.1 (Perkin Elmer, Inc.).

High resolution mass spectra (HRMS) were performed in the central analytical facility of the Department Chemistry and Pharmacy of the Ludwig-Maximilians-University of Munich applying a Thermo Finnigan MAT 95 or Joel MStation Sektorfeld instrument at a core temperature of 250 °C and 70 eV for electron ionisation (EI) or a Thermo Finnigan LTQ FT Ultra Fourier Transform Ion Cyclotron Resonance device at 250 °C for electron spray ionisation (ESI).

Values for specific rotation $[\alpha]_D^{20}$ were measured at a wavelength of λ = 589 nm (Na-D-line) at 20 °C using a Perkin Elmer 241 Polarimeter instrument (layer thickness l = 10 cm) in MeOH. The concentration is stated in g/100 mL.

5.7.1 Synthesis of analogues of Lys01 (4)

HPLC analytical measurements for Lys01 (**4**) analogues were carried out using the following Methods:

Method 1: Zorbax Eclipse Plus, C18 5 µm (4.6 x 150 mm), 10 µL injection volume, 50 °C, 1.2 mL/min flow rate

a) 50 °C, ACN/ion pair reagent pH 3.0 20:80 → 80:20

b) 50 °C, MeOH/phosphate buffer pH 9.0 80:20

c) 35 °C, MeOH/phosphate buffer pH 9.0 80:20

Method 2: column Xbridge Phenyl, 3.5 µm (4.6 x 150mm), 5 µL injection volume, 35 °C

a) 1.0 mL/min flow rate, MeOH/phosphate buffer pH 9.0 80:20

b) 0.8 mL/min flow rate, MeOH/phosphate buffer pH 9.0 70:30

Method 3: column Zorbax SB-Aq, C18 5 µm (4.6 x 150mm), 10 µL injection volume, 50 °C, 1.0 mL/min flow rate, ACN/phosphate buffer pH 5.0 50:50

5.7.1.1 General procedures

General procedure A: Nucleophilic aromatic substitution of aryl halides

To a solution of the corresponding aryl halide (1.0 eq) in *N*-methylpyrrolidone (1.0 mL/mmol aryl halide) under nitrogen atmosphere were added triethylamine (1.5 eq) and the corresponding amine (0.5 eq). The mixture was heated to 110 °C and stirred for 22 – 48 h. Water was added, and the solution basified (pH = 8) with 2 M NaOH aq. solution. The formed precipitate was collected by filtration with suction, washed with water, and dried in a desiccator. If the filtration from water was inconvenient, the aq. suspension was extracted with EtOAc (3 x), the combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was further purified by flash column chromatography.

General procedure B: Reductive methylation of amines

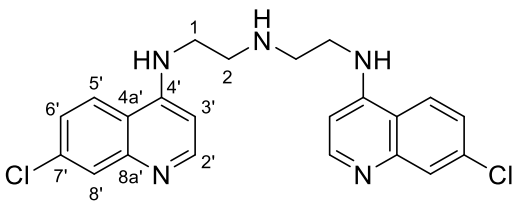
To a solution of the corresponding amine (1.0 eq) in methanol (2.4 mL/mmol amine) was added formaldehyde (25% aq. solution, 2.4 eq) and triethylamine (2.4 eq), and the mixture was stirred at room temperature for 30 min. Sodium triacetoxyborohydride (2.4 eq) was added, and the mixture stirred at room temperature for additional 24 h. Aq. sat. $NaHCO_3$ solution was added, and the mixture was extracted with EtOAc or $CHCl_3$/isopropanol (3:1). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography.

General procedure C: Reductive alkylation of amines

To a solution of the corresponding amine (1.0 eq) in methanol (2.0 mL/mmol amine) was added the corresponding aldehyde or ketone (2.0 eq), acetic acid (0.4 mL/mmol amine), and sodium cyanoborohydride (2.0 eq) at 0 °C. The mixture was stirred at room temperature for 2 – 40 h. Aq. sat. $NaHCO_3$ solution was added, and the mixture was extracted with EtOAc or $CHCl_3$/isopropanol (3:1). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography.

5.7.1.2 Synthetic procedures

***N*^{1}-(7-Chloroquinolin-4-yl)-*N*^{2}-(2-((7-chloroquinolin-4-yl)amino)ethyl)ethane-1,2-diamine (8)**



$C_{22}H_{21}Cl_2N_5$

M_w = 426.35 g/mol

Amine **8** was synthesised according to literature[98], following General procedure A, using 4,7-dichloroquinoline (**9**, 7.92 g, 40.0 mmol, 1.0 eq), triethylamine (8.4 mL, 60 mmol, 1.5 eq), and diethylenetriamine (**10**, 2.15 mL, 20.0 mmol, 0.5 eq). The mixture was stirred for 22 h. The crude product was further purified by flash column chromatography (94:5:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **8** (4.08 g, 9.56 mmol, 48%) as colourless solid. ^{1}H and ^{13}C NMR data are in accordance with the literature[98].

R_f: 0.56 (70:28:2 DCM/MeOH/25% NH_3 aq. solution).

Mp: 248 °C (decomposition). [Ref.[98]: 257 – 262 °C, decomposition.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.37 (d, *J* = 5.4 Hz, 2H, 2'-H), 8.21 (d, *J* = 9.0 Hz, 2H, 5'-H), 7.77 (d, *J* = 2.3 Hz, 2H, 8'-H), 7.39 (dd, *J* = 8.9, 2.3 Hz, 2H, 6'-H), 7.23 (t br, *J* = 6.5 Hz, 2H, 2 ArNH), 6.49 (d, *J* = 5.5 Hz, 2H, 3'-H), 3.35 – 3.20 (m, 4H, 1-H), 2.89 (t, *J* = 6.4 Hz, 4H, 2-H) ppm.

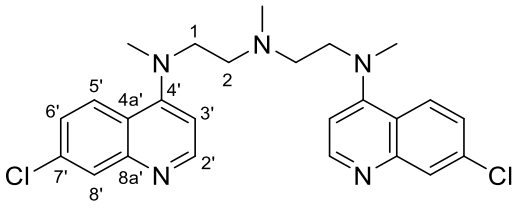
^{13}C NMR (101 MHz, DMSO-d_6): δ = 151.9 (C-2'), 150.2 (C-4'), 149.1 (C-8a'), 133.4 (C-7'), 127.5 (C-8'), 124.0 (C-5'), 123.9 (C-6'), 117.4 (C-4a'), 98.7 (C-3'), 47.1 (C-2), 42.6 (C-1) ppm.

IR (ATR): $\tilde{\nu}$ = 3225, 2960, 1577, 1543, 1452, 1367, 1144, 846, 797 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{22}H_{22}Cl_2N_5$ $[M+H]^+$: 426.1247; found: 426.1246.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

N^1-(7-Chloroquinolin-4-yl)-*N*^{2}-(2-((7-chloroquinolin-4-yl)(methyl)amino)ethyl)-*N*^{1},*N*^{2}-dimethylethane-1,2-diamine (11)



$C_{25}H_{27}Cl_2N_5$

M_w = 468.43 g/mol

Amine **11** was synthesised following General procedure A, using 4,7-dichloroquinoline (**9**, 99 mg, 0.50 mmol, 1.0 eq), triethylamine (0.11 mL, 0.75 mmol, 1.5 eq), and *N*,*N*',*N*''-trimethyldiethylenetriamine (0.042 mL, 0.25 mmol, 0.50 eq). The mixture was stirred for 22 h. The mixture was extracted with EtOAc (3 x 10 mL), and the crude product was further purified

by flash column chromatography (95:4:1 → 93:6:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **11** (334 mg, 0.735 mmol, 49%) as colourless solid.

R_f: 0.72 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61 (d, J = 5.1 Hz, 2H, 2'-H), 7.92 – 7.94 (m, 4H, 8'-H and 5'-H), 7.32 (dd, J = 9.1, 2.2 Hz, 2H, 6'-H), 6.74 (d, J = 5.2 Hz, 2H, 3'-H), 3.33 (t, J = 6.8 Hz, 4H, 1-H), 2.92 (s, 6H, 2 $ArNCH_3$), 2.68 (t, J = 6.8 Hz, 4H, 2-H), 2.24 (s, 3H, NCH_3) ppm.

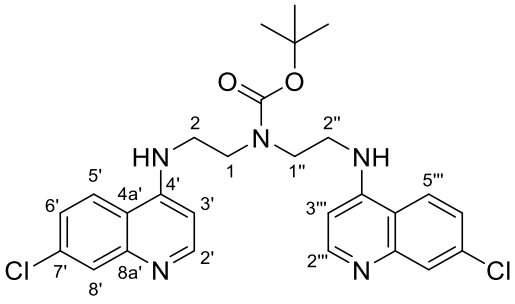
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.1 (C-4'), 151.5 (C-2'), 151.4 (C-8a'), 134.8 (C-7'), 128.8 (C-8' or C-5'), 125.6 (C-8' or C-5'), 125.5 (C-6'), 121.7 (C-4a'), 108.6 (C-3'), 55.5 (C-2), 54.3 (C-1), 43.0 (NCH_3), 40.9 (2 $ArNCH_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3353, 2847, 2803, 1605, 1565, 1417, 876, 818 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{25}H_{28}Cl_2N_5$ $[M+H]^+$: 468.1717; found: 468.1715.

Purity (HPLC): >95% (λ = 210 nm), >98% (λ = 254 nm), Method 1b.

tert-Butyl bis(2-((7-chloroquinolin-4-yl)amino)ethyl)carbamate (13)



$C_{27}H_{29}Cl_2N_5O_2$

M_w = 526.47 g/mol

Amine **8** (256 mg, 0.600 mmol, 1.0 eq) was suspended in a mixture of THF/DMF (7:1, 3 mL). Triethylamine (0.12 mL, 0.84 mmol, 1.4 eq), 4-(dimethylamino)pyridine (7 mg, 0.06 mmol, 0.1 eq), and di-*tert*-butyl dicarbonate (144 mg, 0.660 mmol, 1.1 eq) were added, and the mixture was stirred at room temperature for 14 h. Solvents were removed under reduced pressure, and the residue was directly purified by flash column chromatography (96:3:1 DCM/MeOH/25% NH_3 aq. solution), yielding Boc-derivative **13** (313 mg, 0.595 mmol, 99%) as a light yellow solid.

R_f: 0.52 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 109 – 111 °C.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.27 – 8.25 (m, 2H, 2'-H, 2'''-H), 7.96 (d, *J* = 9.0 Hz, 1H, 5'-H or 5'''-H), 7.90 (d, *J* = 9.0 Hz, 1H, 5'-H or 5'''-H), 7.74 (s, 2H, 8'-H), 7.34 (dd, *J* = 9.0, 2.2 Hz, 2H, 6'-H), 6.50 (d, *J* = 5.6 Hz, 1H, 3'-H or 3'''-H), 6.44 (d, *J* = 5.7 Hz, 1H, 3'-H or 3'''-H), 3.58 – 3. 56 (m, 8H, 1-H, 1'-H, 2-H, 2'-H), 1.28 (s, 9H, $C(CH_3)_3$) ppm.

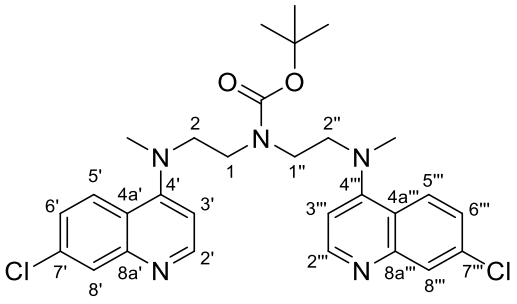
^{13}C NMR (101 MHz, CD_3OD): δ = 158.3 (C-4'), 152.6 (C-2' or C-2'''), 152.4 (C-2' or C-2'''), 152.3 (NCOO), 136.4 (C-7'), 127.8 (C-8'), 126.1 (C-6'), 124.1 (C-5' or C-5'''), 124.0 (C-5' or C-5'''), 118.5 (C-4a'), 99.6 (C-3' or C-3'''), 99.3 (C-3' or C-3'''), 81.9 ($O\underline{C}(CH_3)_3$), 47.5 (C-2 or C-2''), 47.4 (C-2 or C-2''), 43.0 (C-1 or C-1''), 42.0 (C-1 or C-1''), 28.5 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2972, 1689, 1579, 1366, 1232, 1140, 799 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{27}H_{30}Cl_2N_5O_2$ $[M+H]^+$: 526.1772; found: 526.1771.

Purity (HPLC): >94% (λ = 210 nm), >94% (λ = 254 nm), Method 2a.

tert-Butyl bis(2-((7-chloroquinolin-4-yl)(methyl)amino)ethyl)carbamate (14)



$C_{29}H_{33}Cl_2N_5O_2$

M_w = 554.52 g/mol

To a solution of Boc-derivative **13** (526 mg, 1.00 mmol, 1.0 eq) in 1.5 mL DMF under ice-bath cooling, NaH (80 mg, 60% dispersion in paraffin, 2.0 mml, 2.0 eq) was added, and the mixture was stirred for 20 min. Iodomethane (0.125 mL, 2.00 mmol, 2.0 eq) was added at 0 °C, and the mixture was stirred for 0.5 h. The reaction was quenched with aq. sat. ammonium chloride solution (15 mL), and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 15 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (99:0.5:0.5 → 98:1:1 DCM/MeOH/25% NH_3 aq. solution), yielding Boc-derivative **14** (165 mg, 0.297 mmol, 30%) as a colourless waxy oil.

R_f: 0.57 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.62 (d, *J* = 5.1 Hz, 2H, 2'-H, 2'''-H), 8.01 (d, *J* = 2.2 Hz, 2H, 8'-H, 8'''-H), 7.86 – 7.84 (m, 2H, 6'-H, 6'''-H), 7.32 (d, *J* = 8.9 Hz, 2H, 5'-H, 5'''-H), 6.76 (d,

J = 5.1 Hz, 1H, 3'-H or 3'''-H), 6.70 (d, *J* = 5.1 Hz, 1H, 3'-H or 3'''-H), 3.48 – 3.44 (m, 4H, 1-H, 1''-H, or 2-H, 2''-H), 3.24 (s br, 4H, 1-H, 1''-H, or 2-H, 2''-H), 2.89 (s, 3H, NCH_3), 2.86 (s, 3H, NCH_3), 1.39 (s, 9H, $C(CH_3)_3$) ppm.

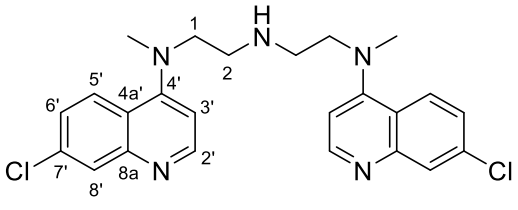
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.0 (C-4' or C-4'''), 156.6 (C-4' or C-4'''), 155.3 (NCOO), 151.5 (C-2' and C-2'''), 150.5 (C-8a' or C-8a''), 150.4 (C-8a' or C-8a'''), 135.1 (C-7' and C-7'''), 129.1 (C-8' or C-8'''), 128.9 (C-8' or C-8'''), 126.1 (C-5' or C-5'''), 125.8 (C-5' or C-5'''), 125.6 (C-6' or C-6'''), 125.2 (C-6' or C-6'''), 121.8 (C-4a' or C-4a'''), 121.7 (C-4a' or C-4a'''), 109.0 (C-3' or C-3'''), 108.9 (C-3' or C-3'''), 80.6 ($O\underline{C}(CH_3)_3$), 54.3 (C-2 or C-2''), 53.7 (C-2 or C-2''), 45.3 (C-1 or C-1''), 45.1 (C-1 or C-1''), 41.5 (double signal, $(NCH_3)_2$), 28.5 ($OC(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3342, 2974, 1687, 1667, 1416, 1150, 876, 819, 769 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{29}H_{34}Cl_2N_5O_2$ $[M+H]^+$: 554.2084; found: 554.2080.

Purity (HPLC): ND.

N^1-(7-Chloroquinolin-4-yl)-*N*^{2}-(2-((7-chloroquinolin-4-yl)(methyl)amino)ethyl)-*N*^{1}-methylethane-1,2-diamine (12)



$C_{24}H_{25}Cl_2N_5$

M_w = 454.40 g/mol

To a solution of Boc-derivative **14** (128 mg, 0.230 mmol, 1.0 eq) in 1.2 mL DCM, 1.2 mL TFA were added under ice-bath cooling. The mixture was stirred at room temperature for 1 h. Volatile residues were removed under reduced pressure, water (20 mL) was added, the mixture was basified (pH = 9) with 2 M NaOH aq. solution and extracted with $CHCl_3$/isopropanol (3:1, 4 x 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (97:2:1 → 96:3:1 DCM/MeOH/25% NH_3 aq. solution), yielding secondary amine **12** (85 mg, 0.19 mmol, 82%) as a colourless waxy solid.

R_f: 0.50 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.64 (d, *J* = 5.1 Hz, 2H, 2'-H), 8.06 (d, *J* = 9.0 Hz, 2H, 5'-H), 8.00 (d, *J* = 2.2 Hz, 2H, 8'-H), 7.32 (dd, *J* = 9.1, 2.2 Hz, 2H, 6'-H), 6.76 (d, *J* = 5.1 Hz, 2H, 3'-H), 3.39 (t, *J* = 6.3 Hz, 4H, 1-H), 2.94 (t, *J* = 6.2 Hz, 4H, 2-H), 2.92 (s, 6H, NCH_3) ppm.

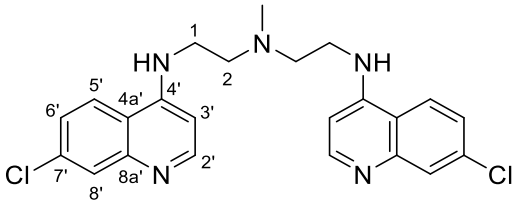
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.3 (C-4'), 151.6 (C-2'), 150.5 (C-8a'), 135.0 (C-7'), 129.0 (C-8'), 125.9 (C-6'), 125.7 (C-5'), 122.0 (C-4a'), 109.0 (C-3'), 55.7 (C-1), 47.1 (C-2), 41.1 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3276, 2834, 1565, 1417, 1376, 1040, 874, 818 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{26}Cl_2N_5$ $[M+H]^+$: 454.1560; found: 454.1559.

Purity (HPLC): >95 % (λ = 210 nm), >95 % (λ = 254 nm), Method 1b.

N^1-(7-Chloroquinolin-4-yl)-*N*^{2}-(2-((7-chloroquinolin-4-yl)amino)ethyl)-*N*^{2}-methylethane-1,2-diamine (Lys01, 4)



$C_{23}H_{23}Cl_2N_5$

M_w = 440.37 g/mol

Amine **4** was synthesised following General procedure B from amine **8** (171 mg, 0.400 mmol, 1.0 eq). The mixture was extracted with EtOAc (3 x 20 mL). The crude product was purified by flash column chromatography (94:5:1 DCM/MeOH/25% NH_3 aq. solution), yielding tertiary amine **4** (113 mg, 0.257 mmol, 64%) as a colourless solid. ^{1}H and ^{13}C NMR data are in accordance with the literature[152].

R_f: 0.37 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 197 – 200 °C (decomposition). [Ref.[152]: 199 – 200 °C.]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.51 (d, *J* = 5.3 Hz, 2H, 2'-H), 7.93 (d, *J* = 2.1 Hz, 2H, 8'-H), 7.40 (d, *J* = 8.9 Hz, 2H, 5'-H), 6.96 (dd, *J* = 8.9, 2.2 Hz, 2H, 6'-H), 6.37 (d, *J* = 5.3 Hz, 2H, 3'-H), 5.49 (t br, *J* = 4.5 Hz, 2H, 2 ArNH), 3.39 (dt, *J* = 6.4, 4.8 Hz, 4H, 1-H), 2.90 – 2.87 (m, 4H, 2-H), 2.46 (s, 3H, CH_3) ppm.

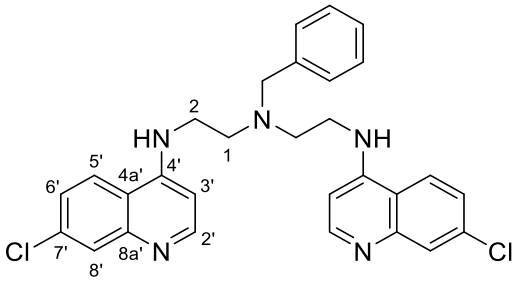
^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.2 (C-2'), 149.5 (C-4'), 149.2 (C-8a'), 135.1 (C-7'), 129.1 (C-8'), 125.6 (C-6'), 120.6 (C-5'), 117.2 (C-4a'), 99.4 (C-3'), 55.6 (C-2), 42.5 (C-1), 40.4 (CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3236, 2956, 1576, 1541, 1370, 1132, 848, 808 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{23}H_{24}Cl_2N_5$ $[M+H]^+$: 440.1404; found: 440.1403.

Purity (HPLC): >98% (λ = 210 nm), >98% (λ = 254 nm), Method 1a.

N^1-Benzyl-N^2-(7-chloroquinolin-4-yl)-N^1-(2-((7-chloroquinolin-4-yl)amino)ethyl)ethane-1,2-diamine (15)



$C_{29}H_{27}Cl_2N_5$

M_w = 516.47 g/mol

Tertiary amine **15** was synthesised following General procedure C, using amine **8** (213 mg, 0.500 mmol, 1.0 eq) and benzaldehyde (0.102 mL, 1.00 mmol, 2 eq). The mixture was stirred for 40 h. Aq. sat. $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with EtOAc (3 x 10 mL). The crude product was purified by flash column chromatography (94:5:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **15** (148 mg, 0.286 mmol, 57%) as a colourless solid.

R_f: 0.62 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 234 – 235 °C (decomposition).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.47 (d, J = 5.3 Hz, 2H, 2'-H), 7.91 (d, J = 2.1 Hz, 2H, 8'-H), 7.43 – 7.35 (m, 5H, $NCH_2C_6\underline{H}_5$), 7.05 (d, J = 8.9 Hz, 2H, 5'-H), 6.65 (dd, J = 8.9, 2.2 Hz, 2H, 6'-H), 6.30 (d, J = 5.3 Hz, 2H, 3'-H), 5.23 (t br, J = 4.3 Hz, 2H, 2 ArNH), 3.72 (s, 2H, $NC\underline{H}_2C_6H_5$), 3.36 – 3.34 (m, 4H, 2-H), 3.02 (dd, J = 7.7, 4.3 Hz, 4H, 1-H) ppm.

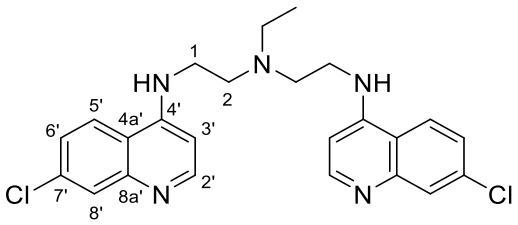
^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.1 (C-2'), 149.4 (C-4' or C-8a'), 149.1 (C-4' or C-8a'), 139.0 ($NCH_2\underline{C}C_5H_5$), 135.2 (C-7'), 129.5 ($NCH_2C\underline{C}_5H_5$), 129.3 ($NCH_2C\underline{C}_5H_5$), 128.9 (C-8'), 128.2 ($NCH_2C\underline{C}_5H_5$), 125.4 (C-6'), 120.5 (C-5'), 117.0 (C-4a'), 99.3 (C-3'), 58.6 ($N\underline{C}H_2C_6H_5$), 52.4 (C-1), 40.6 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3219, 2949, 1570, 1540, 1453, 1430, 1134, 851, 699 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{29}H_{28}Cl_2N_5$ $[M+H]^+$: 516.1717; found: 516.1716.

Purity (HPLC): 100% (λ = 210 nm), >98% (λ = 254 nm), Method 1b.

N^1-(7-Chloroquinolin-4-yl)-N^2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^2-ethylethane-1,2-diamine (16)



$C_{24}H_{25}Cl_2N_5$

M_w = 454.40 g/mol

Tertiary amine **16** was synthesised following General procedure C, using amine **8** (213 mg, 0.500 mmol, 1.0 eq) and acetaldehyde (0.056 mL, 1.0 mmol, 2.0 eq). The mixture was stirred for 17 h. Aq. sat. $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 10 mL). The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **16** (146 mg, 0.321 mmol, 64%) as a colourless solid.

R_f: 0.28 (94:5:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 169 – 170 °C (decomposition).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.49 (d, *J* = 5.3 Hz, 2H, 2'-H), 7.91 (d, *J* = 2.2 Hz, 2H, 8'-H), 7.29 (d, *J* = 8.9 Hz, 2H, 5'-H), 6.85 (dd, *J* = 8.9, 2.2 Hz, 2H, 6'-H), 6.33 (d, *J* = 5.4 Hz, 2H, 3'-H), 5.47 (t br, *J* = 4.5 Hz, 2H, 2 ArNH), 3.37 – 3.33 (m, 4H, 1-H), 2.94 (dd, *J* = 6.4, 5.2 Hz, 4H, 2-H), 2.74 (q, *J* = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 1.20 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

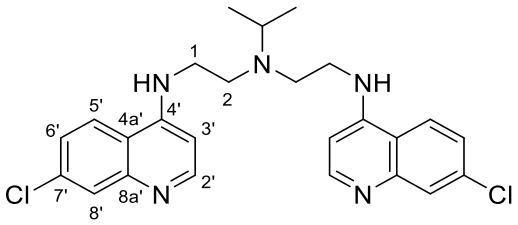
^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.1 (C-2'), 149.5 (C-4'), 149.1 (C-8a'), 135.1 (C-7'), 129.0 (C-8'), 125.5 (C-6'), 120.4 (C-5'), 117.1 (C-4a'), 99.4 (C-3'), 51.9 (C-2), 47.9 ($\underline{C}H_2CH_3$), 40.6 (C-1), 12.4 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3374, 2962, 1578, 1448, 818, 802 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{26}Cl_2N_5$ $[M+H]^+$: 454.1560; found: 454.1560.

Purity (HPLC): >95% (λ = 210 nm), >95% (λ = 254 nm), Method 1b.

N^1-(7-Chloroquinolin-4-yl)-N^2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^2-isopropylethane-1,2-diamine (17)



$C_{25}H_{27}Cl_2N_5$

M_w = 468.43 g/mol

Tertiary amine **17** was synthesised following General procedure C, using amine **8** (213 mg, 0.500 mmol, 1.0 eq) and acetone (0.074 mL, 1.0 mmol, 2.0 eq). The mixture was stirred for 22 h. Aq. sat. $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 10 mL). The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **17** (190 mg, 0.406 mmol, 81%) as a colourless solid.

R_f: 0.27 (94:5:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 142 °C (decomposition).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.48 (d, J = 5.3 Hz, 2H, 2'-H), 7.89 (d, J = 2.2 Hz, 2H, 8'-H), 7.25 (d, J = 9.0 Hz, 2H, 5'-H), 6.79 (dd, J = 9.0, 2.2 Hz, 2H, 6'-H), 6.31 (d, J = 5.3 Hz, 2H, 3'-H), 5.45 (t br, J = 4.7 Hz, 2H, 2 ArNH), 3.29 (q, J = 5.4 Hz, 4H, 1-H), 3.15 (p, J = 6.6 Hz, 1H, C<u>H</u>$(CH_3)_2$), 2.91 (t, J = 5.8 Hz, 4H, 2-H), 1.18 (d, J = 6.6 Hz, 6H, CH(C<u>H</u>$_3)_2$) ppm.

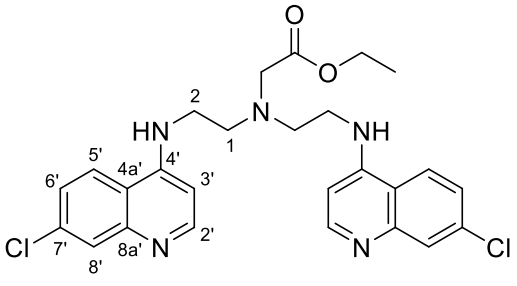
^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.1 (C-2'), 149.5 (C-4' or C-8a'), 149.1 (C-4' or C-8a'), 135.1 (C-7'), 129.0 (C-8'), 125.5 (C-6'), 120.3 (C-5'), 117.1 (C-4a'), 99.4 (C-3'), 50.1 (<u>C</u>H$(CH_3)_2$), 48.0 (C-2), 41.0 (C-1), 18.4 (CH(<u>C</u>H$_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3207, 2964, 1580, 1566, 1452, 1138, 1079, 847, 796 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{25}H_{28}Cl_2N_5$ $[M+H]^+$: 468.1717; found: 468.1716.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1b.

Ethyl bis(2-((7-chloroquinolin-4-yl)amino)ethyl)glycinate (18)



$C_{26}H_{27}Cl_2N_5O_2$

M_w = 512.44 g/mol

Tertiary amine **18** was synthesised following General procedure C, using amine **8** (128 mg, 0.300 mmol, 1.0 eq) and ethyl glyoxylate (0.12 mL, 50% in toluene, 0.60 mmol, 2.0 eq). The mixture was stirred for 7 h. Aq. sat. $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 10 mL). The crude product was purified by flash column chromatography (98:1:1 → 97:2:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **18** (70 mg, 0.14 mmol, 46%) as a colourless solid.

R_f: 0.52 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 165 – 166 °C (decomposition).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.45 (d, *J* = 5.3 Hz, 2H, 2'-H), 7.89 (d, *J* = 2.2 Hz, 2H, 8'-H), 7.59 (d, *J* = 8.9 Hz, 2H, 5'-H), 6.62 (dd, *J* = 8.9, 2.2 Hz, 2H, 6'-H), 6.40 (t br, *J* = 4.1 Hz, 2H, 2 ArNH), 6.26 (d, *J* = 5.4 Hz, 2H, 3'-H), 4.28 (q, *J* = 7.1 Hz, 2H, OCH_2), 3.49 (s, 2H, NCH_2CO), 3.29 (dd, *J* = 5.2, 4.9 Hz, 4H, 2-H), 3.03 (dd, *J* = 6.5, 4.3 Hz, 4H, 1-H), 1.30 (t, *J* = 7.1 Hz, 2H, CH_3) ppm.

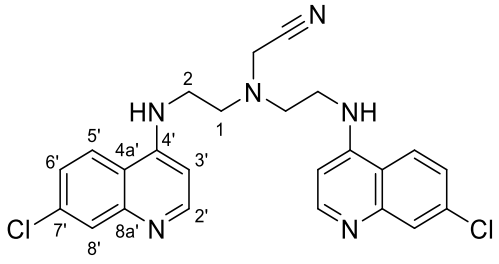
^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.9 (CO), 152.0 (C-2'), 149.9 (C-8a' or C-4'), 149.0 (C-8a' or C-4'), 135.2 (C-7'), 128.5 (C-8'), 125.5 (C-6'), 121.5 (C-5'), 117.3 (C-4a'), 99.0 (C-3'), 61.9 (OCH_2), 54.9 ($N\underline{C}H_2CO$), 53.1 (C-1), 40.7 (C-2), 14.3 (CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3387, 3343, 2830, 1729, 1579, 1522, 1213, 813 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{26}H_{28}Cl_2N_5O_2$ $[M+H]^+$: 512.1615; found: 512.1614.

Purity (HPLC): >90 % (λ = 210 nm), >90 % (λ = 254 nm), Method 1b.

2-(Bis(2-((7-chloroquinolin-4-yl)amino)ethyl)amino)acetonitrile (19)



$C_{24}H_{22}Cl_2N_6$

M_w = 465.38 g/mol

To a solution of amine **8** (426 mg, 1.00 mmol, 1.0 eq) in 4 mL DMF, bromoacetonitrile (0.077 mL, 1.1 mmol, 1.1 eq) and caesium carbonate (652 mg, 2.00 mmol, 2.0 eq) were added at 0 °C. The mixture was stirred at room temperature for 24 h. After the solvent was evaporated under reduced pressure, aq. sat. $NaHCO_3$ solution (40 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 40 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (97:2:1 DCM/MeOH/25% NH_3 aq. solution), yielding nitrile derivative **19** (89 mg, 0.19 mmol, 19%) as an off-white solid.

R_f: 0.80 (80:18:2 DCM/MeOH/25% NH_3 aq. solution).

Mp: 128 – 130 °C (decomposition).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.20 (d, *J* = 5.6 Hz, 2H, 2'-H), 7.60 (d, *J* = 1.5 Hz, 2H, 8'-H), 7.59 (d, *J* = 5.1 Hz, 2H, 5'-H), 6.96 (dd, *J* = 9.0, 2.1 Hz, 2H, 6'-H), 6.36 (d, *J* = 5.7 Hz, 2H, 3'-H), 4.01 (s, 2H, NC$\underline{H}_2$CN), 3.40 (dd, *J* = 6.4, 4.9 Hz, 4H, 2-H), 2.99 (q, *J* = 6.5, 4.9 Hz, 2H, 1-H) ppm.

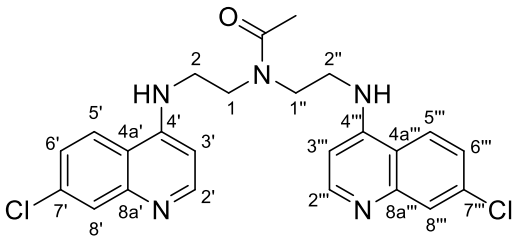
^{13}C NMR (101 MHz, CD_3OD): δ = 152.3 (C-4'), 151.9 (C-2'), 148.9 (C-8a'), 136.3 (C-7'), 127.4 (C-5'), 125.9 (C-6'), 123.4 (C-8'), 118.2 (C-4a'), 116.8 (NCH$_2\underline{C}$N), 99.6 (C-3'), 52.8 (C-1), 43.5 (N$\underline{C}$H$_2$CN), 41.4 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3402, 3292, 2810, 2212, 1579, 1531, 1328, 879, 803 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{23}Cl_2N_6$ $[M+H]^+$: 465.1356; found: 465.1357.

Purity (HPLC): >96% (λ = 210 nm), >97% (λ = 254 nm), Method 1b.

_N_,_N_-Bis(2-((7-chloroquinolin-4-yl)amino)ethyl)acetamide (20)



$C_{24}H_{23}Cl_2N_5O$

M_w = 468.39 g/mol

Amine **8** (179 mg, 0.420 mmol, 1.0 eq) was dissolved in 4 mL acetic anhydride. The solution was stirred at room temperature for 1 h. The excess of acetic anhydride was removed under reduced pressure. Water (10 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 2 x 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (98:1:1 → 96:3:1 DCM/MeOH/25% NH_3 aq. solution), yielding acetylated amine **20** (117 mg, 0.250 mmol, 60%) as a colourless solid.

R_f: 0.35 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 240 – 241 °C (decomposition).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.30 (dd, J = 5.6, 4.5 Hz, 2H, 2'-H, 2'''-H), 7.97 (d, J = 9.0 Hz, 1H, 5'-H or 5'''-H), 7.92 (d, J = 9.0 Hz, 1H, 5'-H or 5'''-H), 7.77 (t, J = 2.1 Hz, 2H, 8'-H, 8'''-H), 7.38 (t, J = 2.3 Hz, 1H, 5'-H or 5'''-H), 7.36 (t, J = 2.3 Hz, 1H, 5'-H or 5'''-H), 6.53 (d, J = 5.6 Hz, 1H, 3'-H or 3'''-H), 6.50 (d, J = 5.6 Hz, 1H, 3'-H or 3'''-H), 3.76 (t, J = 6.1 Hz, 2H, 1-H or 1''-H), 3.72 (t, J = 6.1 Hz, 2H, 1-H or 1''-H), 3.67 – 3.63 (m, 2H, 2-H or 2''-H), 3.57 (t, J = 6.1 Hz, 2H, 2-H or 2''-H), 2.08 (s, 3H, CH_3) ppm.

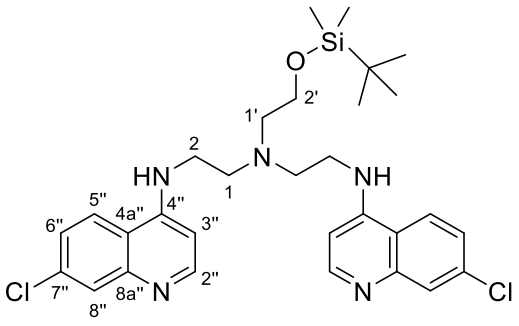
^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.4 ($\underline{C}OCH_3$), 151.2 (C-4' or C-4'''), 151.1 (C-2' or C-2'''), 151.0 (C-2' or C-2'''), 150.9 (C-4' or C-4'''), 148.3 (C-8a' or C-8a'''), 148.1 (C-8a' or C-8a'''), 135.2 (C-7' or C-7'''), 135.1 (C-7' or C-7'''), 126.4 (C-8' or C-8'''), 126.3 (C-8' or C-8'''), 125.0 (C-6' or C-6'''), 124.8 (C-6' or C-6'''), 122.6 (C-5' or C-5'''), 122.5 (C-5' or C-5'''), 117.3 (C-4a' or C-4a'''), 117.2 (C-4a' or C-4a'''), 98.2 (C-3' or C-3'''), 98.1 (C-3' or C-3'''), 47.7 (C-1 or C-1''), 45.1 (C-1 or C-1''), 40.9 (C-2 or C-2''), 40.8 (C-2 or C-2''), 20.0 (CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3346, 1641, 1577, 1544, 1227, 1138, 798 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{24}H_{24}Cl_2N_5O$ $[M+H]^+$: 468.1353; found: 468.1352.

Purity (HPLC): >98% (λ = 210 nm), 100% (λ = 254 nm), Method 1b.

N^1-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-N^2-(7-chloroquinolin-4-yl)-N^1-(2-((7-chloroquinolin-4-yl) amino)ethyl)ethane-1,2-diamine (22)



$C_{30}H_{39}Cl_2N_5OSi$

M_w = 584.66 g/mol

Tertiary amine **22** was synthesised following General procedure C, using amine **8** (426 mg, 1.00 mmol, 1.0 eq) and (*tert*-butyldimethylsilyloxy)acetaldehyde (0.42 mL, 2.0 mmol, 2.0 eq). The mixture was stirred for 2 h. Aq. sat. $NaHCO_3$ solution (30 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 2 x 30 mL). The crude light yellow oil was used for the following step without any further purification.

R_f: 0.59 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.47 (d, J = 5.3 Hz, 2H, 2''-H), 7.90 (d, J = 2.2 Hz, 2H, 8''-H), 7.35 (d, J = 9.0 Hz, 2H, 5''-H), 6.69 (dd, J = 9.0, 2.1 Hz, 2H, 6''-H), 6.31 (d, J = 5.3 Hz, 2H, 3''-H), 5.78 (s br, 2H, 2 ArNH), 3.89 (t, J = 4.8 Hz, 2H, 2'-H), 3.32 (q, J = 5.4 Hz, 4H, 2-H), 3.08 – 3.04 (m, 4H, 1-H), 2.81 (t, J = 4.9 Hz, 2H, 1'-H), 0.88 (s, 9H, ($OSiC(CH_3)_3$), 0.07 (s, 6H, $OSi(CH_3)_2$) ppm.

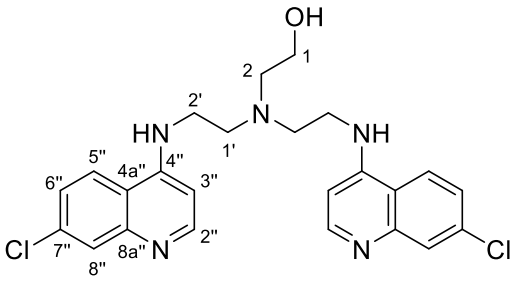
^{13}C NMR (101 MHz, $CDCl_3$): δ = 151.9 (C-2''), 149.6 (C-4'' or C-8a''), 148.9 (C-4'' or C-8a''), 135.0 (C-7''), 128.7 (C-8''), 125.3 (C-6''), 120.7 (C-5''), 117.0 (C-4a''), 99.2 (C-3''), 63.5 (C-2'), 56.2 (C-1'), 53.5 (C-1), 40.9 (C-2), 26.1 ($OSiC(\underline{C}H_3)_3$), 18.5 ($OSi\underline{C}(CH_3)_3$), -5.02 ($OSi(\underline{C}H_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3250, 2927, 2855, 1577, 1079, 834, 805, 775 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{30}H_{40}Cl_2N_5OSi$ $[M+H]^+$: 584.2374; found: 584.2371.

Purity (HPLC): >96% (λ = 210 nm), >96% (λ = 254 nm), Method 2a.

2-(Bis(2-((7-chloroquinolin-4-yl)amino)ethyl)amino)ethan-1-ol (21)



$C_{24}H_{25}Cl_2N_5O$

M_w = 470.40 g/mol

Crude silyl ether **22** was dissolved in a mixture of AcOH/H_2O/THF (3:1:1, 4 ml) and the mixture was stirred at 60 °C for 3.5 h. The solvents were removed under reduced pressure, and the oily residue purified by flash column chromatography (97:2:1 → 96:3:1 DCM/MeOH/25% NH_3 aq. solution) to give primary alcohol **21** (397 mg, 0.844 mmol) as a colourless solid with an 84% overall yield over two steps.

R_f: 0.33 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 211 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.32 (d, J = 5.4 Hz, 2H, 2''-H), 8.05 (d, J = 9.1 Hz, 2H, 5''-H), 7.73 (d, J = 2.3 Hz, 2H, 8''-H), 7.20 (dd, J = 9.0, 2.3 Hz, 2H, 6''-H), 7.09 (t br, J = 5.2 Hz, 2H, 2 ArNH), 6.40 (d, J = 5.4 Hz, 2H, 3''-H), 4.72 (s br, 1H, OH), 3.55 (s br, 2H, 1-H), 3.30 (t, J = 5.9 Hz, 4H, 2'-H), 2.88 (t, J = 6.4 Hz, 4H, 1'-H), 2.72 (t, J = 5.9 Hz, 2H, 2-H) ppm.

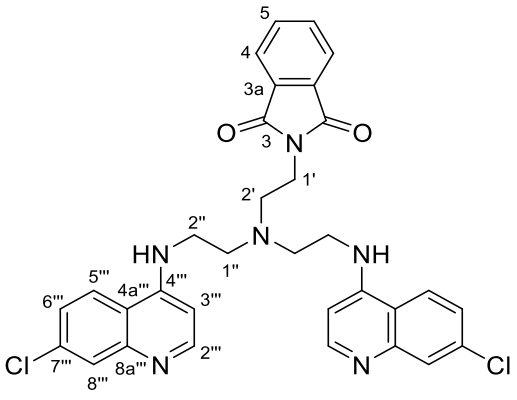
^{13}C NMR (101 MHz, DMSO-d_6): δ = 151.8 (C-2''), 149.9 (C-4''), 148.9 (C-8a''), 133.3 (C-7''), 127.4 (C-8''), 124.0 (C-6''), 123.6 (C-5''), 117.3 (C-4a''), 98.7 (C-3''), 59.4 (C-1), 56.1 (C-2), 52.3 (C-1'), 40.9 (C-2') ppm.

IR (ATR): $\tilde{\nu}$ = 3302, 2803, 1576, 1529, 1334, 810 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{26}Cl_2N_5O$ $[M+H]^+$: 470.1509; found: 470.1508.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1b.

2-(2-(Bis(2-((7-chloroquinolin-4-yl)amino)ethyl)amino)ethyl)isoindoline-1,3-dione (24)



$C_{32}H_{28}Cl_2N_6O_2$

M_w = 599.52 g/mol

Primary alcohol **21** (94 mg, 0.20 mmol, 1.0 eq), phthalimide (44 mg, 0.30 mmol, 1.5 eq), and triphenylphosphine (79 mg, 0.30 mmol, 1.5 eq) were suspended in 1.3 mL THF. Diethyl azodicarboxylate (0.14 mL, 40% in toluene, 0.30 mmol, 1.5 eq) was added dropwise at room temperature, and the mixture was stirred at room temperature for 16 h. Solvents were removed under reduced pressure, and the residue was purified by flash column chromatography (98:1:1 DCM/MeOH/25% NH_3 aq. solution), yielding phthalimide derivative **24** (83 mg, 0.14 mmol, 69%) as a yellow solid.

R_f: 0.67 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 170 – 174 °C (decomposition).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.37 (d, *J* = 5.3 Hz, 2H, 2'''-H), 7.87 (d, *J* = 2.2 Hz, 2H, 8'''-H), 7.65 (d, *J* = 8.9 Hz, 2H, 5'''-H), 7.03 – 7.02 (m, 4H, 4-H and 5-H), 6.54 (dd, *J* = 8.9, 2.2 Hz, 2H, 6'''-H), 6.19 (d, *J* = 5.4 Hz, 2H, 3'''-H), 5.42 (m br, 2H, 2 ArNH), 3.89 – 3.84 (m, 2H, 1'-H), 3.23 (dt, *J* = 7.1, 4.2 Hz, 4H, 2''-H), 3.01 – 2.87 (m, 4H, 1''-H), 2.95 – 2.90 (m, 2H, 2'-H) ppm.

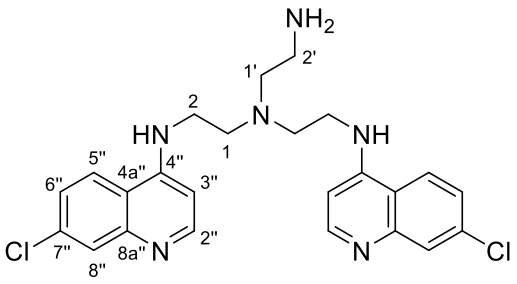
^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.3 (CO), 151.9 (C-2'''), 149.9 (C-4''' or C-8a'''), 149.0 (C-4''' or C-8a'''), 135.1 (C-7'''), 133.5 (C-4 or C-5), 130.4 (C-3a), 128.5 (C-8'''), 125.3 (C-6'''), 122.6 (C-4 or C-5), 122.1 (C-5'''), 117.1 (C-4a'''), 99.4 (C-3'''), 53.7 (C-2'), 51.6 (C-1''), 40.9 (C-2''), 36.2 (C-1') ppm.

IR (ATR): $\tilde{\nu}$ = 3385, 2924, 1701, 1576, 1330, 874, 799, 717 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{32}H_{29}Cl_2N_6O_2$ $[M+H]^+$: 599.1724; found: 599.1722.

Purity (HPLC): >96% (λ = 210 nm), >94% (λ = 254 nm), Method 2a.

N^1-(2-Aminoethyl)-N^2-(7-chloroquinolin-4-yl)-N^1-(2-((7-chloroquinolin-4-yl)amino)ethyl)ethane-1,2-diamine (23)



$C_{24}H_{26}Cl_2N_6$

M_w = 469.42 g/mol

Phthalimide derivative **24** (72 mg, 0.12 mmol, 1.0 eq) was dissolved in 0.8 mL ethanol, and hydrazine monohydrate (18 µL, 79%, 0.30 mmol, 2.5 eq) was added. The mixture was heated to 70 °C for 1 h. The mixture was allowed to cool to room temperature, the solvent removed under reduced pressure. Water (10 mL) was added, and the mixture was acidified (pH = 3) with 2 M HCl aq. solution. The mixture was extracted with EtOAc (10 mL), and the organic extract was discarded. After alkalising the aq. phase (pH = 8) with 2 M NaOH aq. Solution, it was extracted with $CHCl_3$/isopropanol (3:1, 4 x 10 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (94:5:1 DCM/MeOH/25% NH_3 aq. solution), yielding primary amine **23** (32 mg, 0.086 mmol, 57%) as a colourless solid.

R_f: 0.40 (80:19:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 160 °C (decomposition).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.21 (d, J = 5.6 Hz, 2H, 2''-H), 7.68 (d, J = 9.0 Hz, 2H, 5''-H), 7.64 (d, J = 2.2 Hz, 2H, 8''-H), 6.98 (dd, J = 9.0, 2.2 Hz, 2H, 6''-H), 6.36 (d, J = 5.6 Hz, 2H, 3''-H), 3.36 (t, J = 5.9 Hz, 4H, 2-H), 2.89 (dd, J = 6.5, 5.2 Hz, 4H, 1-H), 2.83 (dd, J = 6.6, 5.1 Hz, 2H, 2'-H), 2.75 (dd, J = 7.2, 5.7 Hz, 2H, 1'-H) ppm.

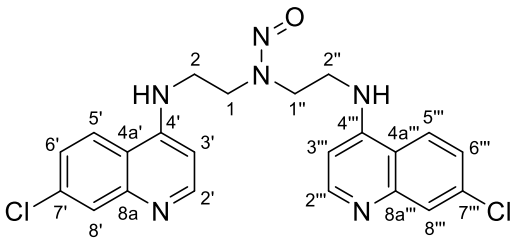
^{13}C NMR (101 MHz, CD_3OD): δ = 152.3 (C-4''), 152.2 (C-8''), 149.3 (C-8a''), 136.2 (C-7''), 127.6 (C-8''), 125.8 (C-6''), 123.6 (C-5''), 118.5 (C-4a''), 99.7 (C-3''), 58.0 (C-1'), 53.6 (C-1), 42.0 (C-2), 40.3 (C-2') ppm.

IR (ATR): $\tilde{\nu}$ = 3348, 3241, 2845, 1576, 1445, 874, 802 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{27}Cl_2N_6$ $[M+H]^+$: 469.1669; found: 469.1672.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1a.

_N_,_N_-Bis(2-((7-chloroquinolin-4-yl)amino)ethyl)nitrosamine (27)



$C_{22}H_{20}Cl_2N_6O$

M_w = 455.35 g/mol

Amine **8** (426 mg, 1.00 mmol, 1.0 eq) was suspended in 20 mL H_2O and the pH adjusted to 4 with 1 M HCl aq. solution. A solution of sodium nitrite (69 mg, 1.0 mmol, 1.0 eq) in 1.5 mL H_2O was added slowly at 0 °C, maintaining pH = 4 using 1 M HCl aq. solution. The mixture was stirred at 70 °C for 0.5 h. After cooling to room temperature, the mixture was basified (pH = 8) with 2 M NaOH aq. Solution, and the resulting suspension was filtered with suction. The solid obtained was washed with water and dried in a desiccator to yield nitrosamine **27** (435 mg, 0.995 mmol, 96%) as a colourless solid.

R_f: 0.43 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 250 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.38 (d, J = 5.4 Hz, 1H, 2'-H or 2'''-H), 8.33 (d, J = 5.4 Hz, 1H, 2'-H or 2'''-H), 8.17 (d, J = 9.0 Hz, 1H, 5'-H or 5'''-H), 8.13 (d, J = 9.0 Hz, 1H, 5'-H or 5'''-H), 7.80 (d, J = 2.2 Hz, 1H, 8'-H or 8'''-H), 7.78 (d, J = 2.2 Hz, 1H, 8'-H or 8'''-H), 7.44 (ddt, J = 8.4, 4.7, 2.3 Hz, 4H, 6'-H, 6'''-H, 2 ArNH), 6.53 (d, J = 5.4 Hz, 1H, 3'-H or 3'''-H), 6.48 (d, J = 5.5 Hz, 1H, 3'-H or 3'''-H), 4.40 (t, J = 6.1 Hz, 2H, 1-H or 1''-H), 2.89 (t, J = 6.5 Hz, 2H, 1-H or 1''-H), 3.69 (t, J = 6.1 Hz, 2H, 2-H or 2''-H), 3.42 (t, J = 6.1 Hz, 2H, 2-H or 2''-H) ppm.

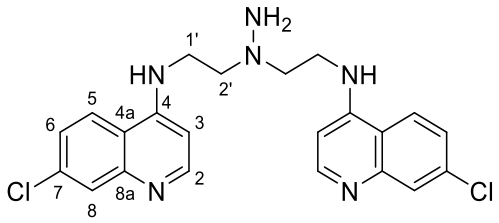
^{13}C NMR (101 MHz, DMSO-d_6): δ = 151.9 (C-2' or C-2'''), 151.8 (C-2' or C-2'''), 149.6 (C-4' or C-4'''), 149.6 (C-4' or C-4'''), 149.1 (C-8a' or C-8a'''), 149.1 (C-8a' or C-8a'''), 133.5 (C-7' or C-7'''), 133.5 (C-7' or C-7'''), 127.6 (C-8' or C-8'''), 127.6 (C-8' or C-8'''), 124.4 (C-6' or C-6'''), 124.3 (C-6' or C-6'''), 123.9 (C-5' and C-5'''), 117.5 (C-4a' or C-4a'''), 117.4 (C-4a' or C-4a'''), 98.8 (C-3' or C-3'''), 98.8 (C-3' or C-3'''), 50.4 (C-1 or C-1''), 42.7 (C-1 or C-1''), 41.0 (C-2 or C-2''), 38.2 (C-2 or C-2'') ppm.

IR (ATR): $\tilde{\nu}$ = 3237, 2934, 1575, 1447, 1367, 1040, 800 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{22}H_{21}Cl_2N_6O$ $[M+H]^+$: 455.1149; found: 455.1147.

Purity (HPLC): >96% (λ = 210 nm), >96% (λ = 254 nm), Method 2a.

_N_,_N_'-(Hydrazine-1,1-diylbis(ethane-2,1-diyl))bis(7-chloroquinolin-4-amine) (25)



$C_{22}H_{22}Cl_2N_6$

M_w = 441.36 g/mol

Nitrosamine **27** (911 mg, 2.00 mmol, 1.0 eq) was suspended in 14 mL anhydrous DCM under nitrogen atmosphere at 0 °C. Diisobutylaluminium hydride (8.0 mL, 1 M in toluene, 8.0 mmol, 4.0 eq) was added dropwise, and the mixture was stirred at room temperature for 1.5 h. The reaction was quenched by dropwise addition of 2 M NaOH aq. solution at 0 °C and was stirred for 20 min. The mixture was extracted with $CHCl_3$/isopropanol (3:1, 4 x 10 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (94:5:1 DCM/MeOH/25% NH_3 aq. solution), yielding hydrazine **25** (650 mg, 1.47 mmol, 74%) as a colourless solid.

R_f: 0.63 (80:19:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 77 – 80 °C (melting), 80 – 83 °C (resolidification), 188 – 190 °C (melting).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.27 (d, J = 5.6 Hz, 2H, 2-H), 7.73 (d, J = 9.0 Hz, 2H, 5-H), 7.70 (d, J = 2.2 Hz, 2H, 8-H), 7.10 (dd, J = 9.0, 2.2 Hz, 2H, 6-H), 6.50 (d, J = 5.7 Hz, 2H, 3-H), 3.52 (t, J = 5.9 Hz, 4H, 1‘-H), 2.97 (t, J = 5.9 Hz, 4H, 2‘-H) ppm.

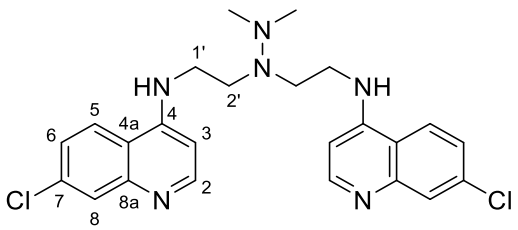
^{13}C NMR (101 MHz, CD_3OD): δ = 152.5 (C-4), 152.3 (C-2), 149.4 (C-8a), 136.3 (C-7), 127.6 (C-8), 125.9 (C-6), 123.7 (C-5), 118.5 (C-4a), 99.7 (C-3), 59.6 (C-2’), 41.7 (C-1’) ppm.

IR (ATR): $\tilde{\nu}$ = 3241, 2924, 1578, 1449, 1331, 803 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{22}H_{23}Cl_2N_6$ $[M+H]^+$: 441.1356; found: 441.1353.

Purity (HPLC): >96% (λ = 210 nm), >97% (λ = 254 nm), Method 1b.

_N_,_N_'-((2,2-Dimethylhydrazine-1,1-diyl)bis(ethane-2,1-diyl))bis(7-chloroquinolin-4-amine) (26)



$C_{24}H_{26}Cl_2N_6$

M_w = 469.42 g/mol

To a solution of hydrazine derivative **25** (88 mg, 0.20 mmol, 1.0 eq) in 0.5 mL methanol, formaldehyde (0.066 mL, 25% aq. solution, 0.60 mmol, 3.0 eq), 0.1 mL acetic acid, and sodium cyanoborohydride (40 mg, 0.60 mmol, 3.0 eq) were added at 0 °C. The mixture was stirred at room temperature for 4 h. Aq. sat. $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 2 x 10 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (98:1:1 DCM/MeOH/25% NH_3 aq. solution), yielding dimethylhydrazine derivative **26** (61 mg, 0.13 mmol, 65%) as a colourless solid.

R_f: 0.67 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 191 – 192 °C.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.25 (d, J = 5.6 Hz, 2H, 2-H), 7.63 (d, J = 2.2 Hz, 2H, 8-H), 7.48 (d, J = 9.0 Hz, 2H, 5-H), 6.98 (dd, J = 9.0, 2.2 Hz, 2H, 6-H), 6.43 (d, J = 5.7 Hz, 2H, 3-H), 3.40 (dd, J = 6.4, 4.9 Hz, 4H, 1'-H), 2.91 (dd, J = 6.4, 4.9 Hz, 4H, 2'-H), 2.46 (s, 6H, $N(CH_3)_2$) ppm.

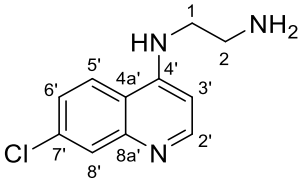
^{13}C NMR (101 MHz, CD_3OD): δ = 152.4 (C-4), 152.2 (C-2), 149.3 (C-8a), 136.2 (C-7), 127.6 (C-8), 125.9 (C-6), 123.1 (C-5), 118.4 (C-4a), 99.7 (C-3), 47.7 (C-2'), 42.1 (C-1'), 40.0 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3372, 3263, 2944, 2849, 1569, 1521, 1448, 1328, 807 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{27}Cl_2N_6$ $[M+H]^+$: 469.1669; found: 469.1666.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1b.

N^1-(7-Chloroquinolin-4-yl)ethane-1,2-diamine (28)



$C_{11}H_{12}ClN_3$

M_w = 221.69 g/mol

Amine **28** was synthesised according to literature[104]. 4,7-Dichloroquinoline (**9**, 396 mg, 2.00 mmol, 1.0 eq) and ethylenediamine (0.669 mL, 10.0 mmol, 5.0 eq) were heated at 80 °C for 1 h and at 140 °C for 2.5 h. After cooling to room temperature, water (7 mL) was added,

the mixture was basified (pH = 10) with 2 M NaOH aq. solution and extracted with $CHCl_3$/isopropanol (3:1, 3 x 10 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (94:5:1 → 93:6:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **28** (313 mg, 1.41 mmol, 71%) as a light yellow solid. ^1H and ^{13}C NMR data are in accordance with the literature[104].

R_f: 0.20 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 134 – 136 °C. [Ref.[104]: 137 – 139 °C.]

^1H NMR (400 MHz, CD_3OD): δ = 8.36 (d, J = 5.6 Hz, 1H, 2'-H), 8.11 (d, J = 9.0 Hz, 1H, 5'-H), 7.78 (d, J = 2.2 Hz, 1H, 8'-H), 7.40 (dd, J = 9.0, 2.2 Hz, 1H, 6'-H), 6.57 (d, J = 5.7 Hz, 1H, 3'-H), 3.45 (t, J = 6.4 Hz, 2H, 1-H), 2.98 (t, J = 6.4 Hz, 2H, 2-H) ppm.

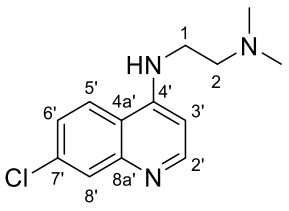
^{13}C NMR (101 MHz, CD_3OD): δ = 152.8 (C-4'), 152.5 (C-2'), 149.7 (C-8a'), 136.4 (C-7'), 127.6 (C-8'), 126.1 (C-6'), 124.3 (C-5'), 118.8 (C-4a'), 99.7 (C-3'), 46.2 (C-1), 40.8 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3355, 3249, 2852, 1742, 1580, 1329, 1142, 951, 800, 758 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{11}H_{13}ClN_3$ $[M+H]^+$: 222.0792; found: 222.0792.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

N^1-(7-Chloroquinolin-4-yl)-N^2,N^2-dimethylethane-1,2-diamine (30)



$C_{13}H_{16}ClN_3$

M_w = 249.74 g/mol

Tertiary amine **30** was synthesised according to literature[105]. 4,7-Dichloroquinoline (**9**, 99 mg, 0.50 mmol, 1.0 eq) and *N*,*N*-dimethylethylenediamine (0.67 mL, 3.5 mmol, 7.0 eq) were heated at 115 °C for 4.5 h. After completion, 2 M NaOH aq. solution (1.5 mL) was added, and the mixture was extracted with DCM (3 x 3 mL). The combined organic layers were dried over Na2SO4, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (99:0.5:0.5 DCM/MeOH/25% NH_3 aq. solution), yielding product **30** (55 mg, 0.22 mmol, 44%) as a colourless solid. ^1H and ^{13}C NMR data are in accordance with the literature[105].

R_f: 0.49 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 120 – 121 °C. [Ref.[154]: 121 – 122.8 °C.]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.52 (d, J = 5.4 Hz, 1H, 2'-H), 7.95 (d, J = 2.1 Hz, 1H, 8'-H), 7.72 (d, J = 8.9 Hz, 1H, 5'-H), 7.36 (dd, J = 8.9, 2.2 Hz, 1H, 6'-H), 6.37 (d, J = 5.4 Hz, 1H, 3'-H), 5.96 (s br, 1H, ArNH), 3.29 (td, J = 6.2, 3.7 Hz, 2H, 1-H), 2.71 – 2.68 (m, 2H, 2-H), 2.31 (s, 6H, $N(CH_3)_2$) ppm.

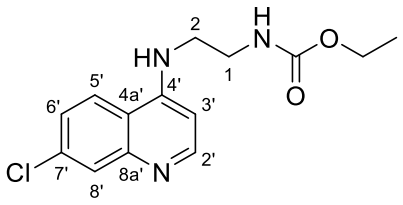
^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.1 (C-2'), 150.1 (C-4' or C-8a'), 149.1 (C-4' or C-8a'), 135.0 (C-7'), 128.7 (C-8'), 125.4 (C-6'), 121.6 (C-5'), 117.4 (C-4a'), 99.3 (C-3'), 57.1 (C-2), 45.2 ($N(CH_3)_2$), 40.0 (C-1) ppm.

IR (ATR): $\tilde{\nu}$ = 3214, 2972, 2764, 1580, 1429, 1137, 802 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{13}H_{17}ClN_3$ $[M+H]^+$: 250.1105; found: 250.1105.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

Ethyl (2-((7-chloroquinolin-4-yl)amino)ethyl)carbamate (31)



$C_{14}H_{16}ClN_3O_2$

M_w = 293.75 g/mol

To a solution of primary amine **28** (295 mg, 1.33 mmol, 1.0 eq) and ethyl chloroformate (0.165 mL, 1.73 mmol, 1.3 eq) in 24 mL DCM, triethylamine (0.241 mL, 1.73 mmol, 1.3 eq) was added dropwise at room temperature. After 1 h, water was added to the reaction mixture and it was extracted with CH_3Cl/isopropanol (3:1, 3 x 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was further purified by flash column chromatography (99:1 DCM/MeOH), yielding carbamate **31** (305 mg, 1.04 mmol, 78%) as a colourless solid.

R_f: 0.8 (78:20:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 172 – 174 °C.

^{1}H NMR (400 MHz, MeOD) δ = 8.36 (d, J = 5.8 Hz, 1H, 2'-H), 8.06 (d, J = 9.0 Hz, 1H, 5'-H), 7.79 (d, J = 2.2 Hz, 1H, 8'-H), 7.43 (dd, J = 9.0, 2.2 Hz, 1H, 6'-H), 6.63 (d, J = 5.8 Hz, 1H, 3'-H), 4.08 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 3.50 (dd, J = 7.5, 5.5 Hz, 2H, 2-H), 3.43 (dd, J = 7.4, 5.6 Hz, 2H, 1-H), 1.21 (t, J = 7.1 Hz, 3H, CH_3) ppm.

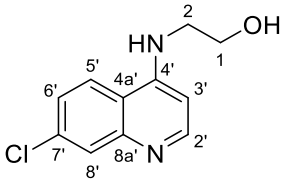
^{13}C NMR (101 MHz, MeOD) δ = 159.8 (NCOO), 153.4 (C-4'), 151.5 (C-2'), 148.6 (C-8a'), 136.9 (C-7'), 126.8 (C-8'), 126.4 (C-6'), 124.4 (C-5'), 118.6 (C-4a'), 99.6 (C-3'), 62.0 ($\underline{C}H_2CH_3$), 44.4 (C-2), 40.2 (C-1), 13.0 (CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3196, 2888, 1574, 1536, 1437, 13336, 1130, 810, 759 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{14}H_{17}ClN_3O_2$ $[M+H]^+$: 294.1004; found: 294.1004.

Purity (HPLC): ND.

2-((7-Chloroquinolin-4-yl)amino)ethan-1-ol (32)



$C_{11}H_{11}ClN_2O$

M_w = 222.67 g/mol

Primary alcohol **32** was synthesised according to literature[104]. A mixture of 4,7-dichloroquinoline (**9**, 990 mg, 5.00 mmol, 1.0 eq), ethanolamine (6.04 mL, 100 mmol, 20 eq), and triethylamine (1.1 mL, 7.5 mmol, 1.5 eq) was stirred under nitrogen atmosphere at 120 °C for 3 h. After cooling to room temperature, cold water (18 mL) was added, and the mixture was cooled in the refrigerator at 5 °C for 1 h. The resulting suspension was filtered, and the precipitate was washed with cold water and cold diethyl ether. After drying in a desiccator overnight, the residue was further purified by flash column chromatography (95:5 DCM/MeOH), yielding product **32** (1.00 g, 4.49 mmol, 90%) as a colourless solid. ^{1}H and ^{13}C NMR data are in accordance with the literature[153].

R_f: 0.77 (80:18:2 DCM/MeOH/25% NH_3 aq. solution).

Mp: 218 – 220 °C. [Ref.[104]: 220 – 222 °C.]

^{1}H NMR (400 MHz, CD_3OD): δ = 8.35 (d, *J* = 5.7 Hz, 1H, 2'-H), 8.09 (d, *J* = 9.0 Hz, 1H, 5'-H), 7.77 (d, *J* = 2.2 Hz, 1H, 8'-H), 7.40 (dd, *J* = 9.0, 2.2 Hz, 1H, 6'-H), 6.57 (d, *J* = 5.7 Hz, 1H, 3'-H), 3.84 (t, *J* = 5.8 Hz, 2H, 1-H), 3.50 (t, *J* = 5.8 Hz, 2H, 2-H) ppm.

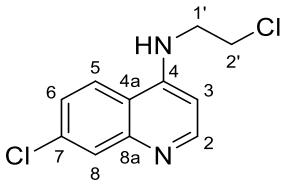
^{13}C NMR (101 MHz, CD_3OD): δ = 152.9 (C-4'), 152.4 (C-2'), 149.7 (C-8a'), 136.4 (C-7'), 127.6 (C-8'), 126.0 (C-6'), 124.3 (C-5'), 118.8 (C-4a'), 99.7 (C-3'), 60.7 (C-1), 46.2 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3307, 2818, 1581, 1538, 1079, 1063, 800, 763 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{11}H_{12}ClN_2O$ $[M+H]^+$: 223.0633; found: 223.0631.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2b.

7-Chloro-*N*-(2-chloroethyl)quinolin-4-amine (33)



$C_{11}H_{10}Cl_2N_2$

M_w = 241.12 g/mol

Alkyl chloride **33** was synthesised according to literature[104]. A mixture of primary alcohol **32** (1.0 g, 4.5 mmol, 1.0 eq), thionyl chloride (6.65 mL, 90.0 mmol, 20 eq), and DMF (80 µL, 1.0 mmol, 0.23 eq) was stirred under nitrogen atmosphere at room temperature for 17 h. The solution was then treated with aq. sat. $NaHCO_3$ solution (15 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure, yielding product **33** (1.02 g, 4.23 mmol, 94%) as a colourless solid.

R_f: 0.74 (90:10 DCM/MeOH).

Mp: 132 – 135 °C. [Ref.[104]: 153 – 154 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.43 (d, *J* = 5.5 Hz, 1H, 2-H), 8.28 (d, *J* = 9.1 Hz, 1H, 5-H), 7.83 (d, *J* = 2.3 Hz, 1H, 8-H), 7.68 (t br, *J* = 5.7 Hz, 1H, NH), 7.50 (dd, *J* = 9.1, 2.2 Hz, 1H, 6-H), 6.61 (d, *J* = 5.5 Hz, 1H, 3-H), 3.86 (t, *J* = 6.2 Hz, 2H, 2'-H), 3.69 (t, *J* = 6.1 Hz, 2H, 1'-H) ppm.

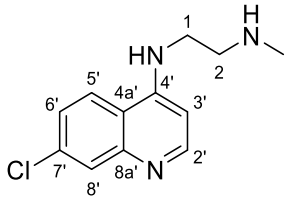
^{13}C NMR (101 MHz, DMSO-d_6): δ = 151.4 (C-2), 150.1 (C-4), 148.4 (C-8a), 133.8 (C-7), 127.1 (C-8), 124.5 (C-6), 124.1 (C-5), 117.3 (C-4a), 98.9 (C-3), 44.1 (C-1'), 42.4 (C-2') ppm.

IR (ATR): $\tilde{\nu}$ = 3203, 2922, 1609, 1576, 1546, 1426, 1248, 1136, 877, 810, 766 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{11}H_{11}Cl_2N_2$ $[M+H]^+$: 241.0294; found: 241.0293.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2b.

N^1-(7-Chloroquinolin-4-yl)-N^2-methylethane-1,2-diamine (29)



$C_{12}H_{14}ClN_3$

M_w = 235.72 g/mol

Secondary amine **29** was synthesised according to literature[104]. A mixture of alkyl chloride **33** (482 mg, 2.00 mmol, 1.0 eq) and methylamine (33% in EtOH, 5.0 mL, 40 mmol, 20 eq) was stirred at 120 °C for 6 h. The mixture was cooled to room temperature and the volatiles were removed under reduced pressure. The crude product was purified by flash column chromatography (92:7:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **29** (130 mg, 0.552 mmol, 28%) as a colourless solid.

R_f: 0.70 (80:18:2 DCM/MeOH/25% NH_3 aq. solution).

Mp: 92 – 94 °C. [Ref.[104]: 110 – 111 °C.]

^{1}H NMR (400 MHz, CD_3OD): δ = 8.37 (d, J = 5.6 Hz, 1H, 2'-H), 8.10 (d, J = 9.0 Hz, 1H, 5'-H), 7.78 (d, J = 2.2 Hz, 1H, 8'-H), 7.40 (dd, J = 9.0, 2.2 Hz, 1H, 6'-H), 6.56 (d, J = 5.6 Hz, 1H, 3'-H), 3.50 (t, J = 6.4 Hz, 2H, 1-H), 2.91 (t, J = 6.4 Hz, 2H, 2-H), 2.45 (s, 3H, CH_3) ppm.

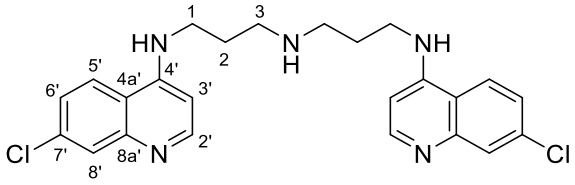
^{13}C NMR (101 MHz, CD_3OD): δ = 152.7 (C-4'), 152.5 (C-2'), 149.7 (C-8a'), 136.4 (C-7'), 127.6 (C-8'), 126.1 (C-6'), 124.4 (C-5'), 118.8 (C-4a'), 99.7 (C-3'), 50.4 (C-2), 43.1 (C-1), 36.0 (CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3264, 1579, 1524, 1475, 1448, 1233, 877, 805, 757 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{12}H_{15}ClN_3$ $[M+H]^+$: 236.0950; found: 236.0947.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

N^1-(7-Chloroquinolin-4-yl)-N^3-(3-((7-chloroquinolin-4-yl)amino)propyl)propane-1,3-diamine (35)



$C_{24}H_{25}Cl_2N_5$

M_w = 454.40 g/mol

Amine **35** was synthesised following General procedure A, using 4,7-dichloroquinoline (**9**, 594 mg, 3.00 mmol, 1.0 eq), triethylamine (0.42 mL, 3.0 mmol, 1.0 eq), and bis(3-aminopropyl)amine (0.214 mL, 1.50 mmol, 0.5 eq). The mixture was stirred for 48 h. The solid residue was further purified by flash column chromatography (95:4:1 → 93:6:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **35** (334 mg, 0.735 mmol, 49%) as colourless solid. ^{1}H and ^{13}C NMR data are in accordance with the literature[98].

R_f: 0.24 (80:19:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 199 – 200 °C. [Ref.[98]: 199 – 201 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.36 (d, J = 5.4 Hz, 2H, 2'-H), 8.21 (d, J = 9.0 Hz, 2H, 5'-H), 7.77 (d, J = 2.2 Hz, 2H, 8'-H), 7.50 (s br, 2H, 2 ArNH), 7.41 (dd, J = 9.0, 2.3 Hz, 2H, 6'-H), 6.45 (d, J = 5.5 Hz, 2H, 3'-H), 3.32 (s br, 4H, 1-H), 2.66 (t, J = 6.6 Hz, 4H, 3-H), 1.82 (p, J = 6.8 Hz, 4H, 2-H) ppm.

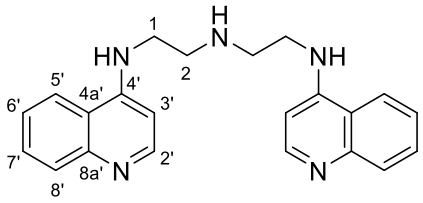
^{13}C NMR (101 MHz, DMSO-d_6): δ = 151.9 (C-2'), 150.1 (C-4'), 149.1 (C-8a'), 133.3 (C-7'), 127.5 (C-8'), 124.0 (C-5' or C-6'), 123.9 (C-5' or C-6'), 117.4 (C-4a'), 98.6 (C-3'), 47.4 (C-3), 41.1 (C-1), 28.0 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3230, 2834, 1576, 1366, 1329, 1137, 802 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{24}H_{26}Cl_2N_5$ $[M+H]^+$: 454.1560; found: 454.1562.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1c.

N^1-(Quinolin-4-yl)-N^2-(2-(quinolin-4-ylamino)ethyl)ethane-1,2-diamine (36)



$C_{22}H_{23}N_5$

M_w = 357.46 g/mol

Amine **36** was synthesised following General procedure A, using 4-bromoquinoline (**37**, 419 mg, 2.00 mmol, 1.0 eq), triethylamine (0.279 mL, 2.00 mmol, 1.0 eq), and diethylenetriamine (0.11 mL, 1.0 mmol, 0.50 eq). The mixture was stirred for 36 h. The solid residue was further purified by flash column chromatography (95:4:1 → 92:7:1

DCM/MeOH/25% NH_3 aq. solution), yielding product **36** (143 mg, 0.399 mmol, 50%) as colourless solid.

R_f: 0.27 (70:29:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 206 – 207 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.37 (d, *J* = 5.4 Hz, 2H, 2'-H), 8.18 (d, *J* = 8.5 Hz, 2H, 5'-H), 7.77 (d, *J* = 8.4 Hz, 2H, 8'-H), 7.59 (t, *J* = 7.7 Hz, 2H, 7'-H), 7.38 (t, *J* = 7.7 Hz, 2H, 6'-H), 7.07 (s br, 2H, 2 ArNH), 6.47 (d, *J* = 5.4 Hz, 2H, 3'-H), 3.37 (s br, 4H, 1-H), 2.91 (t, *J* = 6.5 Hz, 4H, 2-H) ppm.

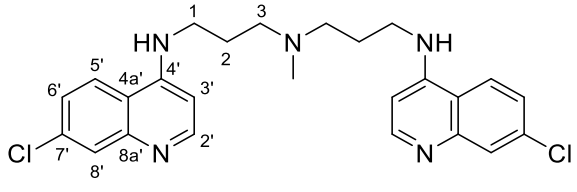
^{13}C NMR (101 MHz, DMSO-d_6): δ = 150.7 (C-2'), 150.0 (C-4'), 148.3 (C-8a'), 129.0 (C-8'), 128.7 (C-7'), 123.7 (C-6'), 121.6 (C-5'), 118.8 (C-4a'), 98.2 (C-3'), 47.2 (C-2), 42.6 (C-1) ppm.

IR (ATR): $\tilde{\nu}$ = 3230, 3061, 1572, 1531, 1334, 1127, 758 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{22}H_{24}N_5$ $[M+H]^+$: 358.2026; found: 358.2023.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

N^1-(7-Chloroquinolin-4-yl)-*N*^{3}-(3-((7-chloroquinolin-4-yl)amino)propyl)-*N*^{3}-methylpropane-1,3-diamine (38)



$C_{25}H_{27}Cl_2N_5$

M_w = 468.43 g/mol

Amine **38** was synthesised following General procedure B from amine **35** (136 mg, 0.300 mmol, 1.0 eq). The mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 10 mL). The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **38** (114 mg, 0.243 mmol, 81%) as a colourless solid. ^{1}H and ^{13}C NMR data are in accordance with the literature[98].

R_f: 0.47 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 136 – 138 °C. [Ref.[98]: 161 – 167 °C.]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.39 (d, *J* = 5.5 Hz, 2H, 2'-H), 7.89 (d, *J* = 2.2 Hz, 2H, 8'-H), 7.61 (d, *J* = 8.9 Hz, 2H, 5'-H), 7.24 (dd, *J* = 9.0, 2.2 Hz, 2H, 6'-H), 6.75 (s br, 2H, 2 ArNH), 6.22

(d, *J* = 5.5 Hz, 2H, 3'-H), 3.36 (q, *J* = 5.7 Hz, 4H, 1-H), 2.66 (t, *J* = 6.4 Hz, 4H, 3-H), 2.44 (s, 3H, NCH_3), 1.98 (t, *J* = 6.6 Hz, 4H, 2-H) ppm.

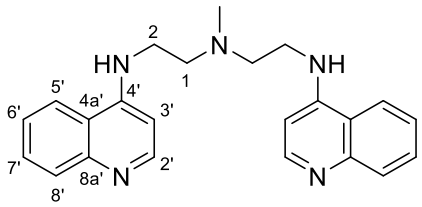
^{13}C NMR (101 MHz, $CDCl_3$): δ = 151.4 (C-2'), 150.5 (C-4'), 148.5 (C-8a'), 135.2 (C-7'), 128.2 (C-8'), 125.4 (C-6'), 121.7 (C-5'), 117.3 (C-4a'), 98.8 (C-3'), 57.0 (C-3), 43.0 (C-3), 42.4 (NCH_3), 25.5 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3231, 2958, 1579, 1578, 1366, 1282, 1137, 864, 844, 802 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{25}H_{28}Cl_2N_5$ $[M+H]^+$: 468.1717; found: 468.1730.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

N^1-Methyl-*N*^{2}-(quinolin-4-yl)-*N*^{1}-(2-(quinolin-4-ylamino)ethyl)ethane-1,2-diamine (39)



$C_{23}H_{25}N_5$

M_w = 371.49 g/mol

Amine **39** was synthesised following General procedure B from amine **36** (182 mg, 0.510 mmol, 1.0 eq). The crude product was purified by flash column chromatography (97:2:1 → 96:3:1 DCM/MeOH/25% NH_3 aq. solution). The product was dissolved in DCM and precipitation occurred upon addition of toluene. The formed precipitate was collected by filtration, washed with toluene, and dried under reduced pressure, yielding product **39** (167 mg, 0.450 mmol, 88%) as colourless waxy solid.

R_f: 0.48 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.53 (d, *J* = 5.3 Hz, 2H, 2'-H), 7.97 – 7.95 (m, 2H, 8'-H), 7.58 – 7.56 (m, 4H, 5'-H and C-7'), 7.06 (ddd, *J* = 8.3, 6.7, 1.3 Hz, 2H, 6'-H), 6.40 (d, *J* = 5.3 Hz, 2H, 3'-H), 5.61 (s br, 2H, 2 ArNH), 3.41 (dt, *J* = 6.5, 4.8 Hz, 4H, 2-H), 2.91 – 2.88 (m, 4H, 1-H), 2.44 (s, 3H, NCH_3) ppm.

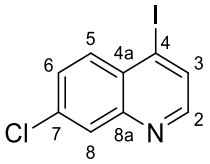
^{13}C NMR (101 MHz, $CDCl_3$): δ = 151.1 (C-2'), 149.7 (C-4'), 148.4 (C-8a'), 130.0 (C-8'), 129.3 (C-7'), 124.9 (C-6'), 119.2 (C-5'), 118.8 (C-4a'), 99.1 (C-3'), 55.8 (C-1), 42.1 (NCH_3), 40.4 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3238, 2951, 1571, 1528, 1335, 809, 728, 694 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{23}H_{26}N_5$ $[M+H]^+$: 372.2183; found: 372.2187.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

7-Chloro-4-iodoquinoline (43)



C_9H_5ClIN

M_w = 289.50 g/mol

Iodoquinoline **43** was synthesised according to literature[109]. To 16 mL HI (57% w/w in H_2O, 10 eq) was added 4,7-dichloroquinoline (**9**, 1.98 g, 10.0 mmol, 1.0 eq) in small portions, till a homogeneous suspension formed. The suspension was heated at 130 °C for 5.5 h. The mixture was cooled to room temperature, and ice-water mixture (25 mL) was added. The mixture was basified with 25% NaOH aq. solution and extracted with DCM (3 x 25 mL). The combined organic layers were washed with NH_4OAc buffer aq. solution (30 mL), 10% $Na_2S_2O_3$ aq. solution (30 mL), and brine (30 mL). The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure to give product **43** (2.67 g, 9.22 mmol, 92%) as colourless solid. ^{1}H and ^{13}C NMR data are in accordance with the literature[109].

R_f: 0.43 (DCM).

Mp: 122 – 123 °C. [Ref.[109]: 120 – 122 °C.]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.44 (d, J = 4.7 Hz, 1H, 2-H), 8.06 (d, J = 2.1 Hz, 1H, 8-H), 7.99 – 7.97 (m, 2H, 3-H and 5-H), 7.56 (dd, J = 9.0, 2.0 Hz, 1H, 6-H) ppm.

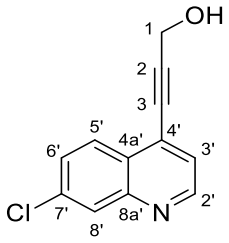
^{13}C NMR (101 MHz, $CDCl_3$): δ = 150.8 (C-2), 148.3 (C-8a), 136.7 (C-7), 133.3 (C-3 or C-5), 132.9 (C-3 or C-5), 129.3 (C-6), 129.2 (C-4), 129.0 (C-8), 111.7 (C-4a) ppm.

IR (ATR): $\tilde{\nu}$ = 1548, 1480, 1287, 1075, 873, 828, 798 cm^{-1}.

HRMS (ESI): m/z = calculated for C_9H_6ClIN $[M+H]^+$: 289.9227; found: 289.9227.

Purity (HPLC): >94% (λ = 210 nm), >97% (λ = 254 nm), Method 3.

3-(7-Chloroquinolin-4-yl)prop-2-yn-1-ol (44)



$C_{12}H_8ClNO$

M_w = 217.65 g/mol

To a suspension of 7-chloro-4-iodoquinoline (**43**, 1.64 g, 5.10 mmol, 1.0 eq), $Pd(PPh_3)_2Cl_2$ (179 mg, 0.225 mmol, 0.05 eq), and CuI (48 mg, 0.25 mmol, 0.05 eq) in 6.6 mL DMF under nitrogen atmosphere, propargyl alcohol (0.445 mL, 7.65 mmol, 1.5 eq) and triethylamine (2.84 mL, 20.4 mmol, 4.0 eq) were added. The mixture was stirred at room temperature for 1 h. Aq. sat. $NaHCO_3$ solution (35 mL) was added, and the mixture was extracted with DCM (3 x 70 mL). The combined organic layers were washed with brine (70 mL), dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (1:1 EtOAc/isohexanes), yielding alkyne **44** (1.11 g, 5.10 mmol, quant.) as colourless solid.

R_f: 0.57 (9:1 DCM/MeOH).

Mp: 150 – 152 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.84 (d, J = 4.5 Hz, 1H, 2'-H), 8.13 – 8.11 (m, 2H, 5'-H and 8'-H), 7.50 (dd, J = 8.8, 2.2 Hz, 1H, 6'-H), 7.43 (d, J = 4.5 Hz, 1H, 3'-H), 4.68 (s, 2H, 2-H), 2.87 (s br, 1H, OH) ppm.

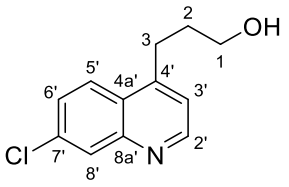
^{13}C NMR (101 MHz, $CDCl_3$): δ = 150.7 (C-2'), 148.3 (C-8a'), 136.2 (C-7'), 129.6 (C-4'), 128.7 (C-5'), 128.5 (C-6'), 127.4 (C-8'), 126.3 (C-4a'), 124.0 (C-3'), 97.9 (C-2), 80.9 (C-3), 51.6 (C-1) ppm.

IR (ATR): $\tilde{\nu}$ = 2924, 2358, 1741, 1577, 1355, 1029, 874, 825 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_9ClNO$ $[M+H]^+$: 218.0367; found: 218.0366.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2b.

3-(7-Chloroquinolin-4-yl)propan-1-ol (41)



$C_{12}H_{12}ClNO$

M_w = 221.69 g/mol

Alkyne **44** (1.09 g, 5.00 mmol, 1.0 eq) was dissolved in 66 mL EtOAc and 3.3 mL MeOH. The solution was hydrogenated over palladium on charcoal (10 wt%, 532 mg, 0.500 mmol, 10 mol%) at atmospheric pressure for 6 h. The catalyst was removed by filtration over a celite pad. The solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (97:3 DCM/MeOH), yielding alcohol **41** (777 mg, 3.50 mmol, 70%) as a light yellow oil. ^{1}H and ^{13}C NMR data are in accordance with the literature[155].

R_f: 0.58 (9:1 DCM/MeOH).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.79 (d, *J* = 4.5 Hz, 1H, 2'-H), 8.10 (d, *J* = 2.2 Hz, 1H, 8'-H), 8.01 (d, *J* = 9.0 Hz, 1H, 5'-H), 7.50 (dd, *J* = 9.0, 2.2 Hz, 1H, 6'-H), 7.26 (d, *J* = 4.8 Hz, 1H, 3'-H), 3.76 (t, *J* = 6.1 Hz, 2H, 1-H), 3.20 – 3.16 (m, 2H, 3-H), 2.05 – 1.98 (m, 2H, 3-H), 1.85 (s br, 1H, OH) ppm.

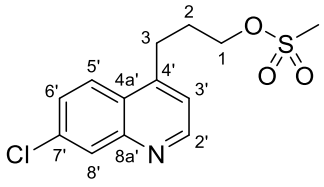
^{13}C NMR (101 MHz, $CDCl_3$): δ = 151.3 (C-2'), 148.9 (C-8a'), 148.4 (C-4'), 135.2 (C-7'), 129.2 (C-8'), 127.5 (C-6'), 126.2 (C-4a'), 125.2 (C-5'), 121.2 (C-3'), 62.0 (C-1), 32.9 (C-2), 28.4 (C-3) ppm.

IR (ATR): $\tilde{\nu}$ = 3260, 2938, 2871, 1588, 1573, 1423, 1059, 1035, 879, 836, 821 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{13}ClNO$ $[M+H]^+$: 222.0681; found: 222.0678.

Purity (HPLC): >95% (λ = 210 nm), >96% (λ = 254 nm), Method 2b.

3-(7-Chloroquinolin-4-yl)propyl methanesulfonate (49)



$C_{13}H_{14}ClNO_2S$

M_w = 299.04 g/mol

Alcohol **41** (266 mg, 1.20 mmol, 1.0 eq) and triethylamine (0.50 mL, 3.6 mmol, 3.0 eq) were dissolved in 8 mL anhydrous THF under nitrogen atmosphere at 0 °C, and methanesulfonyl chloride (0.30 mL, 3.6 mmol, 3.0 eq) was added dropwise. The resulting solution was stirred at room temperature for 0.5 h, to be then quenched with aq. sat. $NaHCO_3$ solution (6.5 mL). The mixture was extracted with DCM (3 x 7 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (99:2 DCM/MeOH), yielding methanesulfonate **49** (363 mg, 1.20 mmol, quant.) as a brown oil.

R_f: 0.88 (90:10 DCM/MeOH).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.83 (d, J = 4.5 Hz, 1H, 2'-H), 8.15 (d, J = 2.2 Hz, 1H, 8'-H), 7.96 (d, J = 9.0 Hz, 1H, 5'-H), 7.55 (dd, J = 9.0, 2.2 Hz, 1H, 6'-H), 7.28 (d, J = 4.6 Hz, 1H, 3'-H), 4.32 (t, J = 6.0 Hz, 2H, 1-H), 3.25 – 3.22 (m, 2H, 3-H), 3.04 (s, 3H, CH_3), 2.23 – 2.20 (m, 2H, 2-H) ppm.

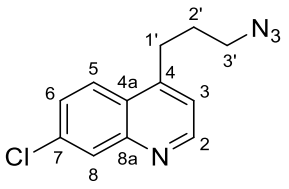
^{13}C NMR (101 MHz, CD_3OD): δ = 151.1 (C-2), 148.6 (C-8a), 147.2 (C-4), 135.6 (C-7), 129.1 (C-8), 128.0 (C-6), 125.8 (C-4a), 124.8 (C-5), 121.3 (C-3), 68.8 (C-3'), 37.7 (CH_3), 29.5 (C-2'), 28.1 (C-1') ppm.

IR (ATR): $\tilde{\nu}$ = 3425, 1604, 1590, 1345, 1167, 1038, 925, 833, 771 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{13}H_{15}ClNO_2S$ $[M+H]^+$: 300.0456; found: 300.0454.

Purity (HPLC): ND.

4-(3-Azidopropyl)-7-chloroquinoline (50)



$C_{12}H_{11}ClN_4$

M_w = 246.70 g/mol

To a solution of methanesulfonate **49** (360 mg, 1.20 mmol, 1.0 eq) in 9 mL DMF was added sodium azide (156 mg, 2.40 mmol, 2.0 eq) and the mixture was stirred at 70 °C for 3.5 h. After cooling to room temperature, the mixture was quenched with water (10 mL) and extracted with EtOAc (2 x 15 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (1:1 EtOAc/isohexanes), yielding azide derivative **50** (280 mg, 1.13 mmol, 93%) as a light yellow waxy solid.

R_f: 0.74 (90:10 DCM/MeOH).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.83 (d, *J* = 4.5 Hz, 1H, 2-H), 8.21 (d, *J* = 9.0 Hz, 1H, 5-H), 8.07 (d, *J* = 2.2 Hz, 1H, 8-H), 7.67 (dd, *J* = 9.0, 2.2 Hz, 1H, 6-H), 7.43 (d, *J* = 4.4 Hz, 1H, 3-H), 3.46 (t, *J* = 6.8 Hz, 2H, 3'-H), 3.16 – 3.14 (m, 2H, 1'-H), 1.95 – 1.91 (m, 2H, 2'-H) ppm.

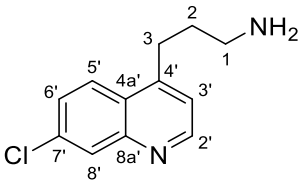
^{13}C NMR (101 MHz, DMSO-d_6): δ = 151.6 (C-2), 148.3 (C-8a), 147.5 (C-4), 133.8 (C-7), 128.8 (C-8), 127.0 (C-6), 126.2 (C-5), 125.6 (C-4a), 121.5 (C-3), 50.2 (C-3'), 28.8 (C-2'), 28.2 (C-1') ppm.

IR (ATR): $\tilde{\nu}$ = 2932, 2092, 1606, 1590, 1422, 1241, 882, 834 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{12}ClNO$ $[M+H]^+$: 247.0745; found: 247.0745.

Purity (HPLC): >97% (λ = 210 nm), >95% (λ = 254 nm), Method 2b.

3-(7-Chloroquinolin-4-yl)propan-1-amine (48)



$C_{12}H_{13}ClN_2$

M_w = 220.70 g/mol

The azide **50** (173 mg, 0.700 mmol, 1.0 eq) was taken up in 7 mL THF/H_2O (10:1), and triphenylphosphine (223 mg, 0.840 mmol, 1.2 eq) was added to the mixture, which was stirred at room temperature for 16 h. The solvents were removed under reduced pressure, and the residue was purified by flash column chromatography (92:7:1 DCM/MeOH/25% NH_3 aq. solution) to give primary amine **48** (126 mg, 0.571 mmol, 82%) as a brown oil.

R_f: 0.55 (80:18:2 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.75 (d, *J* = 4.5 Hz, 1H, 2'-H), 8.20 (d, *J* = 9.1 Hz, 1H, 5'-H), 8.01 (d, *J* = 2.2 Hz, 1H, 8'-H), 7.61 (dd, *J* = 9.1, 2.2 Hz, 1H, 6'-H), 7.43 (d, *J* = 4.6 Hz, 1H, 3'-H), 3.19 – 3.16 (m, 2H, 3-H), 2.77 (dd, *J* = 7.8, 6.5 Hz, 2H, 1-H), 1.92 – 1.88 (m, 2H, 2-H) ppm.

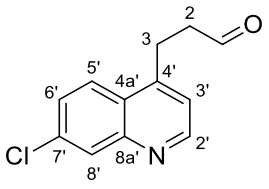
^{13}C NMR (101 MHz, CD_3OD): δ = 152.3 (C-2'), 151.5 (C-4'), 149.3 (C-8a'), 136.6 (C-7'), 128.6 (C-6' and C-8'), 127.4 (C-4a'), 127.1 (C-5'), 122.5 (C-3'), 42.3 (C-1), 34.2 (C-2), 30.4 (C-3) ppm.

IR (ATR): $\tilde{\nu}$ = 3281, 2932, 2867, 1588, 1567, 1498, 1307, 1095, 881, 834, 771 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{14}ClN_2$ $[M+H]^+$: 221.0841; found: 221.0840.

Purity (HPLC): >96% (λ = 210 nm), >96% (λ = 254 nm), Method 2b.

3-(7-Chloroquinolin-4-yl)propanal (47)



$C_{12}H_{10}ClNO$

M_w = 219.67 g/mol

To a solution of alcohol **41** (776 mg, 3.50 mmol, 1.0 eq) in 5 mL DCM at 0 °C was added 1,1-dihydro-1,1,1-triacetoxy-1,2-benziodoxol-3(1*H*)-one (Dess-Martin periodinane, 1.63 g, 3.85 mmol, 1.1 eq). The mixture was stirred at room temperature for 1 h. Few drops of water were added, and the resulting suspension was filtered on a hydrophobic filter paper. The solvent was removed under reduced pressure, and the crude residue purified by flash column chromatography (97:3 DCM/MeOH), yielding aldehyde **47** (584 mg, 2.66 mmol, 76%) as a light yellow oil.

R_f: 0.67 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, CD_3OD): δ = 9.88 (s, 1H, CHO), 8.81 (d, *J* = 4.5 Hz, 1H, 2'-H), 8.16 (d, *J* = 2.2 Hz, 1H, 8'-H), 7.95 (d, *J* = 9.0 Hz, 1H, 5'-H), 7.55 (dd, *J* = 9.0, 2.2 Hz, 1H, 6'-H), 7.27 (d, *J* = 4.6 Hz, 1H, 3'-H), 3.40 (t, *J* = 7.5 Hz, 2H, 3-H), 2.96 (td, *J* = 7.5, 1.0 Hz, 2H, 2-H) ppm.

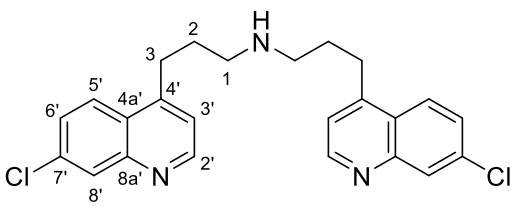
^{13}C NMR (101 MHz, CD_3OD): δ = 199.8 (CHO), 150.7 (C-2'), 148.2 (C-8a'), 147.3 (C-4'), 135.6 (C-7'), 128.8 (C-8'), 128.0 (C-6'), 125.7 (C-4a'), 124.6 (C-5'), 120.9 (C-3'), 43.4 (C-2), 24.0 (C-3) ppm.

IR (ATR): $\tilde{\nu}$ = 3189, 2930, 1722, 1589, 1499, 1116, 1064, 881, 837, 824, 744 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{11}ClNO$ $[M+H]^+$: 220.0524; found: 220.0522.

Purity (HPLC): >88% (λ = 210 nm), >87% (λ = 254 nm), Method 2b.

Bis(3-(7-chloroquinolin-4-yl)propyl)amine (40)



$C_{24}H_{23}Cl_2N_3$

M_w = 424.37 g/mol

Amine **40** was synthesised following General procedure C, using primary amine **48** (265 mg, 1.20 mmol, 1.0 eq) and aldehyde **47** (527 mg, 2.40 mmol, 2 eq). The mixture was stirred at room temperature for 22 h. Aq. sat. $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 10 mL). The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **40** (117 mg, 0.276 mmol, 23%) as a colourless solid.

R_f: 0.53 (90:9:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 70 – 73 °C.

^{1}H NMR (400 MHz, CD_3OD): δ = 8.74 (d, J = 4.6 Hz, 2H, 2'-H), 8.19 (d, J = 9.1 Hz, 2H, 5'-H), 8.01 (d, J = 2.2 Hz, 2H, 8'-H), 7.60 (dd, J = 9.0, 2.2 Hz, 2H, 6'-H), 7.42 (d, J = 4.6 Hz, 2H, 3'-H), 3.18 – 3.15 (m, 4H, 3-H), 2.72 (t, J = 7.4 Hz, 4H, 1-H), 1.96 (p, 4H, 2-H) ppm.

^{13}C NMR (101 MHz, CD_3OD): δ = 152.3 (C-2'), 151.3 (C-4'), 149.3 (C-8a'), 136.6 (C-7'), 128.7 (C-6' or C-8'), 128.6 (C-6' or C-8'), 127.4 (C-4a'), 127.1 (C-5'), 122.5 (C-3'), 50.2 (C-1), 31.0 (C-2), 30.7 (C-3) ppm.

IR (ATR): $\tilde{\nu}$ = 2931, 2806, 1588, 1498, 1093, 1072, 869, 840, 765 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{24}Cl_2N_3$ $[M+H]^+$: 424.1342; found: 424.1343.

Purity (HPLC): >98% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

5.7.2 Synthesis of SARS-CoV-2 RNA ligands

Compounds **52** – **97** were synthesised by the technicians Karl Sauvageot-Witzku and Anja Rausch and the undergraduate student Korbinian Lohr under my supervision. Compounds **207** – **247** were synthesised during the master's thesis of the Erasmus student Ludovica Bellino under my supervision.

HPLC analytical measurements for potential ligand of 5_SL1 were carried out using the following Methods:

Method 1: Xbridge Phenyl, 3.5 µm (4.6 x 150mm), 35 °C, 0.8 mL/min flow rate

a) 10 µL injection volume, MeOH/phosphate buffer pH 9.0 30:70 → 70:30

b) 5.0 µL injection volume, MeOH/phosphate buffer pH 9.0 20:80 → 90:10

c) 10 µL injection volume, MeOH/phosphate buffer pH 9.0 70:30

d) 10 µL injection volume, MeOH/phosphate buffer pH 9.0 80:20

e) 10 µL injection volume, MeOH/water 65:35

Method 2: Zorbax Eclipse Plus, C18 5 µm (4.6 x 150 mm), 10 µL injection volume

a) 50 °C, 1.5 mL/min flow rate, MeOH/water 70:30

b) 35 °C, 1.2 mL/min flow rate, MeOH/water 65:35

Method 3: Xbridge Phenyl, 3.5 µm (4.6 x 150mm), 35 °C

a) 2.0 µL injection volume, 0.6 mL/min flow rate, ACN/0.1% TFA in water 30:70

b) 5.0 µL injection volume, 0.8 mL/min flow rate, ACN/0.1% TFA in water 15:85

c) 2.0 µL injection volume, 0.6 mL/min flow rate, MeOH/0.1% TFA in water 20:80 → 80:20

d) 10 µL injection volume, 0.8 mL/min flow rate, MeOH/0.1% TFA in water 70:30

e) 3.0 µL injection volume, 0.6 mL/min flow rate, MeOH/0.1% TFA in water 65:35

f) 10 µL injection volume, 0.8 mL/min flow rate, MeOH/0.1% TFA in water 80:20

Method 4: Zorbax Eclipse Plus, C18 5 µm (4.6 x 150 mm), 10 µL injection volume, 50 °C, 1.5 mL/min flow rate, ACN/ion pair reagent pH 3.5 5:95 → 0:100

5.7.2.1 General procedures

General procedure A: *N*-Methylthiourea synthesis

The respective amine (1.0 eq), methyl isothiocyanate (2.0 eq), and triethylamine (2.0 eq) were dissolved in toluene (2.5 mL/mmol amine), and the solution was stirred at 90 °C for 3 – 18 h. The mixture was cooled to room temperature. The formed precipitate was filtered off, washed with DCM, and discarded. The filtrate was evaporated to dryness to give the respective products.

General procedure B: *N*-Methylurea synthesis

Methylaminoformyl chloride (1.0 eq) was added portion wise at 5 °C to pyridine (0.3 mL/mmol methylaminoformyl chloride) under nitrogen atmosphere. After portion wise addition of the respective amine (1.0 eq), the mixture was stirred at 50 °C for 2 h. After cooling to room temperature, water was slowly added to the mixture, which was then diluted with DCM. The precipitate was collected by filtration with suction and washed with DCM and water. The solid was dried under reduced pressure to give the final product.

General procedure C: *O*-Demethylation of methoxybenzothiazoles

Boron tribromide (11 eq) was added dropwise to a suspension of the corresponding methoxybenzothiazole (1.0 eq) in DCM (15 mL/mmol methoxybenzothiazole) at 0 °C. The mixture was allowed to warm up to room temperature and stirred for 16 h. The mixture was then cooled to 0 °C and quenched with methanol (2.5 mL/mmol benzothiazole). The mixture was neutralised through the addition of aq. sat. $NaHCO_3$ solution. The formed precipitate was filtered off and discarded. The aqueous and organic phases of the filtrate were separated. The aq. phase was extracted with EtOAc (3 x 50 mL). All organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was treated with DCM, the formed precipitate was collected, washed with DCM, and dried under reduced pressure. If required, the product was further purified by flash column chromatography.

General procedure D: *N*-Acetylation of 2-aminobenzothiazoles

The corresponding 2-aminobenzothiazole (1.0 eq) was dissolved in DCM (9.0 mL/mmol 2-aminobenzothiazole) under nitrogen atmosphere. Subsequently, triethylamine (2.0 eq) and Ac_2O (1.5 eq) were added dropwise at 0 °C. The mixture was stirred at room temperature for 18 h. Water was added, and the mixture was extracted with DCM (3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by recrystallisation or flash column chromatography.

General procedure E: Amide synthesis from 2-aminobenzothiazoles

The corresponding 2-aminobenzothiazole (1.0 eq) was dissolved in DCM (9.0 mL/mmol 2-aminobenzothiazole) or THF (4.5 mL/mmol 2-aminobenzothiazole) under nitrogen atmosphere. Subsequently, triethylamine (1.1 eq) and the corresponding acyl chloride (1.1 eq) were added dropwise at 0 °C. The mixture was stirred at room temperature for 1 – 72 h. The solvent was removed under reduced pressure, and the residue taken up in water. The precipitate of the resulting suspension was filtered off, washed with water, and the filtrate discarded. If the filtration from water was inconvenient, the aq. suspension was extracted with EtOAc (3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by crystallisation from EtOH or flash column chromatography.

General procedure F: Nucleophilic substitution of chloroacetamides with amines

The corresponding chloroacetamide (1.0 eq) was dissolved in DMF (1.4 mL/mmol chloroacetamide). The corresponding amine (2.2 eq) or the corresponding amine (1.1 eq) and triethylamine (1.1 eq), with respect to the availability of the amines, were added to the solution at room temperature, and the mixture was stirred for 1 – 24 h. For amines, whose synthesis was time consuming, were used 1.0 eq of amine and 1.1 eq of the corresponding chloroacetamide. The reaction was quenched by addition of water, and the mixture was

extracted with a suitable organic solvent (3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by filtration, crystallisation, or flash column chromatography.

General procedure G: Reduction of nitroarenes to primary amines

To a solution of the corresponding nitroarene (1.0 eq) in MeOH (50 mL/mmol nitroarene) was added palladium on charcoal (0.05 eq, 10 wt%) and one drop of AcOH. The mixture was hydrogenated at atmospheric pressure for 1 – 3 h. Then, the catalyst was removed by filtration through a celite pad and the pad washed with methanol. The filtrate was evaporated under reduced pressure, and the residue was further purified by flash column chromatography.

General procedure H: *N*-Boc deprotection with TFA

The corresponding *tert*-butyl carbamate (1.0 eq) was dissolved in DCM (10 mL/mmol carbamate) and TFA (10 mL/mmol carbamate) was added at 0 °C. The mixture was stirred for 1 h and then concentrated under reduced pressure. The excess of TFA was neutralised with aq. sat. $NaHCO_3$ solution, and the mixture was extracted with EtOAc (3 x), DCM (3 x), or $CHCl_3$/isopropanol (3:1, 3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. If required, the crude product was purified by flash column chromatography.

General procedure I: Reductive alkylation of primary amines with $NaBH_3CN$

Amine **148** or **149** (160 mg, 0.500 mmol, 1.0 eq) and 3-hydroxybenzaldehyde (123 mg, 1.00 mmol, 2.0 eq) were suspended in 2 mL MeOH, and the suspension was stirred at room temperature under nitrogen atmosphere. After 1 h stirring, $NaBH_3CN$ (66 mg, 1.0 mml, 2.0 eq) was added at 0 °C, and the mixture was stirred for 1 h. Aq. sat. $NaHCO_3$ solution (20 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 20 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/25% NH_3 aq. solution).

General procedure J: *N*-Alkylation of aminophenols with alkyl iodides

The corresponding alkyl iodide (2.0 eq) was added to a suspension of 3-((2,2-diethoxyethyl)amino)phenol (1.0 eq) and Na_2CO_3 (2.0 eq) in ACN (10 mL/mmol aminophenol), and the mixture was stirred at 80 °C for 4 h. Water was added, and the mixture was extracted with EtOAc (3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (1:4 EtOAc/isohexanes).

General procedure K: Nosyl protection of phenols and alcohols

The corresponding phenol (1.0 eq) or alcohol (1.0 eq) was dissolved in ACN (2.3 mL/mmol phenol). 2-Nosyl chloride (1.5 eq) and triethylamine (2.0 eq) were added at 0 °C, and the mixture was stirred for 1 h. The reaction was quenched with HCl (2 M aq. solution), the mixture was diluted with water and extracted with DCM (3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography.

General procedure L: Acetal deprotection

The corresponding acetal (1.0 eq) was dissolved in 1,4-dioxane (5.0 mL/mmol acetal) and HCl (3.0 eq, 37% aq. solution) was added. The mixture was stirred at 40 °C for 1 h. The mixture was cooled to 0 °C, neutralised with aq. sat. $NaHCO_3$ solution, and the mixture was extracted with EtOAc (3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 EtOAc/isohexanes).

General procedure M: Reductive alkylation of primary amines with $NaBH(OAc)_3$

The corresponding aldehyde (1.0 eq), the corresponding primary amine (1.0 eq) and 3 Å molecular sieve (0.05 g/10 mL solvent) were suspended in anhydrous 1,2-dichloroethane (20 mL/mmol aldehyde) under nitrogen atmosphere. $NaBH(OAc)_3$ (1.4 eq) was added, and the mixture was stirred at room temperature for 3 h. After filtration on celite and washing with $CHCl_3$/isopropanol (3:1, 3 x), the filtrate was washed with NaOH (1 M aq. solution). The separated aq. phase was extracted with $CHCl_3$/isopropanol (3:1, 3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (97:2:1 DCM/MeOH/25% NH_3 aq. solution).

General procedure N: Nosyl deprotection

The corresponding *O*-nosyl-phenol (1.0 eq) and Cs_2CO_3 (1.5 eq) were dissolved in DMF (10 mL/mmol nosyl-phenol) under nitrogen atmosphere, and benzenethiol (2.5 eq) was added. The mixture was stirred at room temperature for 1 h. Water was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/25% NH_3 aq. solution).

General procedure O: *O*-Tosylation of alcohols and phenols

The corresponding phenol (1.0 eq) was dissolved in DCM (1.5 mL/mmol phenol) under nitrogen atmosphere. *p*-Toluenesulfonyl chloride (3.0 eq) and triethylamine (3.0 eq) were added at 0 °C. The mixture was stirred at room temperature for 1 h. Ice-water was added, and

the mixture was extracted with DCM (3 x). The organic layers were combined and washed with 2 M HCl aq. solution (2 x). The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography.

General procedure P: *N*-Alkylation of aromatic amines with alkyl bromides

The respective alkyl bromide (1.1 – 3.0 eq) was added to a solution of the corresponding aromatic amine (1.0 eq) and $NaHCO_3$ (1.1 – 3.0 eq) in DMF (1.25 mL/mmol aromatic amine). The reaction mixture was stirred at 100 – 120 °C for 4 – 19 h. After cooling to room temperature, the reaction was quenched by addition of water, and the mixture was extracted with a suitable organic solvent (3 x). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography.

General procedure Q: *N*-Boc deprotection with HCl in dioxane

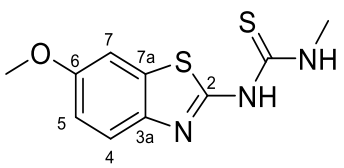
The corresponding *tert*-butyl carbamate (1.0 eq) was suspended in 4 N HCl in dioxane (20 eq). DCM (6 mL/mmol carbamate) was added, and the mixture was stirred at room temperature for 4 – 16 h. The reaction mixture was neutralised with saturated $NaHCO_3$ aq. solution and extracted with chloroform $CHCl_3$/isopropanol (3:1, 3 x). Concentration of the organic layer under reduced pressure was followed by purification by flash column chromatography (88:10:2 → 76:20:4 DCM/MeOH/25% NH_3 aq. solution).

General procedure R: Reduction of *tert*-butyl carbamates to *N*-methyl amines with lithium aluminium hydride

A solution of the corresponding *tert*-butyl carbamate (1.0 eq) in THF (5 mL/mmol carbamate) was added to a suspension $LiAlH_4$ (6.0 eq) in THF (9 mL/mmol $LiAlH_4$) at 0 °C under nitrogen atmosphere. The reaction mixture was left stirring for at 55 °C 16 h. After cooling to 0 °C, the mixture was quenched with water and then filtered. The filtrate was extracted with EtOAc (3 x) or $CHCl_3$/isopropanol (3:1, 3 x). Concentration of the organic layer under reduced pressure was followed by purification by flash column chromatography.

5.7.2.2 Synthetic procedures

1-(6-Methoxybenzo[*d*]thiazol-2-yl)-3-methylthiourea (54)



$C_{10}H_{11}N_3OS_2$

M_w = 253.34 g/mol

Thiourea **54** was prepared following General procedure A, using 2-amino-6-methoxybenzothiazole (**55**, 251 mg, 1.35 mmol, 1.0 eq). The mixture was stirred for 18 h to give thiourea **54** (65 mg, 0.26 mmol, 19%) as an off-white solid.

Mp: 213 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.76 (s, 1H, CSNH), 9.93 (s, 1H, CSNH), 7.57 (d, *J* = 8.5 Hz, 1H, 4-H), 7.51 (d, *J* = 2.6 Hz, 1H, 7-H), 7.00 (dd, *J* = 8.8, 2.6 Hz, 1H, 5-H), 3.79 (s, 3H, OCH_3), 3.07 (d, *J* = 4.4 Hz, 3H, NCH_3) ppm.

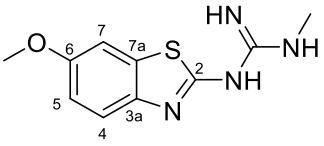
^{13}C NMR (101 MHz, DMSO-d_6): δ = 179.4 (CS), 156.1 (C-6), 142.7 (C-3a or C-7a), 131.0 (C-2, C-3a or C-7a), 130.9 (C-2, C-3a or C-7a), 120.0 (C-4), 114.5 (C-5), 105.3 (C-7), 55.7 (OCH_3), 31.5 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3178, 1558, 1531, 1471, 1435, 1213, 1063, 1026, 857, 823, 739, 677 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{10}H_{10}N_3OS_2$ $[M-H]^-$: 252.0270; found: 252.0273.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2a.

1-(6-Methoxybenzo[*d*]thiazol-2-yl)-3-methylguanidine (52)



$C_{10}H_{12}N_4OS$

M_w = 236.29 g/mol

In a closed vial were added thiourea **54** (200 mg, 0.789 mmol, 1.0 eq), PbO (405 mg, 1.82 mmol), and NH_3 (1.6 mL, 7 N in MeOH, 14 eq). The mixture was heated to 100 °C for 4 h. After cooling to room temperature, 2 mL EtOH were added, the mixture was heated to 60 °C, and the precipitate was collected by filtration from the warm mixture. The precipitate was then washed with diethyl ether to give guanidine derivative **52** (65 mg, 0.28 mmol, 35%) as colourless solid.

Mp: 197 – 198 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.58 (s, 3H, 3 NH), 7.35 (d, *J* = 8.8 Hz, 1H, 4-H), 7.28 (d, *J* = 2.6 Hz, 1H, 7-H), 6.84 (dd, *J* = 8.8, 2.7 Hz, 1H, 5-H), 3.74 (s, 3H, OCH_3), 2.76 (d, *J* = 4.8 Hz, 3H, NCH_3) ppm.

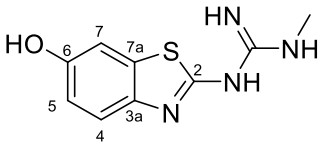
^{13}C NMR (101 MHz, DMSO-d_6): δ = 172.2 (CN), 154.8 (C-6), 146.0 (C-3a), 131.3 (C-7a), 118.8 (C-4), 113.0 (C-5), 105.0 (C-7), 55.5 (OCH_3), 27.6 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3120, 1615, 1584, 1451, 1435, 1397, 1220, 1027, 828, 820, 781, 742 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{10}H_{11}N_2O_2S$ $[M-H]^-$: 235.0659; found: 235.0659.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

1-(6-Hydroxybenzo[*d*]thiazol-2-yl)-3-methylguanidine (53)



$C_9H_{10}N_4OS$

M_w = 222.28 g/mol

6-Hydroxybenzothiazole **53** was prepared following General procedure C from 6-methoxybenzothiazole **52** (200 mg, 0.846 mmol, 1.0 eq). The crude product was treated with DCM/diethyl ether (2:1, 20 mL), and the insoluble solid was filtered off, washed with diethyl ether, and dried under reduced pressure to give **53** (148 mg, 0.666 mmol, 79%) as an off-white solid.

Mp: 232 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.18 (s, 1H, OH), 7.56 (s, 2H, 2 NH), 7.25 (d, J = 8.6 Hz, 1H, 4-H), 7.02 (d, J = 2.5 Hz, 1H, 7-H), 6.69 (dd, J = 8.6, 2.5 Hz, 1H, 5-H), 6.52 (s, 1H, NH), 2.75 (d, J = 4.8 Hz, 3H, NCH_3) ppm.

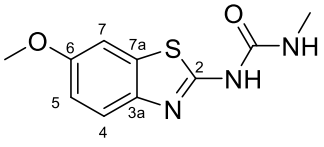
^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.2 (CN), 157.2 (C-6), 152.8 (C-3a), 144.9 (C-2), 131.3 (C-7a), 118.8 (C-4), 113.7 (C-5), 106.5 (C-7), 27.6 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1601, 1490, 1453, 1381, 1277, 1230, 1048, 898, 875, 836, 791, 723 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_9H_9N_4OS$ $[M-H]^-$: 221.0502; found: 221.0502.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 3b.

1-(6-Methoxybenzo[*d*]thiazol-2-yl)-3-methylurea (7)



$C_{10}H_{11}N_3O_2S$

M_w = 237.28 g/mol

N-Methylurea **7** was prepared following General procedure B, using 2-amino-6-methoxybenzothiazole (**55**, 4.0 g, 21 mmol, 1.0 eq). Off-white solid (3.7 g, 16 mmol, 72%).

Mp: 320 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.62 (s, 1H, NH), 7.55 – 7.41 (m, 2H, 4-H, 7-H), 6.94 (dd, J = 8.8, 2.6 Hz, 1H, 5-H), 6.60 (d, J = 5.0 Hz, 1H, NH), 3.78 (s, 3H, OCH_3), 2.71 (d, J = 4.7 Hz, 3H, NCH_3) ppm.

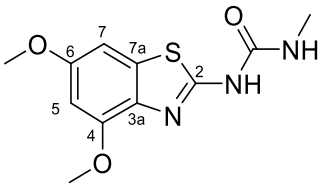
^{13}C NMR (101 MHz, DMSO-d_6): δ = 158.0 (CO), 155.5 (C-6), 154.4 (C-2), 143.2 (C-3a), 132.6 (C-7a), 120.1 (C-4), 114.1 (C-5), 104.8 (C-7), 55.6 (OCH_3), 26.3 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1710, 1605, 1576, 1544, 1465, 1267, 1214, 1031, 845, 811, 711, 671 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{10}H_{10}N_3O_2S$ $[M-H]^-$: 236.0499; found: 236.0499.

Purity (HPLC): >98% (λ = 210 nm), >98% (λ = 254 nm), Method 3a.

1-(4,6-Dimethoxybenzo[*d*]thiazol-2-yl)-3-methylurea (56)



$C_{11}H_{13}N_3O_3S$

M_w = 267.30 g/mol

N-Methylurea **56** was prepared following General procedure B, using 2-aminobenzothiazole **57** (190 mg, 0.904 mmol, 1.0 eq). Colourless solid (46 mg, 0.17 mmol, 19%).

Mp: 208 – 209 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.34 (s, 1H), 6.89 (d, J = 2.2 Hz, 1H, 7-H), 6.54 (d, J = 2.2 Hz, 1H, 5-H), 6.29 (s, 1H, NH), 3.96 (s, 3H, (C-4)OCH_3), 3.87 (s, 3H, (C-6)OCH_3), 2.87 (d, J = 4.6 Hz, 3H, NCH_3) ppm.

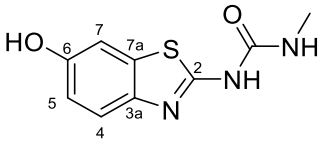
^{13}C NMR (101 MHz, $CDCl_3$): δ = 160.3 (C-2), 157.3 (C-6), 155.1 (CO), 150.7 (C-4), 133.9 (C-3a or C-7a), 132.8 (C-3a or C-7a), 98.2 (C-5), 96.2 (C-7), 56.1 ((C-4)O$\underline{C}H_3$ or (C-6)O$\underline{C}H_3$), 56.0 ((C-4)O$\underline{C}H_3$ or (C-6)O$\underline{C}H_3$), 27.0 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3296, 1698, 1598, 1544, 1459, 1419, 1292, 1149, 1041, 812, 745, 680 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{11}H_{13}N_3O_3S$ $[M]^{\cdot+}$: 267.0673; found: 267.0669.

Purity (HPLC): >99% (λ = 210 nm), >98% (λ = 254 nm), Method 1a.

1-(6-Hydroxybenzo[*d*]thiazol-2-yl)-3-methylurea (58)



$C_9H_9N_3O_2S$

M_w = 223.25 g/mol

6-Hydroxybenzothiazole **58** was prepared following General procedure C from 6-methoxybenzothiazole **7** (500 mg, 2.11 mmol, 1.0 eq). Grey solid (292 mg, 1.31 mmol, 62%).

Mp: 347 °C (decomposition), 281 – 283 °C (melting).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.54 (s, 1H, NH), 9.38 (s, 1H, NH), 7.39 (d, *J* = 8.6 Hz, 1H, 4-H), 7.18 (d, *J* = 2.5 Hz, 1H, 7-H), 6.79 (dd, *J* = 8.6, 2.5 Hz, 1H, 5-H), 6.67 (d, *J* = 5.0 Hz, 1H, OH), 2.70 (d, *J* = 4.6 Hz, 3H, NCH_3) ppm.

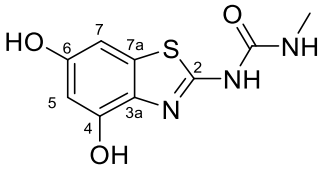
^{13}C NMR (101 MHz, DMSO-d_6): δ = 157.2 (C-2), 154.5 (CO), 153.4 (C-6), 142.2 (C-3a), 132.5 (C-7a), 120.1 (C-4), 114.5 (C-5), 106.5 (C-7), 26.3 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1668, 1561, 1537, 1442, 1428, 1283, 1240, 1130, 845, 823, 716, 664 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_9H_8N_3O_2S$ $[M-H]^-$: 222.0342; found: 222.0342.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 4.

1-(4,6-Dihydroxybenzo[*d*]thiazol-2-yl)-3-methylurea (59)



$C_9H_9N_3O_3S$

M_w = 239.25 g/mol

4,6-Dihydroxybenzothiazole **59** was prepared following General procedure C from 4,6-dimethoxybenzothiazole **56** (64 mg, 0.24 mmol, 1.0 eq). The crude product was purified by

flash column chromatography (DCM → 9:1 DCM/EtOH) to give **59** (25 mg, 0.084 mmol, 35%) as a light yellow solid.

Mp: 234 – 236 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.37 (s, 1H, NH), 9.50 (s, 1H, (C-6)OH), 9.19 (s, 1Hz, (C-4)OH), 7.07 (s, 1H, NH), 6.61 (d, J = 2.3 Hz, 1H, 7-H), 6.28 (d, J = 2.2 Hz, 1H, 5-H), 2.72 (d, J = 4.6 Hz, 3H, NCH_3) ppm.

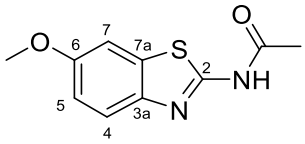
^{13}C NMR (101 MHz, DMSO-d_6): δ = 154.9 (CO or C-6), 154.4 (CO or C-6), 149.3 (C-4), 133.1 (C-3a or C-7a), 131.6 (C-3a or C-7a), 100.8 (C-5), 97.2 (C-7), 26.3 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1661, 1553, 1445, 1294, 1260, 1168, 1138, 1125, 1002, 953, 836, 743 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_9H_8N_3O_3S$ $[M\text{-}H]^-$: 238.0291; found: 238.0292.

Purity (HPLC): 100% (λ = 210 nm), >99% (λ = 254 nm), Method 3b.

N-(6-Methoxybenzo[*d*]thiazol-2-yl)acetamide (60)



$C_{10}H_{10}N_2O_2S$

M_w = 222.26 g/mol

Acetamide **60** was prepared following General procedure D, using 2-amino-6-methoxybenzothiazole (**55**, 250 mg, 1.66 mmol, 1.0 eq). Purification by flash column chromatography (9.5:0.5 DCM/MeOH) gave the final product that was recrystallised from DCM to give **60** (236 mg, 1.06 mmol, 79%) as a colourless solid.

Mp: 230 – 231 °C. [Ref.[157]: 226 – 228 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.19 (s, 1H, NH), 7.62 (d, J = 8.8 Hz, 1H, 4-H), 7.54 (d, J = 2.6 Hz, 1H, 7-H), 7.01 (dd, J = 8.8, 2.6 Hz, 1H, 5-H), 3.80 (s, 3H, OCH_3), 2.17 (s, 3H, $(CO)CH_3$) ppm.

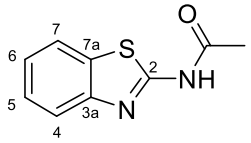
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.1 (CO), 156.1 (C-2 or C-6), 155.9 (C-2 or C-6), 142.6 (C-3a), 132.7 (C-7a), 121.1 (C-4), 114.8 (C-5), 104.7 (C-7), 55.6 (OCH_3), 22.7 ($(CO)\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2168, 1685, 1601, 1546, 1437, 1283, 1236, 1060, 849, 812, 723, 700 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{10}H_9N_2O_2S$ $[M\text{-}H]^-$: 221.0390; found: 221.0391.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2a.

N-(Benzo[*d*]thiazol-2-yl)acetamide (61)



$C_9H_8N_2OS$

M_w = 192.04 g/mol

Acetamide **61** was prepared following General procedure D, using 2-aminobenzothiazole (**63**, 250 mg, 1.66 mmol, 1.0 eq). Purification by recrystallisation from DCM gave acetamide **61** (110 mg, 0.572 mmol, 34%) as a colourless solid. ^{1}H and ^{13}C NMR data is in accordance with literature[156].

Mp: 182 – 183 °C. [Ref.[156]: 184 – 187 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.32 (s, 1H, NH), 7.99 – 7.92 (m, 1Hz, 4-H), 7.73 (dt, J = 8.1, 0.8 Hz, 1H, 7-H), 7.42 (ddd, J = 8.3, 7.2, 1.3 Hz, 1H, 5-H), 7.33 – 7.25 (m, 1H, 6-H), 2.20 (s, 3H, CH_3) ppm.

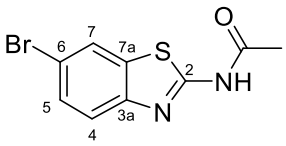
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.4 (CO), 158.0 (C-2), 148.5 (C-3a), 131.4 (C-7a), 126.1 (C-5), 123.5 (C-6), 121.7 (C-4), 120.5 (C-7), 22.8 (CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 2929, 1692, 1545, 1442, 1365, 1271, 1228, 999, 865, 770, 752, 725, 677 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_9H_7N_2OS$ [M-H]$^-$: 191.0284; found: 191.0284.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2b.

N-(6-Bromobenzo[*d*]thiazol-2-yl)acetamide (62)



$C_9H_7BrN_2OS$

M_w = 271.13 g/mol

Acetamide **62** was prepared following General procedure D, using 2-amino-6-bromobenzothiazole (**64**, 250 mg, 1.09 mmol, 1.0 eq). Purification by flash column chromatography (9.5:0.5 DCM/EtOH) gave **62** (94 mg, 0.35 mmol, 32%) as a colourless solid.

Mp: 158 – 160 °C. [Ref.[158]: 150 – 153 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.42 (s, 1H, NH), 8.23 (d, *J* = 2.0 Hz, 1H, 7-H), 7.66 (d, *J* = 8.6 Hz, 1H, 4-H), 7.55 (dd, *J* = 8.6, 2.1 Hz, 1H, 5-H), 2.20 (s, 3H, OCH_3) ppm.

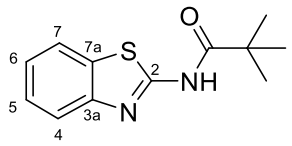
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.7 (CO), 158.8 (C-2), 147.7 (C-6), 133.7 (C-3a), 129.1 (C-5), 124.2 (C-7), 122.1 (C-4), 115.4 (C-7a), 22.8 (OCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1689, 1588, 1553, 1434, 1255, 1226, 1089, 991, 882, 809, 753, 717, 680 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_9H_8{}^{79}BrN_2OS$ $[M+H]^+$: 272.9534; found: 272.9534.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2b.

N-(Benzo[*d*]thiazol-2-yl)pivalamide (65)



$C_{12}H_{14}N_2OS$

M_w = 234.32 g/mol

Amide **65** was prepared following General procedure E, using 2-aminobenzothiazole (**63**, 250 mg, 1.66 mmol, 1.0 eq) and pivaloyl chloride in DCM for 72 h. After extraction, the residue was purified by flash column chromatography (9.75:0.25 DCM/EtOH) to give pivalamide **65** (144 mg, 0.616 mmol, 37%) as a low-melting beige solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.04 (s, 1H, NH), 7.97 (ddd, *J* = 7.9, 1.3, 0.6 Hz, 1H, 4-H), 7.73 (dt, *J* = 8.1, 0.9 Hz, 1H, 7-H), 7.43 (ddd, *J* = 8.2, 7.2, 1.3 Hz, 1H, 5-H), 7.30 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H, 6-H), 1.27 (s, 9H, $C(CH_3)_3$) ppm.

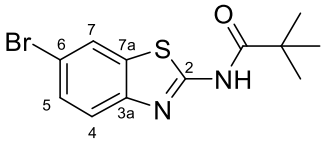
^{13}C NMR (101 MHz, DMSO-d_6): δ = 177.5 (CO), 158.8 (C-2), 148.5 (C-3a), 131.5 (C-7a), 126.1 (C-5), 123.4 (C-6), 121.6 (C-4), 120.3 (C-7), 39.1 ($\underline{C}(CH_3)_3$), 26.5 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2168, 1687, 1670, 1526, 1442, 1277, 1256, 1141, 943, 757, 730, 670 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{12}H_{15}N_2OS$ $[M+H]^+$: 235.0900; found: 235.0902.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2b.

N-(6-Bromobenzo[*d*]thiazol-2-yl)pivalamide (66)



$C_{12}H_{13}BrN_2OS$

M_w = 213.21 g/mol

Amide **66** was prepared following General procedure E, using 2-amino-6-bromobenzothiazole (**64**, 250 mg, 1.09 mmol, 1.0 eq) and pivaloyl chloride in DCM for 72 h. After extraction, the residue was purified by flash column chromatography (9.75:0.25 DCM/EtOH) to give pivalamide **66** (62 mg, 0.20 mmol, 18%) as light beige solid.

Mp: 129 – 130 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.15 (s, 1H, NH), 8.24 (d, J = 2.0 Hz, 1H, 7-H), 7.66 (d, J = 8.6 Hz, 1H, 4-H), 7.57 (dd, J = 8.6, 2.0 Hz, 1H, 5-H), 1.27 (s, 9H, $C(CH_3)_3$) ppm.

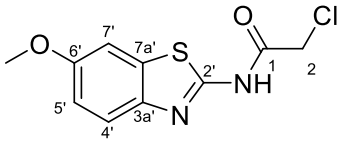
^{13}C NMR (101 MHz, DMSO-d_6): δ = 177.8 (CO), 159.6 (C-2), 147.7 (C-3a), 133.7 (C-7a), 129.1 (C-5), 124.2 (C-7), 121.9 (C-4), 115.3 (C-6), 39.1 ($\underline{C}(CH_3)_3$), 26.5 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2168, 1684, 1589, 1531, 1433, 1271, 1255, 1137, 1078, 945, 879, 816 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{12}H_{14}{}^{79}BrN_2OS$ $[M+H]^+$: 213.0005; found: 213.0005.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2b.

2-Chloro-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (67)



$C_{10}H_9ClN_2O_2S$

M_w = 256.71 g/mol

Chloroacetamide **67** was prepared as described in literature[159] from 2-amino-6-methoxybenzothiazole (**55**, 5.0 g, 27 mmol, 1.0 eq), using 2-chloroacetyl chloride (2.35 mL, 29.6 mmol, 1.1 eq) and triethylamine (4.13 mL, 29.6 mmol, 1.1 eq). Light brown solid (6.08 g, 23.7 mmol, 88%).

Mp: 172 – 173 °C. [Ref.[160]: 169 – 172 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.58 (s br, 1H, NH), 7.66 (d, J = 8.8 Hz, 1H, 7'-H), 7.59 (d, J = 2.6 Hz, 1H, 4'-H), 7.04 (dd, J = 8.8, 2.6 Hz, 1H, 5'-H), 4.44 (s, 2H, 2-H), 3.81 (s, 3H, OCH_3) ppm.

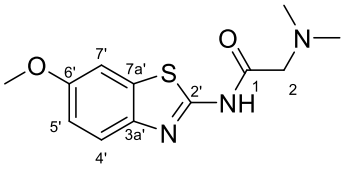
^{13}C NMR (101 MHz, DMSO-d_6): δ = 166.1 (C-1), 156.8 (C-6'), 155.9 (C-2'), 143.0 (C-3a'), 133.3 (C-7a'), 121.8 (C-4'), 115.6 (C-5'), 105.3 (C-7'), 56.1 (OCH_3), 43.0 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 1661, 1613, 1546, 1460, 1405, 1261, 1216, 1071, 858, 808, 785, 729 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{10}H_{10}ClN_2O_2S$ $[M+H]^+$: 257.0147; found: 257.0148.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-(Dimethylamino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (68)



$C_{12}H_{15}N_3O_2S$

M_w = 265.33 g/mol

2-(Dimethylamino)acetamide **68** was prepared following General procedure F, using chloroacetamide **67** (513 mg, 2.00 mmol, 1.0 eq) and dimethylamine (2.2 mL, 2 M in THF, 4.4 mmol, 2.2 eq). The mixture was stirred for 1 h, and the extraction was conducted with diethyl ether. The residue was suspended in DCM, the solid collected by filtration and washed with DCM to give product **68** (108 mg, 0.407 mmol, 42%) as a light yellow solid (108 mg, 0.407 mmol, 42%).

Mp: 114 – 115 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.90 (s, 1H, NH), 7.63 (d, J = 8.8 Hz, 1H, 4'-H), 7.56 (d, J = 2.6 Hz, 1H, 7'-H), 7.02 (dd, J = 8.8, 2.6 Hz, 1H, 5'-H), 3.80 (s, 3H, OCH_3), 3.27 (s, 2H, 2-H), 2.29 (s, 6H, $N(CH_3)_2$) ppm.

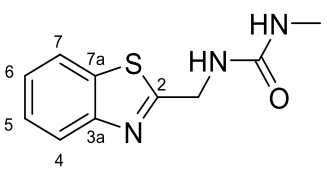
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.4 (C-1), 156.1 (C-2' or C-6'), 155.5 (C-2' or C-6'), 142.6 (C-3a'), 132.8 (C-7a'), 121.1 (C-4'), 114.9 (C-5'), 104.7 (C-7'), 61.3 (C-2), 55.6 (OCH_3), 45.1 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1661, 1613, 1546, 1460, 1434, 1261, 1071, 1037, 858, 808, 785, 729 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{12}H_{16}N_3O_2S$ $[M+H]^+$: 266.0958; found: 266.0958.

Purity (HPLC): >99% (λ = 210 nm), >98% (λ = 254 nm), Method 2a.

1-(Benzo[*d*]thiazol-2-ylmethyl)-3-methylurea (70)



$C_{10}H_{11}N_3OS$

M_w = 221.28 g/mol

N-Methylurea **70** was prepared following General procedure B, using 1,3-benzothiazol-2-ylmethylamine (**69**, 250 mg, 1.45 mmol, 1.0 eq). Off-white solid (120 mg, 0.542 mmol, 37%).

Mp: 186 – 187 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.04 (d, *J* = 8.0 Hz, 1H, 4-H), 7.91 (d, *J* = 8.1 Hz, 1H, 7-H), 7.48 (t, *J* = 7.6 Hz, 1H, 5-H), 7.39 (t, *J* = 7.7 Hz, 1H, 6-H), 6.88 (s, 1H, NH), 6.14 (s, 1H, NH), 4.58 (d, *J* = 6.1 Hz, 2H, NHC$\underline{H}_2$), 2.59 (d, *J* = 4.6 Hz, 3H, NHC$\underline{H}_3$) ppm.

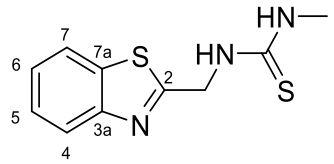
^{13}C NMR (101 MHz, DMSO-d_6): δ = 174.1 (C-2), 158.4 (CO), 153.0 (C-3a), 134.5 (C-7a), 126.0 (C-5), 124.7 (C-6), 122.2 (C-4, C-7), 42.1 ($NHCH_2$), 26.5 ($NHCH_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 1627, 1590, 1509, 1422, 1313, 1283, 1259, 1152, 1011, 750, 723, 680 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{10}H_{11}N_3OS$ $[M]^{\cdot+}$: 221.0618; found: 221.0624.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

1-(Benzo[*d*]thiazol-2-ylmethyl)-3-methylthiourea (71)



$C_{10}H_{11}N_3S_2$

M_w = 237.34 g/mol

Thiourea **71** was prepared following General procedure A, using 1,3-benzothiazol-2-ylmethylamine (**69**, 200 mg, 1.16 mmol, 1.0 eq). The mixture was stirred for 3 h to give thiourea **71** (208 mg, 0.876 mmol, 76%) as a brick red solid.

Mp: 140 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.27 (s br, 1H, NH), 8.08 – 8.01 (m, 1H, 4-H), 7.97 – 7.90 (m, 1H, 7-H), 7.80 (s br, 1H, NH), 7.49 (ddd, *J* = 8.2, 7.2, 1.3 Hz, 1H, 5-H), 7.40 (ddd, *J* = 8.4, 7.2, 1.2 Hz, 1H, 6-H), 5.09 (s, 2H, NHC$\underline{H}_2$), 2.88 (s, 3H, NHC$\underline{H}_3$) ppm.

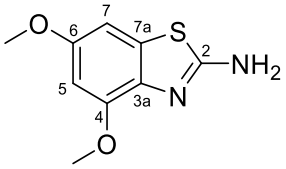
^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.1 (CO), 172.0 (C-2), 152.7 (C-3a), 134.6 (C-7a), 126.0 (C-5), 124.9 (C-6), 122.2 (double signal, C-4, C-7), 45.8 ($NHCH_2$), 31.2 ($NHCH_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3203, 1549, 1510, 1438, 1315, 1221, 1183, 1095, 939, 758, 733, 707 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{10}H_{12}N_3S_2$ $[M+H]^+$: 238.0468; found: 238.0466.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

4,6-Dimethoxybenzo[*d*]thiazol-2-amine (57)



$C_9H_{10}N_2O_2S$

M_w = 210.25 g/mol

Aminobenzothiazole **57** was prepared as described in literature[116] from 2,4-dimethoxyaniline (**74**, 2.0 g, 13 mmol, 1.0 eq), using ammonium thiocyanate (1.74 g, 22.8 mmol, 1.8 eq) and bromine (0.779 mL, 15.2 mmol, 1.2 eq). Colourless solid (1.31 g, 6.22 mmol, 49%).

Mp: 177 – 178 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.71 (d, *J* = 2.3 Hz, 1H, 7-H), 6.45 (d, *J* = 2.3 Hz, 1H, 5-H), 5.16 (s br, 2H, NH_2), 3.93 (s, 3H, (C-4)OCH_3), 3.81 (s, 3H, (C-6)OCH_3) ppm.

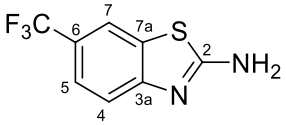
^{13}C NMR (101 MHz, $CDCl_3$): δ = 163.1 (C-2), 156.7 (C-6), 151.3 (C-4), 135.9 (C-3a), 133.1 (C-7a), 97.5 (C-5), 96.1 (C-7), 56.0 ((C-4)O<u>C</u>H_3 or (C-6)O<u>C</u>H_3), 55.9 ((C-4)O<u>C</u>H_3 or (C-6)O<u>C</u>H_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3088, 1620, 1548, 1303, 1259, 1215, 1142, 1046, 820, 812, 792, 729 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_9H_{11}N_2O_2S$ $[M+H]^+$: 211.0536; found: 211.0534.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

6-(Trifluoromethyl)benzo[*d*]thiazol-2-amine (72)



$C_8H_5F_3N_2S$

M_w = 218.20 g/mol

Aminobenzothiazole **72** was prepared as described in literature[117] from 4-(trifluoromethyl)aniline (**75**, 200 mg, 1.24 mmol, 1.0 eq), using ammonium thiocyanate (189 mg, 2.48 mmol, 2.0 eq) and bromine (0.127 mL, 2.48 mmol, 2.0 eq). Bright yellow solid (100 mg, 0.458 mmol, 37%).

Mp: 117 – 119 °C. [Ref.[117]: 113.7 – 115.5 °C.]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.86 (dt, *J* = 1.7, 0.7 Hz, 1H, 7-H), 7.62 – 7.52 (m, 2H, 4-H, 5-H), 5.59 (s br, 2H, NH_2) ppm.

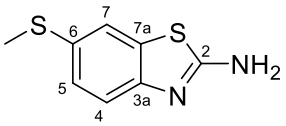
^{13}C NMR (101 MHz, $CDCl_3$): δ = 167.9 (C-2), 154.7 (C-3a), 131.9 (C-7a), 125.9, 124.8, 124.5, 124.2, 123.5 (q, *J* = 3.8 Hz, C-5), 123.2, 119.2 (C-4), 118.6 (q, *J* = 4.2 Hz, C-7) ppm.

IR (ATR): $\tilde{\nu}$ = 1638, 1526, 1314, 1283, 1250, 1079, 1049, 884, 822, 796, 718, 693 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_8H_6F_3N_2S$ $[M+H]^+$: 219.0199; found: 219.0201.

Purity (HPLC): ND.

6-(Methylthio)benzo[*d*]thiazol-2-amine (73)



$C_8H_8N_2S_2$

M_w = 196.29 g/mol

Aminobenzothiazole **73** was prepared following the same procedure described for aminobenzothiazole **72**[117], using 4-(methylthio)aniline (**76**, 200 mg, 1.44 mmol, 1.0 eq), ammonium thiocyanate (219 mg, 2.87 mmol, 2.0 eq), and bromine (0.147 mL, 2.87 mmol, 2.0 eq). Grey solid (93 mg, 0.46 mmol, 33%).

Mp: 141 – 143 °C. [Ref.[161]: 148 – 149 °C.]

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.52 (d, *J* = 1.9 Hz, 1H, 7-H), 7.45 (d, *J* = 8.4 Hz, 1H, 4-H), 7.28 (dd, *J* = 8.4, 1.9 Hz, 1H, 5-H), 2.50 (s, 3H, CH_3) ppm.

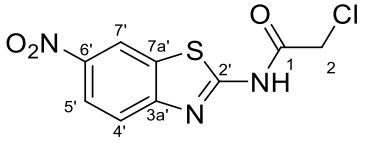
^{13}C NMR (126 MHz, $CDCl_3$): δ = 165.8 (C-2), 149.4 (C-3a), 132.2 (C-6 or C-7a), 132.0 (C-6 or C-7a), 126.9 (C-5), 120.5 (C-7), 119.3 (C-4), 17.8 (CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1629, 1525, 1450, 1433, 1418, 1295, 1268, 1098, 893, 869, 811, 702 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_8H_9N_2S_2$ $[M+H]^+$: 197.0202; found: 197.0202.

Purity (HPLC): ND.

2-Chloro-*N*-(6-nitrobenzo[*d*]thiazol-2-yl)acetamide (80)



$C_9H_6ClN_3O_3S$

M_w = 271.68 g/mol

Chloroacetamide **80** was prepared as described in literature[159] from 2-amino-6-nitrobenzothiazole (**77**, 5.0 g, 26 mmol, 1.0 eq), using 2-chloroacetyl chloride (2.24 mL, 28.2 mmol, 1.1 eq) and triethylamine (3.93 mL, 28.2 mmol, 1.1 eq). Beige solid (6.39 g, 23.5 mmol, 92%).

Mp: 218 °C (decomposition). [Ref.[162]: 188 – 189 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.12 (s, 1H, NH), 9.07 (d, *J* = 2.4 Hz, 1H, 7'-H), 8.28 (dd, *J* = 8.9, 2.4 Hz, 1H, 5'-H), 7.92 (d, *J* = 9.0 Hz, 1H, 4'-H), 4.52 (s, 2H, 2-H) ppm.

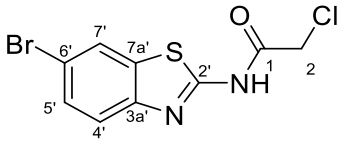
^{13}C NMR (101 MHz, DMSO-d_6): δ = 166.7 (C-1), 163.1 (C-2'), 153.3 (C-6'), 143.2 (C-3a'), 132.2 (C-7a'), 121.9 (C-5'), 120.9 (C-4'), 119.2 (C-7'), 42.6 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 1712, 1527, 1491, 1442, 1329, 1275, 1154, 1041, 828, 807, 754, 721 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_9H_5ClN_3O_2S$ $[M\text{-}H]^-$: 269.9745; found: 269.9746.

Purity (HPLC): >92% (λ = 210 nm), >94% (λ = 254 nm), Method 1e.

N-(6-Bromobenzo[*d*]thiazol-2-yl)-2-chloroacetamide (81)



$C_9H_6BrClN_2OS$

M_w = 305.57 g/mol

Chloroacetamide **81** was prepared following General procedure E[159] from 2-amino-6-bromobenzothiazole (**64**, 1.25 g, 5.46 mmol, 1.0 eq), using 2-chloroacetyl chloride (0.477 mL, 6.00 mmol, 1.1 eq) and triethylamine (0.837 mL, 6.00 mmol, 1.1 eq). Old pink solid (1.34 g, 4.40 mmol, 81%).

Mp: 224 – 226 °C. [Ref.[162]: 210 – 211 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.81 (s, 1H, NH), 8.28 (d, *J* = 2.0 Hz, 1H, 7'-H), 7.71 (d, *J* = 8.6 Hz, 1H, 4'-H), 7.59 (dd, *J* = 8.6, 2.1 Hz, 1H, 5'-H), 4.47 (s, 2H, 2-H) ppm.

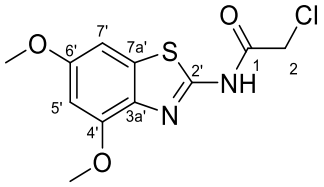
^{13}C NMR (101 MHz, DMSO-d_6): δ = 165.4 (C-1), 157.6 (C-2'), 146.9 (C-6'), 132.9 (C-3a'), 128.5 (C-5'), 123.6 (C-7'), 121.6 (C-4'), 115.0 (C-7a'), 41.7 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3171, 1665, 1594, 1535, 1441, 1406, 1271, 996, 856, 806, 788, 749, 726 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_9H_7{}^{79}BrClN_2O_2S$ $[M+H]^+$: 304.9146; found: 304.9146.

Purity (HPLC): >99% (λ = 210 nm), >98% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(4,6-dimethoxybenzo[*d*]thiazol-2-yl)acetamide (82)



$C_{11}H_{11}ClN_2O_3S$

M_w = 286.73 g/mol

Chloroacetamide **82** was prepared following General procedure E, using 2-aminobenzothizole **57** (683 mg, 3.25 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 16 h. Filtration and recrystallisation from EtOH gave product **82** (823 mg, 2.87 mmol, 89%) as an off-white solid.

Mp: 218 – 219 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.70 (s br, 1H, NH), 7.14 (d, J = 2.3 Hz, 1H, 7'-H), 6.60 (d, J = 2.3 Hz, 1H, 5'-H), 4.41 (s, 2H, 2-H), 3.88 (s, 3H, (C-4')OCH_3), 3.80 (s, 3H, (C-6')OCH_3) ppm.

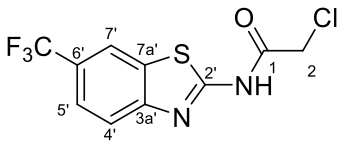
^{13}C NMR (101 MHz, DMSO-d_6): δ = 165.4 (C-1), 157.5 (C-2' or C-6'), 157.3 (C-2' or C-6'), 152.2 (C-4'), 133.5 (C-3a'), 132.7 (C-7a'), 98.2 (C-5'), 95.8 (C-7'), 55.9 ((C-4')O$\underline{C}H_3$ or (C-6')O$\underline{C}H_3$), 55.7 ((C-4)O$\underline{C}H_3$ or (C-6)O$\underline{C}H_3$), 42.4 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 2940, 1709, 1552, 1459, 1303, 1249, 1217, 1165, 1049, 1042, 828, 800 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{11}H_{11}ClN_2O_3S$ $[M]^{\cdot+}$: 286.0174; found: 286.0171.

Purity (HPLC): >97% (λ = 210 nm), >92% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(6-(trifluoromethyl)benzo[*d*]thiazol-2-yl)acetamide (83)



$C_{10}H_6ClF_3N_2OS$

M_w = 294.68 g/mol

Chloroacetamide **83** was prepared following General procedure E, using 2-aminobenzothiazole **72** (100 mg, 0.458 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for

3 h. Filtration and recrystallisation from EtOH gave product **83** (102 mg, 0.347 mmol, 76%) as a light yellow solid. ^{1}H and ^{13}C NMR data is in accordance with literature[163].

Mp: 180 – 181 °C. [Ref.[163]: 179 – 180 °C.]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (dt, *J* = 1.4, 0.8 Hz, 1H, 7'-H), 7.90 (dq, *J* = 8.6, 0.7 Hz, 1H, 4'-H), 7.75 – 7.68 (m, 1H, 5'-H), 4.35 (s, 2H, 2-H) ppm.

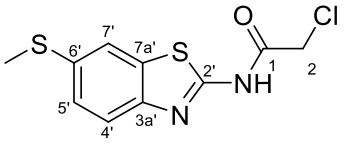
^{13}C NMR (101 MHz, $CDCl_3$): δ = 164.9 (C-1), 159.1 (C-2'), 150.7 (C-3a'), 132.5 (C-7a'), 127.0, 126.7, 125.7, 123.8 (q, *J* = 3.6 Hz, C-5'), 123.0, 121.8 (C-4'), 119.4 (q, *J* = 4.2 Hz, C-7'), 42.2 ppm.

IR (ATR): $\tilde{\nu}$ = 1670, 1555, 1311, 1285, 1155, 1136, 1083, 1051, 885, 826, 744, 667 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_8H_6ClF_3N_2OS$ $[M]^{\cdot+}$: 293.9836; found: 293.9835.

Purity (HPLC): >97% (λ = 210 nm), >96% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(6-(methylthio)benzo[*d*]thiazol-2-yl)acetamide (84)



$C_{10}H_9ClN_2OS_2$

M_w = 272.77 g/mol

Chloroacetamide **84** was prepared following General procedure E, using 2-aminobenzothizole **73** (88 mg, 0.45 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 3 h. Filtration and recrystallisation from EtOH gave product **84** (112 mg, 0.441 mmol, 91%) as a yellow solid.

Mp: 155 – 156 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.71 (dd, *J* = 6.8, 0.5 Hz, 1H, 4'-H), 7.70 (s br, 1H, 7'-H), 7.43 – 7.36 (m, 1H, 5'-H), 4.31 (s, 2H. 2-H), 2.55 (s, 3H, SCH_3) ppm.

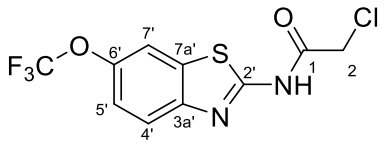
^{13}C NMR (101 MHz, $CDCl_3$): δ = 164.6 (C-1), 156.2 (C-2'), 146.3 (C-3a'), 135.0 (C-6'), 133.4 (C-7a'), 126.6 (C-5'), 121.6 (C-4'), 119.4 (C-7'), 42.2 (C-2), 17.0 (SCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1679, 1593, 1544, 1437, 1395, 1328, 1262, 1176, 985, 802, 711, 685 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{10}H_9ClN_2OS_2$ $[M]^{\cdot+}$: 271.9840; found: 271.9852.

Purity (HPLC): >98% (λ = 210 nm), >98% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(6-(trifluoromethoxy)benzo[*d*]thiazol-2-yl)acetamide (85)



$C_{10}H_6ClF_3N_2O_2S$

M_w = 310.68 g/mol

Chloroacetamide **85** was prepared following General procedure E, using 2-amino-6-(trifluoromethoxy)benzothiazole (**78**, 100 mg, 0.427 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 1 h. Filtration and recrystallisation from EtOH gave product **85** (95 mg, 0.30 mmol, 71%) as an off-white solid.

Mp: 182 – 183 °C. [Ref.[162]: 188 – 189 °C.]

^{1}H NMR (400 MHz, MeOD): δ = 7.87 (dd, *J* = 2.3, 1.1 Hz, 1H, 7'-H), 7.81 (d, *J* = 8.9 Hz, 1H, 4'-H), 7.36 (dtd, *J* = 8.9, 1.9, 0.9 Hz, 1H, 5'-H), 4.36 (s, 2H, C-2) ppm.

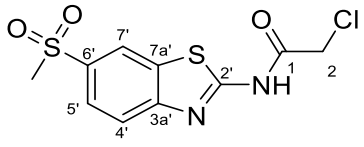
^{13}C NMR (101 MHz, MeOD): δ = 168.0 (C-1), 160.6 (C-2'), 148.9 (C-6'), 146.7, 134.5, 123.3, 123.0 (C-4'), 121.2 (C-5'), 120.8, 115.6 (C-7'), 43.0 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 1666, 1612, 1540, 1454, 1407, 1283, 1256, 1159, 1121, 817, 790, 731 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{10}H_6ClF_3N_2O_2S$ $[M]^{\cdot+}$: 309.9786; found: 309.9784.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(6-(methylsulfonyl)benzo[*d*]thiazol-2-yl)acetamide (86)



$C_{10}H_9ClN_2O_3S_2$

M_w = 304.76 g/mol

Chloroacetamide **86** was prepared following General procedure E, using 2-amino-6-(methylsulfonyl)benzothiazole (**79**, 114 mg, 0.550 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 1 h. After extraction, the residue was purified by flash column chromatography (1:2 EtOAc/isohexanes) to give product **86** (97 mg, 0.32 mmol, 64%) as a pale yellow solid.

Mp: 170 – 171 °C. [Ref.[162]: 173 – 174 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.02 (s, 1H, NH), 8.67 (d, *J* = 1.2 Hz, 1H, 7'-H), 7.96 (m, 2H, 4'-H, 5'-H), 4.51 (s, 2H, 2-H), 3.25 (s, 3H, CH_3) ppm.

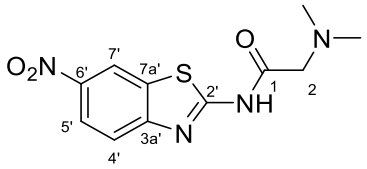
^{13}C NMR (101 MHz, DMSO-d_6): δ = 166.5 (C-1), 161.6 (C-2'), 152.0 (C-3a'), 135.7 (C-6'), 132.0 (C-7a'), 125.0 (C-5'), 122.3 (C-7'), 121.1 (C-4'), 44.0 (CH_3), 42.6 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 1699, 1535, 1286, 1266, 1142, 1096, 1047, 968, 812, 782, 748, 734 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{10}H_8ClN_2O_3S_2$ [M-H]$^-$: 302.9670; found: 302.9670.

Purity (HPLC): 100% (λ = 210 nm), >98% (λ = 254 nm), Method 1e.

2-(Dimethylamino)-*N*-(6-nitrobenzo[*d*]thiazol-2-yl)acetamide (87)



$C_{11}H_{12}N_4O_3S$

M_w = 280.30 g/mol

2-(Dimethylamino)acetamide **87** was prepared following General procedure F, using chloroacetamide **80** (300 mg, 1.10 mmol, 1.0 eq) and dimethylamine (1.21 mL, 2 M in THF, 2.43 mmol, 2.2 eq). The mixture was stirred for 18 h, the formed precipitate was filtered and washed with DCM to give product **87** (268 mg, 0.956 mmol, 87%) as a yellow solid.

Mp: 209 – 210 °C. [Ref.[164]: 204 – 206 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.99 (d, *J* = 2.4 Hz, 1H, C-7'), 8.25 (dd, *J* = 8.9, 2.5 Hz, 1H, C-5'), 7.84 (d, *J* = 9.0 Hz, 1H, C-4'), 3.45 (s, 2H, C-2), 2.39 (s, 6H, $N(CH_3)_2$) ppm.

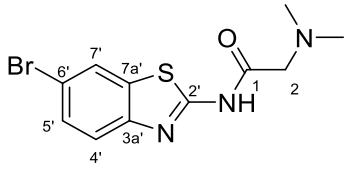
^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.1 (C-1), 164.4 (C-2'), 153.8 (C-6'), 142.7 (C-3a'), 132.3 (C-7a'), 121.7 (C-5'), 120.3 (C-4'), 118.9 (C-7'), 60.8 (C-2), 44.7 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1703, 1509, 1499, 1509, 1439, 1332, 1275, 1127, 862, 825, 720, 681 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{11}H_{11}N_4O_3S$ [M-H]$^-$: 279.0557; found: 279.0557.

Purity (HPLC): >88% (λ = 210 nm), >92% (λ = 254 nm), Method 3a.

N-(6-Bromobenzo[*d*]thiazol-2-yl)-2-(dimethylamino)acetamide (88)



$C_{11}H_{12}BrN_3OS$

M_w = 314.20 g/mol

2-(Dimethylamino)acetamide **88** was prepared following General procedure F, using chloroacetamide **81** (100 mg, 0.327 mmol, 1.0 eq) and dimethylamine (0.36 mL, 2 M in THF, 0.72 mmol, 2.2 eq). The mixture was stirred for 18 h, and the extraction was conducted with diethyl ether. The residue was recrystallised from isohexanes to give product **88** (39 mg, 0.12 mmol, 37%) as a beige solid.

Mp: 97 – 98 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.24 (d, J = 1.9 Hz, 1H, 7'-H), 7.67 (d, J = 8.6 Hz, 1H, 4'-H), 7.56 (dd, J = 8.6, 2.1 Hz, 1H, 5'-H), 3.30 (s, 2H, 2-H), 2.29 (s, 6H, $N(CH_3)_2$) ppm.

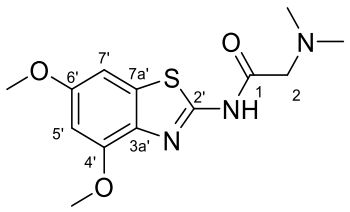
^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.0 (C-1), 158.5 (C-2'), 147.7 (C-6'), 133.7 (C-3a'), 129.1 (C-5'), 124.2 (C-7'), 122.1 (C-4'), 115.4 (C-7a'), 61.3 (C-2), 45.0 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1705, 1592, 1534, 1521, 1441, 1266, 1143, 1044, 849, 809, 734, 708 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{11}H_{12}{}^{79}BrN_3OS$ $[M]^{\cdot+}$: 312.9878; found: 312.9879.

Purity (HPLC): >94% (λ = 210 nm), >98% (λ = 254 nm), Method 1a.

N-(4,6-Dimethoxybenzo[*d*]thiazol-2-yl)-2-(dimethylamino)acetamide (89)



$C_{13}H_{17}N_3O_3S$

M_w = 295.36 g/mol

2-(Dimethylamino)acetamide **89** was prepared following General procedure F, using chloroacetamide **82** (200 mg, 0.697 mmol, 1.0 eq) and dimethylamine (0.767 mL, 2 M in THF, 1.53 mmol, 2.2 eq). The mixture was stirred for 18 h, and the extraction was conducted with diethyl ether. The residue was purified by flash column chromatography (99.5:0.5 DCM/MeOH), yielding product **89** (142 mg, 0.481 mmol, 69%) as an off-white solid.

Mp: 133 – 134 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.00 (s, 1H, NH), 7.11 (d, J = 2.3 Hz, 1H, 7'-H), 6.59 (d, J = 2.3 Hz, 1H, 5'-H), 3.88 (s, 3H, (C-4')OCH_3), 3.80 (s, 3H, (C-6')OCH_3), 3.24 (s, 2H, 2-H), 2.28 (s, 6H, $N(CH_3)_2$) ppm.

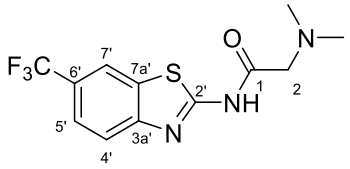
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.2 (C-1), 157.3 (C-6'), 153.8 (C-2'), 152.2 (C-4'), 133.5 (C-3a' or C-7a'), 132.7 (C-3a' or C-7a'), 98.0 (C-5'), 95.7 (C-7'), 61.2 (C-2), 55.8 ((C-4')O<u>C</u>H$_3$ or (C-6')O<u>C</u>H$_3$), 55.7 ((C-4')O<u>C</u>H$_3$ or (C-6')O<u>C</u>H$_3$), 45.1 (N(CH$_3$)$_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1677, 1604, 1535, 1458, 1258, 1146, 1041, 976, 814, 787, 752, 737 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{13}H_{18}N_3O_3S$ $[M+H]^+$: 296.1062; found: 296.1064.

Purity (HPLC): >99% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

2-(Dimethylamino)-*N*-(6-(trifluoromethyl)benzo[*d*]thiazol-2-yl)acetamide (90)



$C_{12}H_{12}F_3N_3OS$

M_w = 303.30 g/mol

2-(Dimethylamino)acetamide **90** was prepared following General procedure F, using chloroacetamide **83** (102 mg, 0.346 mmol, 1.0 eq) and dimethylamine (0.381 mL, 2 M in THF, 0.761 mmol, 2.2 eq). The mixture was stirred for 18 h, and the extraction was conducted with diethyl ether. The residue was purified by flash column chromatography (99:1 DCM/MeOH), yielding product **90** (28 mg, 0.093 mmol, 27%) as a colourless solid.

Mp: 238 – 239 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.25 (s, 1H, NH), 8.48 (dt, *J* = 2.0, 0.8 Hz, 1H, 7'-H), 7.89 (dt, *J* = 8.4, 0.8 Hz, 1H, 4'-H), 7.73 (dd, *J* = 8.8, 1.9 Hz, 1H, 5'-H), 3.37 (s, 2H, 2-H), 2.33 (s, 6H, N(CH$_3$)$_2$) ppm.

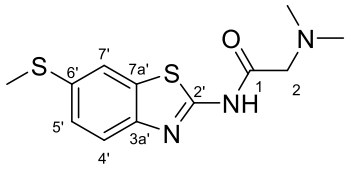
^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.1 (C-1), 161.1 (C-2'), 151.3 (C-6'), 132.1 (C-3a'), 126.0, 123.8, 123.5, 123.3, 122.9 (q, *J* = 3.6 Hz, C-5'), 120.9 (C-4'), 119.8 (q, *J* = 4.0 Hz, C-7'), 61.2 (C-2), 45.0 (N(CH$_3$)$_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1708, 1541, 1324, 1273, 1157, 1129, 1109, 969, 888, 817, 791, 687 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{12}H_{12}N_3O_2F_3$ $[M]^{\cdot+}$: 303.0648; found: 303.0646.

Purity (HPLC): >91% (λ = 210 nm), >87% (λ = 254 nm), Method 1a.

2-(Dimethylamino)-*N*-(6-(methylthio)benzo[*d*]thiazol-2-yl)acetamide (91)



$C_{12}H_{15}N_3OS_2$

M_w = 381.39 g/mol

2-(Dimethylamino)acetamide **91** was prepared following General procedure F, using chloroacetamide **84** (106 mg, 0.389 mmol, 1.0 eq) and dimethylamine (0.427 mL, 2 M in THF, 0.855 mmol, 2.2 eq). The mixture was stirred for 18 h, and the extraction was conducted with diethyl ether. The residue was purified by flash column chromatography (99:1 DCM/MeOH), yielding product **91** (75 mg, 0.27 mmol, 69%) as a brown solid.

Mp: 109 – 110 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.54 (s, 1H, NH), 7.70 (dd, *J* = 1.9, 0.6 Hz, 1H, 7'-H), 7.68 (dd, *J* = 8.5, 0.6 Hz, 1H, 4'-H), 7.37 (dd, *J* = 8.5, 1.9 Hz, 1H, 5'-H), 3.22 (s, 2H, 2-H), 2.54 (s, 3H, SCH_3), 2.41 (s, 6H, $N(CH_3)_2$) ppm.

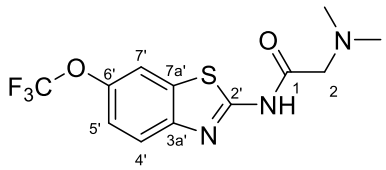
^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.7 (C-1), 156.8 (C-2'), 146.80 (C-3a'), 134.1 (C-6' or C-7a'), 133.5 (C-6' or C-7a'), 126.5 (C-5'), 121.3 (C-4'), 119.7 (C-7'), 62.6 (C-2), 46.2 ($N(CH_3)_2$), 17.2 (SCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3217, 1708, 1593, 1525, 1444, 1266, 1053, 969, 861, 805, 741, 717, 677 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{16}N_3O_2S_2$ $[M+H]^+$: 282.0730; found: 282.0730.

Purity (HPLC): >96% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

2-(Dimethylamino)-*N*-(6-(trifluoromethoxy)benzo[*d*]thiazol-2-yl)acetamide (92)



$C_{12}H_{12}F_3N_3O_2S$

M_w = 319.30 g/mol

2-(Dimethylamino)acetamide **92** was prepared following General procedure F, using chloroacetamide **85** (86 mg, 0.28 mmol, 1.0 eq) and dimethylamine (0.306 mL, 2 M in THF, 0.612 mmol, 2.2 eq). The mixture was stirred for 18 h, and the extraction was conducted with

diethyl ether. The residue was purified by flash column chromatography (95:5 DCM/MeOH), yielding product **92** (28 mg, 0.15 mmol, 53%) as an off-white solid.

Mp: 120 – 122 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.11 (dd, J = 2.6, 1.1 Hz, 1H, 7'-H), 7.81 (d, J = 8.8 Hz, 1H, 4'-H), 7.41 (ddd, J = 9.5, 2.9, 1.3 Hz, 1H, 5'-H), 3.31 (s, 2H, 2-H), 2.30 (s, 6H, $N(CH_3)_2$) ppm.

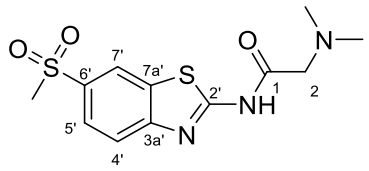
^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.0 (C-1), 159.3 (C-2'), 147.6 (C-6'), 144.0 (m, C-3a', CF_3), 132.7 (C-7'), 121.5 (C-4'), 119.8 (C-5'), 115.0 (C-7'), 61.3 (C-2), 45.0 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 2694, 1702, 1554, 1460, 1262, 1196, 1156, 964, 871, 856, 808, 668 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{12}H_{12}F_3N_3O_2$ $[M]^{\cdot+}$: 319.0597; found: 319.0597.

Purity (HPLC): >97% (λ = 210 nm), >93% (λ = 254 nm), Method 1a.

2-(Dimethylamino)-*N*-(6-(methylsulfonyl)benzo[*d*]thiazol-2-yl)acetamide (93)



$C_{12}H_{15}N_3O_3S_2$

M_w = 313.39 g/mol

2-(Dimethylamino)acetamide **93** was prepared following General procedure F, using chloroacetamide **86** (67 mg, 0.22 mmol, 1.0 eq) and dimethylamine (0.242 mL, 2 M in THF, 0.484 mmol, 2.2 eq). The mixture was stirred for 2 h, and the extraction was conducted with EtOAc. The crude product was purified by flash column chromatography (97:3 DCM/MeOH), yielding product **93** (44 mg, 0.14 mmol, 64%) as a pale yellow solid.

Mp: 232 – 233 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.61 (dd, J = 1.7, 0.8 Hz, 1H, 7'-H), 7.95 – 7.88 (m, 2H, 4'-H, 5'-H), 3.37 (s, 2H, 2-H), 3.24 (s, 3H), 2.33 (s, 6H) ppm.

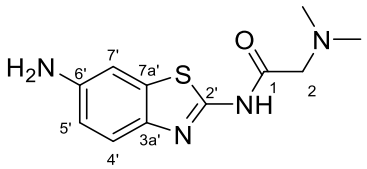
^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.3 (C-1), 162.2 (C-2'), 152.2 (C-3a'), 135.2 (C-6'), 132.1 (C-7a'), 124.8 (C-5'), 122.0 (C-4' or C-7'), 120.7 (C-4' or C-7'), 61.2 (C-2), 44.9 ($N(CH_3)_2$), 44.1 (SO_2CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1687, 1511, 1298, 1266, 1145, 1099, 1057, 973, 825, 777, 750, 732 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{12}H_{15}N_3O_3S_2$ $[M]^{\cdot+}$: 313.0550; found: 313.0550.

Purity (HPLC): >99% (λ = 210 nm), >98% (λ = 254 nm), Method 3b.

N-(6-Aminobenzo[*d*]thiazol-2-yl)-2-(dimethylamino)acetamide (94)



$C_{11}H_{14}N_4OS$

M_w = 250.32 g/mol

6-Aminobenzothiazole **94** was prepared following General procedure G from 6-nitrobenzothiazole **87** (100 mg, 0.357 mmol, 1.0 eq), using palladium on charcoal (19 mg, 0.018 mmol, 10 wt%, 0.05 eq). The mixture was stirred for 3 h. The crude product was purified by flash column chromatography (98:2 DCM/MeOH), yielding product **94** (55 mg, 0.22 mmol, 62%) as an old pink sold.

Mp: 151 – 152 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.66 (s, 1H, NH), 7.40 (d, *J* = 8.6 Hz, 1H, 4'-H), 7.00 (d, *J* = 2.2 Hz, 1H, 7'-H), 6.70 (dd, *J* = 8.6, 2.2 Hz, 1H, 5'-H), 5.16 (s, 2H, $ArNH_2$), 3.22 (s, 2H, 2-H), 2.28 (s, 6H, $N(CH_3)_2$) ppm.

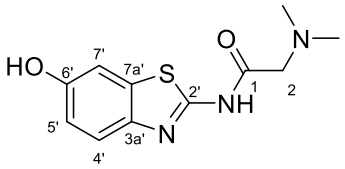
^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.9 (C-1), 152.5 (C-2'), 145.8 (C-3a'), 139.5 (C-6'), 133.0 (C-7a'), 120.9 (C-4'), 114.4 (C-5'), 104.1 (C-7'), 61.3 (C-2), 45.1 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 2780, 1689, 1618, 1542, 1281, 1261, 1131, 1032, 992, 854, 804, 687 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{11}H_{15}N_4OS$ $[M+H]^+$: 251.0962; found: 251.0961.

Purity (HPLC): >99% (λ = 210 nm), >98% (λ = 254 nm), Method 1a.

2-(Dimethylamino)-*N*-(6-hydroxybenzo[*d*]thiazol-2-yl)acetamide (95)



$C_{11}H_{13}N_3O_2S$

M_w = 251.30 g/mol

6-Hydroxybenzothiazole **95** was prepared following General procedure C from 6-methoxybenzothiazole **68** (120 mg, 0.452 mmol, 1.0 eq). Off-white solid (70 mg, 0.28 mmol, 62%).

Mp: 199 – 200 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.53 (d, *J* = 8.7 Hz, 1H, 4'-H), 7.27 (d, *J* = 2.4 Hz, 1H, 7'-H), 6.88 (dd, *J* = 8.7, 2.5 Hz, 1H, 5'-H), 3.26 (s, 2H, 2-H), 2.29 (s, 6H, $N(CH_3)_2$) ppm.

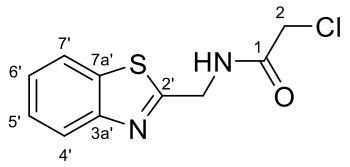
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.2 (C-1), 154.5 (C-2' or C-6'), 154.2 (C-2' or C-6'), 141.5 (C-3a'), 132.8 (C-7a'), 121.1 (C-5'), 115.2 (C-4'), 106.5 (C-7'), 61.3 (C-2), 45.1 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1681, 1602, 1544, 1264, 1223, 1214, 1043, 978, 864, 827, 816, 749, 705 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{11}H_{14}N_3O_2S$ $[M+H]^+$: 252.0802; found: 252.0800.

Purity (HPLC): >80% (λ = 210 nm), >80% (λ = 254 nm), Method 3c.

N-(Benzo[*d*]thiazol-2-ylmethyl)-2-chloroacetamide (97)



$C_{10}H_9ClN_2OS$

M_w = 240.71 g/mol

Chloroacetamide **97** was prepared following General procedure E, using benzo[*d*]thiazol-2-ylmethanamine (**69**, 100 mg, 0.578 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 1 h. Filtration and recrystallisation from EtOH gave product **97** (127 mg, 0.526 mmol, 91%) as a light brown solid.

Mp: 125 – 126 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.02 (dd, *J* = 8.3, 1.4 Hz, 1H, 4'-H), 7.87 (dd, *J* = 8.0, 1.3 Hz, 1H, 7'-H), 7.55 (s, 1H, NH), 7.50 (ddd, *J* = 8.3, 7.3, 1.3 Hz, 1H, 5'-H), 7.41 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H, 6'-H), 4.93 (d, *J* = 5.7 Hz, 2H, (C-2')CH_2), 4.16 (s, 2H, 2-H) ppm.

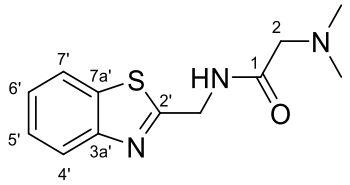
^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.1 (C-2'), 166.4 (C-1), 152.4 (C-3a'), 135.1 (C-7a'), 126.6 (C-5'), 125.7 (C-6'), 123.1 (C-4'), 122.0 (C-7'), 42.6 (C-2), 42.0 ((C-2')$\underline{C}H_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3290, 1671, 1542, 1400, 1332, 1226, 1168, 1016, 791, 755, 733, 697 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{10}H_9ClN_2OS$ $[M]^{\cdot+}$: 240.0119; found: 240.0117.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

N-(Benzo[*d*]thiazol-2-ylmethyl)-2-(dimethylamino)acetamide (96)



$C_{12}H_{15}N_3OS$

M_w = 249.33 g/mol

2-(Dimethylamino)acetamide **96** was prepared following General procedure F, using chloroacetamide **97** (60 mg, 0.25 mmol, 1.0 eq) and dimethylamine (0.274 mL, 2 M in THF, 0.548 mmol, 2.2 eq). The mixture was stirred for 18 h, and the extraction was conducted with diethyl ether. The residue was solubilised in DCM, treated with isohexanes, and the formed suspension was cooled in the refrigerator at 5 °C for 2 h. The formed precipitate was collected by filtration and washed with isohexanes to give product **96** (34 mg, 0.13 mmol, 54%) as a dark yellow solid.

Mp: 117 – 118 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.77 (t, *J* = 6.2 Hz, 1H, NH), 8.06 (ddd, *J* = 7.9, 1.3, 0.6 Hz, 1H, 4'-H), 7.99 – 7.89 (m, 1H, 7'-H), 7.49 (ddd, *J* = 8.2, 7.2, 1.3 Hz, 1H, 5'-H), 7.41 (ddd, *J* = 8.3, 7.2, 1.3 Hz, 1H, 6'-H), 4.68 (d, *J* = 6.2 Hz, 2H, (C-2')CH_2), 2.98 (s, 2H, C-2), 2.27 (s, 6H, $N(CH_3)_2$) ppm.

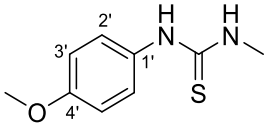
^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.5 (C-2'), 170.5 (C-1), 152.7 (C-3a), 134.5 (C-7a), 126.1 (C-5'), 124.9 (C-6'), 122.3 (C-4' or C-7'), 122.2 (C-4' or C-7'), 62.7 (C-2), 45.6 ($N(CH_3)_2$), 41.0 ((C-2')$\underline{C}H_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3311, 1666, 1521, 1418, 1263, 1167, 1051, 1012, 761, 729 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{12}H_{15}N_3OS$ $[M]^{\cdot+}$: 249.0931; found: 249.0930.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

1-(4-Methoxyphenyl)-3-methylthiourea (100)



$C_9H_{12}N_2OS$

M_w = 196.27 g/mol

Methyl isothiocyanate (226 mg, 3.00 mmol, 1.0 eq) was dissolved in 3 mL EtOH and *p*-anisidine (**99**, 377 mg, 3.00 mmol, 1.0 eq) was added to the solution. The mixture was stirred at room temperature for 4.5 h. The formed precipitate was filtered and washed with EtOH to give methylthiourea **100** (432 mg, 2.20 mmol, 73%) as a colourless solid.

R_f: 0.40 (1:4 EtOAc/isohexanes).

Mp: 152 – 153 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.17 – 7.10 (m, 2H, 3'-H), 6.96 – 6.90 (m, 2H, 2'-H), 3.81 (d, J = 0.9 Hz, 3H, OCH_3), 3.10 (d, J = 1.2 Hz, 3H, NCH_3) ppm.

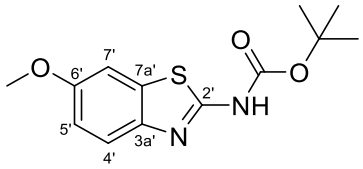
^{13}C NMR (101 MHz, $CDCl_3$): δ = 182.3 (NCSN), 159.2 (C-4'), 128.5 (C-1'), 128.1 (C-3'), 115.5 (C-2'), 55.7 (C-OCH_3), 32.2 (C-NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3288, 3157, 1540, 1514, 1504, 1293, 1242, 1164, 1026, 840, 831, 773 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{13}H_{15}N_2O_3S$ $[M-H]^-$: 195.0597; found: 195.0598.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

tert-Butyl (6-methoxybenzo[*d*]thiazol-2-yl)carbamate (103)



$C_{13}H_{16}N_2O_3S$

M_w = 280.34 g/mol

To a solution of 2-amino-6-methoxybenzothiazole (**55**, 557 mg, 3.00 mmol, 1.0 eq) in 1 mL THF/DMF (7:1) were added triethylamine (0.59 mL, 4.2 mmol, 1.4 eq), 4-(dimethylamino)pyridine (37 mg, 0.3 mmol, 0.1 eq), and di-*tert*-butyl decarbonate (720 mg, 3.30 mmol, 1.1 eq). The mixture was stirred at room temperature for 4 h. The solvents were removed under reduced pressure, and the residue purified by flash column chromatography (1:3 EtOAc/isohexanes) to give Boc-derivative **103** (582 mg, 2.08 mmol, 69%) as an off-white solid. ^{1}H and ^{13}C NMR data is in accordance with literature[165].

R_f: 0.40 (1:4 EtOAc/isohexanes).

Mp: 182 – 183 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, J = 8.9 Hz, 1H, 4'-H), 7.26 (d, J = 2.6 Hz, 1H, 7'-H), 6.99 (dd, J = 8.9, 2.6 Hz, 1H, 5'-H), 3.87 (s, 3H, OCH_3), 1.58 (s, 9H, $OC(CH_3)_3$) ppm.

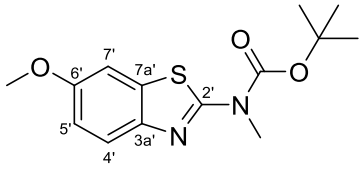
^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.6 (NCOO), 152.7 (C-2'), 156.7 (C-6'), 142.4 (C-3a'), 132.6 (C-7a'), 121.3 (C-4'), 114.8 (C-5'), 104.4 (C-7'), 83.4 ($O\underline{C}(CH_3)_3$), 56.0 (OCH_3), 28.5 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2975, 1708, 1475, 1275, 1246, 1220, 1151, 1052, 1027, 825, 814, 752 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{13}H_{15}N_2O_3S$ $[M-H]^-$: 279.0808; found: 279.0808.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1e.

tert-Butyl (6-methoxybenzo[*d*]thiazol-2-yl)(methyl)carbamate (102)



$C_{14}H_{18}N_2O_3S$

M_w = 294.37 g/mol

To solution of Boc-derivative **103** (505 mg, 1.80 mmol, 1.0 eq) in 4.5 mL DMF under ice-bath cooling, NaH (79 mg, 60% dispersion in paraffin, 2.0 mml, 1.1 eq) was added, and the mixture was stirred for 20 min. Iodomethane (0.123 mL, 1.98 mmol, 1.1 eq) was added at 0 °C, and the mixture was stirred for 0.5 h. The reaction was quenched with aq. sat. ammonium chloride solution (30 mL), and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 30 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (1:6 EtOAc/isohexanes), yielding methylated Boc-derivative **102** (404 mg, 1.37 mmol, 76%) as a colourless solid.

R_f: 0.15 (1:6 EtOAc/isohexanes).

Mp: 100 – 101 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.69 (d, J = 8.8 Hz, 1H, 4'-H), 7.23 (d, J = 2.6 Hz, 1H, 7'-H), 6.99 (dd, J = 8.9, 2.6 Hz, 1H, 5'-H), 3.86 (s, 3H, OCH_3), 3.63 (s, 3H, NCH_3), 1.61 (s, 9H, $OC(CH_3)_3$) ppm.

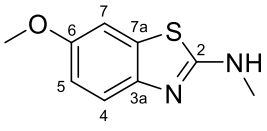
^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.8 (NCOO), 156.6 (C-6'), 153.4 (C-2'), 143.4 (C-3a'), 134.6 (C-7a'), 121.6 (C-4'), 114.6 (C-5'), 104.1 (C-7'), 83.8 ($O\underline{C}(CH_3)_3$), 56.0 (OCH_3), 34.8 (NCH_3), 28.4 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 1693, 1605, 1520, 1472, 1416, 1349, 1227, 1148, 1059, 1028, 844, 812 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{14}H_{18}N_2O_3S$ $[M]^{\cdot+}$: 294.1033; found: 294.1040.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

6-Methoxy-*N*-methylbenzo[*d*]thiazol-2-amine (101)



$C_9H_{10}N_2OS$

M_w = 194.25 g/mol

Protocol from thiourea **100**:

Phenyl-3-methylthiourea **100** (343 mg, 1.75 mmol, 1.0 eq) was dissolved in 7 mL acetic acid and a solution of bromine (0.179 mL, 3.50 mmol, 2.0 eq) in 1 mL acetic acid was added dropwise at 10 °C. The mixture was stirred at room temperature for 3 h. The mixture was neutralised with conc. ammonia aq. solution and extracted with EtOAc (3 x 40 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was further purified by flash column chromatography (97:2:1 DCM/MeOH/25% NH_3 aq. solution), yielding *N*-methylbenzothiazole-2-amine **101** (146 mg, 0.752 mmol, 43%) as a colourless solid.

Protocol from Boc-derivative **102**:

N-Methylbenzothiazole-2-amine **101** was prepared following General procedure H from Boc-derivative **102** (383 mg, 1.30 mmol, 1.0 eq). The mixture was extracted with EtOAc (3 x 50 mL) to give product **101** (205 mg, 1.06 mmol, 81%) as a colourless solid.

R_f: 0.30 (97:3 DCM/MeOH).

Mp: 165 – 166 °C.

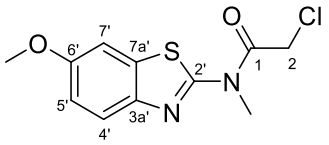
^{1}H NMR (400 MHz, CD_3OD): δ = 7.32 (dd, *J* = 8.7, 0.8 Hz, 1H, 4-H), 7.18 (d, *J* = 2.6 Hz, 1H, 7-H), 6.86 (ddd, *J* = 8.8, 2.6, 0.7 Hz, 1H, 5-H), 3.78 (s, 3H, OCH_3), 3.00 (s, 3H, OCH_3) ppm.

^{13}C NMR (101 MHz, CD_3OD): δ = 168.7 (C-2), 156.7 (C-6), 147.4 (C-3a), 132.2 (C-7a), 119.2 (C-4), 114.4 (C-5), 106.5 (C-7), 56.3 (OCH_3), 31.1 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 2925, 1613, 1470, 1415, 1260, 1219, 1122, 1037, 831, 814, 705, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_9H_{11}N_2OS$ $[M+H]^+$: 195.0587; found: 195.0586.
Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)-*N*-methylacetamide (104)



$C_{11}H_{11}ClN_2O_2S$

M_w = 270.73 g/mol

Chloroacetamide **104** was prepared following General procedure E, using *N*-methylbenzothiazole-2-amine **101** (185 mg, 0.950 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 2 h. After filtration, the residue was purified by flash column chromatography (1:4 EtOAc/isohexanes) to give product **104** (218 mg, 0.805 mmol, 85%) as colourless solid.

R_f: 0.36 (1:4 EtOAc/isohexanes).

Mp: 138 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.74 (d, *J* = 8.9 Hz, 1H, 4'-H), 7.27 (d, *J* = 2.6 Hz, 1H, 7'-H), 7.05 (dd, *J* = 8.9, 2.5 Hz, 1H, 5'-H), 4.43 (s, 2H, 2-H), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, NCH_3) ppm.

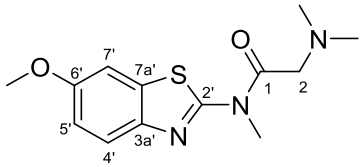
^{13}C NMR (126 MHz, $CDCl_3$): δ = 166.4 (C-1), 157.5 (C-2' or C-6'), 157.2 (C-2' or C-6'), 142.4 (C-3a'), 134.8 (C-7a'), 122.4 (C-4'), 115.6 (C-5'), 103.9 (C-7'), 56.0 (OCH_3), 42.4 (C-2), 35.6 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 2991, 2934, 1669, 1510, 1428, 1295, 1054, 1025, 837, 797, 728 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{11}H_{12}ClN_2O_2S$ $[M+H]^+$: 271.0303; found: 271.0306.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-(Dimethylamino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)-*N*-methylacetamide (98)



$C_{13}H_{17}N_3O_2S$

M_w = 279.36 g/mol

2-(Dimethylamino)acetamide **98** was prepared following General procedure F, using chloroacetamide **104** (81 mg, 0.30 mmol, 1.0 eq) and dimethylamine (0.33 mL, 2 M in THF,

0.66 mmol, 2.2 eq). The mixture was stirred for 2 h, and the extraction was conducted with EtOAc (3 x 5 mL). The crude product was purified by flash column chromatography (99:1 DCM/MeOH), yielding product **98** (65 mg, 0.23 mmol, 78%) as a beige solid.

R_f: 0.13 (1:2 EtOAc/isohexanes).

Mp: 120 – 121 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.70 (d, J = 8.8 Hz, 1H, 4'-H), 7.54 (d, J = 2.5 Hz, 1H, 7'-H), 7.03 (dd, J = 8.8, 2.6 Hz, 1H, 5'-H), 3.81 (s, 3H, OCH_3), 3.72 (s, 3H, NCH_3), 3.52 (s, 2H, 2-H), 2.30 (s, 6H, $N(CH_3)_2$) ppm.

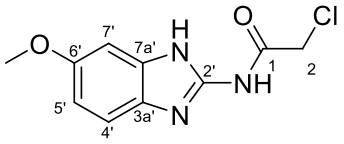
^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.6 (C-1), 157.5 (C-2'), 156.2 (C-6'), 141.8 (C-3a'), 133.9 (C-7a'), 121.5 (C-4'), 114.9 (C-5'), 104.3 (C-7'), 61.6 (C-2), 55.6 (OCH_3), 45.0 ($N(CH_3)_2$), 34.5 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1668, 1464, 1435, 1280, 1259, 1229, 1059, 1033, 853, 815, 735, 673 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{13}H_{18}N_3O_2S$ $[M+H]^+$: 280.1115; found: 280.1113.

Purity (HPLC): 98 % (λ = 210 nm), 97 % (λ = 254 nm), Method 1a.

2-Chloro-*N*-(6-methoxy-1*H*-benzo[*d*]imidazol-2-yl)acetamide (110)



$C_{10}H_{10}ClN_3O_3$

M_w = 239.66 g/mol

Chloroacetamide **110** was prepared following General procedure E, using 5-methoxy-1*H*-benzimidazol-2-ylamine (**109**, 490 mg, 3.00 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 2 h. After extraction, the residue was purified by flash column chromatography (98:2 DCM/MeOH) to give product **110** (394 mg, 1.46 mmol, 49%) as a beige solid.

Mp: 170 °C (decomposition), 224 – 225 °C (melting).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.96 (s br, 2H, 2 NH), 7.32 (d, J = 8.6 Hz, 1H, 4'-H), 6.99 (d, J = 2.4 Hz, 1H, 7'-H), 6.74 (dd, J = 8.7, 2.5 Hz, 1H, 5'-H), 4.35 (s, 2H, 2-H), 3.75 (s, 3H, OCH_3) ppm.

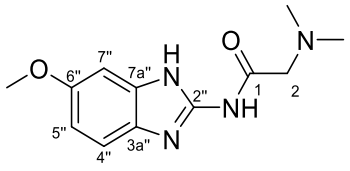
^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.0 (C-1), 155.3, 146.7, 114.2, 110.1, 97.8, 55.4 (OCH_3), 43.4 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3227, 2835, 1621, 1580, 1499, 1359, 1157, 1123, 1035, 791, 770, 734 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{10}H_{11}N_3O_2S$ $[M+H]^+$: 240.0535; found: 240.0534.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-(Dimethylamino)-*N*-(6-methoxy-1*H*-benzo[*d*]imidazol-2-yl)acetamide (105)



$C_{12}H_{16}N_4O_2$

M_w = 248.29 g/mol

2-(Dimethylamino)acetamide **105** was prepared following General procedure F, using chloroacetamide **110** (21 mg, 0.088 mmol, 1.0 eq) and dimethylamine (96 µL, 2 M in THF, 0.19 mmol, 2.2 eq). The mixture was stirred for 18 h, and the extraction was conducted with EtOAc. The crude product was purified by flash column chromatography (9:1 DCM/MeOH), yielding product **105** (13 mg, 0.051 mmol, 58%) as a light brown solid.

Mp: 178 – 180 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.48 (s br, 1H, CONH), 7.31 (d, J = 8.6 Hz, 1H, 4''-H), 6.99 (s br, 1H, 7''-H), 6.71 (dd, J = 8.6, 2.4 Hz, 1H, 5''-H), 3.74 (s, 3H, OCH_3), 3.22 (s, 2H, 2-H), 2.31 (s, 6H, $N(CH_3)_2$) ppm.

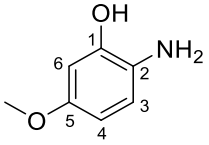
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.6 (C-1), 155.0 (C-6''), 109.7 (C-5''), 61.6 (C-2), 55.4 (OCH_3), 45.1 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 2933, 2827, 2168, 1702, 1643, 1588, 1483, 1429, 1198, 1146, 836, 786 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{12}H_{16}N_4O_2$ $[M]^{\cdot+}$: 248.1268; found: 248.1268.

Purity (HPLC): >98% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

2-Amino-5-methoxyphenol (112)



$C_7H_9NO_2$

M_w = 139.15 g/mol

To a solution of 5-methoxy-2-nitrophenol (**111**, 338 mg, 2.00 mmol, 1.0 eq) in 2.3 mL EtOAc/EtOH (1:1) was added palladium on charcoal (85 mg, 10 wt%, 0.080 mmol, 0.04 eq). The mixture was hydrogenated at atmospheric pressure for 3 h. Then, the catalyst was removed by filtration through a celite pad and the pad washed with methanol. The filtrate was evaporated under reduced pressure, yielding 2-amino-5-methoxyphenol (**112**, 260 mg, 1.87 mmol, 93%) as a red solid. Due to its instability, product **112** was used immediately for the next step.

Mp: 127 – 129 °C.

^{1}H NMR (400 MHz, CD_3OD): δ = 6.67 (dd, J = 8.4, 0.8 Hz, 1H, 3-H), 6.36 (d, J = 2.7 Hz, 1H, 6-H), 6.26 (ddd, J = 8.4, 2.6, 0.7 Hz, 1H, 4-H), 3.67 (d, J = 0.7 Hz, 3H, OCH_3) ppm.

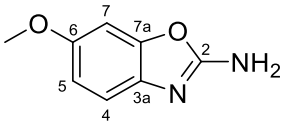
^{13}C NMR (101 MHz, CD_3OD): δ = 155.3 (C-5), 147.8 (C-1), 129.0 (C-2), 118.3 (C-3), 105.4 (C-4), 103.0 (C-6), 56.0 (OCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3354, 3285, 1609, 1581, 1521, 1448, 1256, 1200, 1162, 847, 768, 713 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_7H_8NO_2$ $[M-H]^-$: 138.0560; found: 138.0561.

Purity (HPLC): ND.

6-Methoxybenzo[*d*]oxazol-2-amine (113)



$C_8H_8N_2O_2$

M_w = 164.16 g/mol

Aminophenol **112** (209 mg, 1.50 mmol, 1.0 eq) and di(1*H*-imidazol-1-yl)methanimine (483 mg, 3.00 mmol, 2.0 eq) were dissolved in 3 mL ACN under nitrogen atmosphere. The solution was heated at 80 °C for 18 h. The solvents were removed under reduced pressure, and the residue was purified by flash column chromatography (1:1 EtOAc/isohexanes) to give benzoxazole **113** (138 mg, 0.841 mmol, 56%) as an old pink solid.

R_f: 0.43 (2:1 EtOAc/isohexanes).

Mp: 172 – 173 °C. [Ref.[166]: 168 – 170 °C.]

^{1}H NMR (400 MHz, CD_3OD): δ = 7.09 (d, J = 8.5 Hz, 1H, 4-H), 6.92 (d, J = 2.4 Hz, 1H, 7-H), 6.75 (dd, J = 8.5, 2.4 Hz, 1H, 5-H), 3.78 (d, J = 0.7 Hz, 3H, OCH_3) ppm.

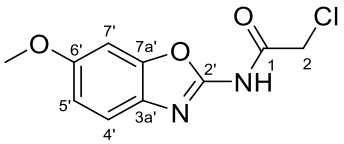
^{13}C NMR (101 MHz, CD_3OD): δ = 164.3 (C-2), 156.8 (C-6), 150.1 (C-7a), 137.1 (C-3a), 116.0 (C-4), 111.3 (C-5), 96.8 (C-7), 56.5 (OCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3426, 3042, 2956, 1661, 1482, 1434, 1391, 1136, 1023, 934, 819, 707 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_8H_9N_2O_2$ $[M+H]^+$: 165.0659; found: 165.0659.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(6-methoxybenzo[*d*]oxazol-2-yl)acetamide (114)



$C_{10}H_9ClN_2O_3$

M_w = 240.64 g/mol

Chloroacetamide **114** was prepared following General procedure E, using aminobenzoxazole **113** (197 mg, 1.20 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 1 h. After filtration, the residue was purified by flash column chromatography (1:1 EtOAc/isohexanes) to give product **114** (213 mg, 0.885 mmol, 74%) as an off-white solid.

R_f: 0.61 (1:2 EtOAc/isohexanes).

Mp: 160 – 161 °C.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.43 (d, *J* = 8.7 Hz, 1H, 4'-H), 7.12 (d, *J* = 2.4 Hz, 1H, 7'-H), 6.93 (dd, *J* = 8.8, 2.4 Hz, 1H, 5'-H), 4.34 (s, 2H, 2-H), 3.83 (s, 3H, OCH_3) ppm.

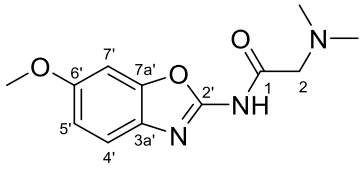
^{13}C NMR (101 MHz, CD_3OD): δ = 166.3 (C-1), 159.2 (C-6'), 157.4 (C-2'), 149.7 (C-7a'), 135.0 (C-3a'), 119.3 (C-4'), 113.5 (C-5'), 96.6 (C-7'), 56.5 (OCH_3), 43.9 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 2298, 1728, 1575, 1491, 1280, 1145, 1119, 1029, 845, 813, 806, 762 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{10}H_8ClN_5O_3$ $[M-H]^-$: 239.0228; found: 239.0230.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-(Dimethylamino)-*N*-(6-methoxybenzo[*d*]oxazol-2-yl)acetamide (106)



$C_{12}H_{15}N_3O_3$

M_w = 249.27 g/mol

2-(Dimethylamino)acetamide **106** was prepared following General procedure F, using chloroacetamide **114** (229 mg, 0.950 mmol, 1.0 eq) and dimethylamine (1.0 mL, 2 M in THF, 2.1 mmol, 2.2 eq). The mixture was stirred for 4 h, and the extraction was conducted with EtOAc (3 x 5 mL). The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/triethylamine), yielding product **106** (156 mg, 0.626 mmol, 67%) as an off-white solid.

R_f: 0.38 (96:3:1 DCM/MeOH/triethylamine).

Mp: 117 – 119 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.21 (s, 1H, CONH), 7.46 (d, J = 8.7 Hz, 1H, 4'-H), 7.28 (d, J = 2.4 Hz, 1H, 7'-H), 6.90 (dd, J = 8.7, 2.4 Hz, 1H, 5'-H), 3.80 (s, 3H, OCH_3), 3.25 (s, 2H, 2-H), 2.30 (s, 6H, $N(CH_3)_2$) ppm.

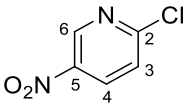
^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.4 (C-1), 156.7 (C-6'), 154.1 (C-2'), 148.6 (C-7a'), 134.0 (C-3a'), 118.3 (C-4'), 111.9 (C-5'), 95.8 (C-7'), 62.1 (C-2), 55.9 (OCH_3), 45.0 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1693, 1631, 1585, 1293, 1195, 1141, 1123, 1027, 839, 795, 712, 658 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{12}H_{16}N_5O_3$ $[M+H]^+$: 250.1187; found: 250.1187.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

2-Chloro-5-nitropyridine (117)



$C_5H_3ClN_2O_2$

M_w = 158.54 g/mol

2-Chloro-5-nitropyridine (**117**) was prepared as described in literature[120] from 2-hydroxy-5-nitropyridine (**116**, 981 mg, 7.00 mmol, 1.0 eq), using phosphorus oxychloride (12.4 mL, 133 mmol, 19 eq). Light yellow solid (985 mg, 6.21 mmol, 89%).

R_f: 0.79 (DCM).

Mp: 102 °C. [Ref.[120]: 110.2 – 108 °C.]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.24 (dd, J = 2.8, 0.7 Hz, 3H, 6-H), 8.49 – 8.41 (m, 3H, 4-H), 7.55 (dd, J = 8.7, 0.7 Hz, 3H, 3-H) ppm.

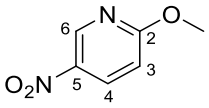
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.3 (C-2), 145.6 (C-6), 143.5 (C-5), 133.7 (C-4), 125.0 (C-3) ppm.

IR (ATR): $\tilde{\nu}$ =3093, 3048, 1660, 1587, 1560, 1345, 1253, 1108, 1012, 944, 853, 748 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_5H_3ClN_2O_2$ $[M]^{\cdot+}$: 157.9878; found: 157.9877.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-Methoxy-5-nitropyridine (118)



$C_6H_6N_2O_3$

M_w = 154.13 g/mol

2-Methoxy-5-nitropyridine (**118**) was prepared as described in literature[121] from 2-chloropyridine **117** (920 mg, 5.80 mmol, 1.0 eq), using potassium *tert*-butoxide (846 mg, 7.54 mmol, 1.3 eq). Colourless solid (810 mg, 5.26 mmol, 91%).

R_f: 0.80 (DCM).

Mp: 100 – 101 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.11 – 9.06 (m, 1H, 6-H), 8.38 – 8.31 (m, 1H, 4-H), 6.82 (dd, *J* = 9.2, 0.6 Hz, 1H. 3-H), 4.05 (d, *J* = 0.7 Hz, 3H, OCH_3) ppm.

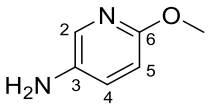
^{13}C NMR (101 MHz, $CDCl_3$): δ = 167.5 (C-2), 144.0 (C-6), 139.6 (C-5), 134.0 (C-4), 111.4 (C-3), 55.0 (OCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1603, 1574, 1502, 1487, 1347, 1314, 1298, 1118, 999, 845, 766, 661 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_6H_6N_2O_3$ $[M]^{\cdot+}$: 154.0373; found: 154.0371.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

6-Methoxypyridin-3-amine (119)



$C_6H_8N_2O$

M_w = 124.14 g/mol

Aminopyridine **119** was prepared following General procedure G from nitropyridine **118** (801 mg, 5.20 mmol, 1.0 eq), using palladium on charcoal (277 mg, 0.260 mmol, 10 wt%, 0.05 eq). The mixture was stirred for 1.5 h. The crude product was purified by flash column chromatography (3:1 EtOAc/isohexanes), yielding product **119** (580 mg, 4.67 mmol, 90%) as a brown oil. ^{1}H and ^{13}C NMR data is in accordance with literature[121].

R_f: 0.24 (DCM).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.66 (dd, *J* = 3.0, 0.7 Hz, 2H, 2-H), 7.03 (dd, *J* = 8.7, 3.0 Hz, 2H, 4-H), 6.60 (dd, *J* = 8.7, 0.8 Hz, 2H, 5-H), 3.86 (s, 6H, OCH_3) ppm.

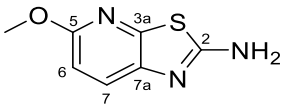
^{13}C NMR (101 MHz, $CDCl_3$): δ = 158.2 (C-6), 136.8 (C-3), 133.0 (C-2), 127.8 (C-4), 110.9 (C-5), 53.5 (OCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3337, 3219, 1577, 1488, 1407, 1268, 1245, 1027, 824, 737 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_6H_8N_2O$ $[M]^{\cdot+}$: 124.0631; found: 124.0631.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

5-Methoxythiazolo[5,4-*b*]pyridin-2-amine (115)



$C_7H_7N_3OS$

M_w = 181.21 g/mol

Thiazolopyridin-2-amine **115** was prepared as described in literature[122] from aminopyridine **119** (472 mg, 3.80 mmol, 1.0 eq), using potassium thiocyanate (1.11 g, 11.4 mmol, 3.0 eq) and bromine (0.234 mL, 4.56 mmol, 1.2 eq). Yellow solid (594 mg, 3.28 mmol, 86%).

R_f: 0.19 (98:2 DCM/MeOH).

Mp: 186 – 189 °C.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.57 (d, *J* = 8.6 Hz, 1H, 6-H), 6.68 (d, *J* = 8.7 Hz, 1H, 7-H), 3.89 (s, 3H, OCH_3) ppm.

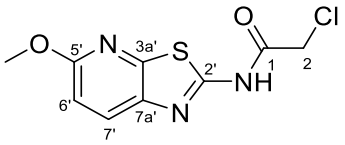
^{13}C NMR (101 MHz, CD_3OD): δ = 167.0 (C-2), 161.6 (C-5), 151.9 (C-3a), 141.7 (C-7a), 128.7 (C-6), 109.1 (C-7), 54.3 (OCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 2946, 1643, 1588, 1536, 1463, 1418, 1374, 1256, 1029, 819, 705 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_7H_8N_3OS$ $[M+H]^{+}$: 182.0383; found: 182.0384.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(5-methoxythiazolo[5,4-*b*]pyridin-2-yl)acetamide (120)



$C_9H_8ClN_3O_2S$

M_w = 257.69 g/mol

Chloroacetamide **120** was prepared following General procedure E, using thiazolopyridin-2-amine **115** (598 mg, 3.30 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 2 h. The extraction gave product **120** (847 mg, 3.29 mmol, quant.) as an orange solid.

R_f: 0.74 (95:5 DCM/MeOH).

Mp: 166 – 169 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.90 (d, J = 8.7 Hz, 1H, 7'-H), 6.85 (d, J = 8.8 Hz, 1H, 6'-H), 4.31 (s, 2H, C-2), 4.00 (s, 3H, OCH_3) ppm.

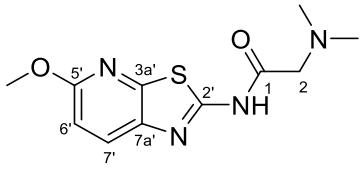
^{13}C NMR (126 MHz, $CDCl_3$): δ = 164.5 (C-1), 162.3 (C-5'), 154.3 (C-2'), 152.1 (C-3a'), 136.0 (C-7a'), 130.8 (C-7'), 110.6 (C-6'), 54.3 (OCH_3), 42.2 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 1711, 1591, 1569, 1471, 1375, 1265, 1169, 1022, 806, 731, 692 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_9H_9ClN_3O_2S$ $[M+H]^+$: 258.0099; found: 258.0100.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

2-(Dimethylamino)-*N*-(5-methoxythiazolo[5,4-*b*]pyridin-2-yl)acetamide (107)



$C_{11}H_{14}ClN_4O_2S$

M_w = 266.32 g/mol

2-(Dimethylamino)acetamide **107** was prepared following General procedure F, using crude chloroacetamide **120** (438 mg, 1.70 mmol, 1.0 eq) and dimethylamine (1.87 mL, 2 M in THF, 3.74 mmol, 2.2 eq). The mixture was stirred for 2 h, and the extraction was conducted with EtOAc (3 x 20 mL). The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/triethylamine), yielding product **107** (292 mg, 1.10 mmol, 65%) as a beige solid.

R_f: 0.40 (96:3:1 DCM/MeOH/triethylamine).

Mp: 115 – 117 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.99 (s, 1H, CONH), 8.02 (d, J = 8.7 Hz, 1H, 6'-H), 6.90 (d, J = 8.8 Hz, 1H, 7'-H), 3.91 (s, 3H, OCH_3), 3.29 (s, 2H, 2-H), 2.30 (s, 6H, $N(CH_3)_2$) ppm.

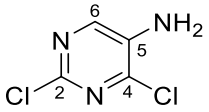
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.6 (C-1), 161.1 (C-5'), 154.1 (C-2'), 151.3 (C-3a'), 136.8 (C-7a'), 131.0 (C-6'), 109.6 (C-7'), 61.2 (C-2), 53.8 (OCH_3), 45.0 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1703, 1587, 1526, 1463, 1444, 1371, 1255, 1015, 826, 818, 739, 692 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{11}H_{14}ClN_4O_2S$ $[M+H]^+$: 267.0911; found: 267.0913.

Purity (HPLC): >96% (λ = 210 nm), >92% (λ = 254 nm), Method 1a.

2,4-Dichloropyrimidin-5-amine (122)



$C_4H_3Cl_2N_3$

M_w = 163.99 g/mol

2,4-Dichloro-5-nitropyrimidine (**121**, 1.16 g, 6.00 mmol, 1.0 eq) and $SnCl_2$ (6.78 g, 30.0 mmol, 5.0 eq) were suspended in 12 mL EtOH. The mixture was refluxed at 80 °C for 1 h. After cooling in an ice-bath, cold water (15 mL) was added, the mixture was neutralised with $NaHCO_3$ sat. aq. solution and extracted with EtOAc (3 x 20 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was further purified by flash column chromatography (1:4 EtOAc/isohexanes), yielding pyrimidin-5-amine **122** (888 mg, 5.41 mmol, 90%) as a red solid.

R_f: 0.25 (1:3 EtOAc/isohexanes).

Mp: 108 – 111 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (s, 1H, C-6), 3.82 (s br, 2H, NH) ppm.

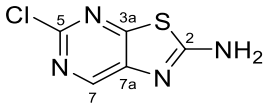
^{13}C NMR (101 MHz, $CDCl_3$): δ = 148.1 (C-2, C-4 or C-5), 146.2 (C-2, C-4 or C-5), 144.5 (C-1), 137.2 (C-2, C-4 or C-5) ppm.

IR (ATR): $\tilde{\nu}$ = 3228, 2923, 2472, 1631, 1517, 1408, 1186, 1124, 875, 731, 664 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_4H_3Cl_2N_3$ $[M]^{\cdot+}$: 162.96.99; found: 162.96.98.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

5-Chlorothiazolo[5,4-*d*]pyrimidin-2-amine (123)



$C_5H_3ClN_4S$

M_w = 186.62 g/mol

Aminopyrimidine **122** (868 mg, 5.29 mmol, 1.0. eq) and potassium thiocyanate (1.03 g, 10.6 mmol, 2.0 eq) were dissolved in 13 mL acetic acid, and the solution was stirred at 95 °C for 3 h. After cooling in an ice-bath, the mixture was neutralised with conc. ammonia aq. solution and extracted with EtOAc (3 x 80 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was further purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding thiazolopyrimidin-2-amine **123** (751 mg, 4.02 mmol, 76%) as a pale yellow solid.

R_f: 0.46 (2:1 EtOAc/isohexanes).

Mp: 250 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.53 (s, 1H, 7-H), 8.29 (s, 2H, NH_2) ppm.

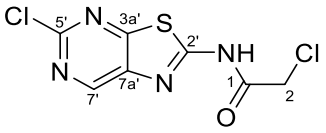
^{13}C NMR (101 MHz, DMSO-d_6): δ = 166.3 (C-3a), 166.1 (C-2), 149.6 (C-5), 144.9 (C-7a), 143.9 (C-7) ppm.

IR (ATR): $\tilde{\nu}$ = 1661, 1586, 1526, 1512, 1373, 1354, 1333, 1229, 1118, 898, 782, 710 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_5H_2ClN_4S$ $[M-H]^-$: 184.9694; found: 184.9694.

Purity (HPLC): >98% (λ = 210 nm), >98% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(5-chlorothiazolo[5,4-*d*]pyrimidin-2-yl)acetamide (127)



$C_7H_4Cl_2N_4OS$

M_w = 263.11 g/mol

Chloroacetamide **127** was prepared following General procedure E, using thiazolopyrimidin-2-amine **123** (373 mg, 2.00 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 2 h. After extraction, the residue was purified by flash column chromatography (1:2 EtOAc/isohexanes) to give product **127** (394 mg, 1.50 mmol, 75%) as a beige solid.

R_f: 0.35 (1:2 EtOAc/isohexanes).

Mp: 142 – 144 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.97 (s, 1H, 7'-H), 4.37 (s, 2H, 2-H) ppm.

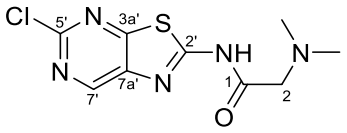
^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.9 (C-3a'), 165.3 (C-1), 157.7 (C-2'), 155.3 (C-5'), 150.1 (C-7'), 139.4 (C-7a'), 42.1 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 1705, 1584, 1515, 1373, 1255, 1220, 1155, 1109, 812, 777, 739, 707 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_7H_3Cl_2N_4OS$ $[M-H]^-$: 260.9410; found: 260.9411.

Purity (HPLC): ND.

N-(5-Chlorothiazolo[5,4-*d*]pyrimidin-2-yl)-2-(dimethylamino)acetamide (126)



$C_9H_{10}ClN_5OS$

M_w = 271.73 g/mol

2-(Dimethylamino)acetamide **126** was prepared following General procedure F, using chloroacetamide **127** (342 mg, 1.30 mmol, 1.0 eq) and dimethylamine (1.43 mL, 2 M in THF, 2.86 mmol, 2.2 eq). The mixture was stirred for 2 h, and the extraction was conducted with EtOAc (3 x 20 mL). The crude product was purified by flash column chromatography (96:3:1 DCM/MeOH/triethylamine), yielding product **126** (185 mg, 0.681 mmol, 52%) as an off-white solid.

R_f: 0.42 (89:10:1 DCM/MeOH/triethylamine).

Mp: 180 – 181 °C (decomposition), 248 – 250 °C (melting).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.84 (s, 1H, H-7'), 3.69 (s, 2H, 2-H), 2.61 (s, 6H, $N(CH_3)_2$) ppm.

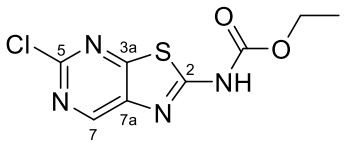
^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.9 (C-1), 166.4 (C-3a), 163.1 (C-9), 151.4 (C-5'), 147.2 (C-7'), 141.0 (C-7a'), 61.0 (C-2), 44.1 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3005, 1581, 1566, 1450, 1367, 1317, 1190, 955, 780, 767, 711, 666 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_9H_{11}ClN_5OS$ $[M+H]^+$: 272.0368; found: 272.0373.

Purity (HPLC): >97% (λ = 210 nm), >97% (λ = 254 nm), Method 3c.

Ethyl (5-chlorothiazolo[5,4-*d*]pyrimidin-2-yl)carbamate (125)



$C_8H_7ClN_4O_2S$

M_w = 258.69 g/mol

To a suspension of thiazolopyrimidin-2-amine **123** (700 mg, 3.75 mmol, 1.0 eq) in 8 mL 1,4-dioxane, ethyl chloroformate (0.43 mL, 4.5 mmol, 1.2 eq) and pyridine (0.36 mL, 4.5 mmol, 1.2 eq) were added at 0 °C. The mixture was stirred at the same temperature for 2 h. Water (80 mL) was added, and the mixture was extracted with EtOAc (3 x 80 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was further purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding carbamate **125** (813 mg, 3.14 mmol, 84%) as a colourless solid.

R_f: 0.20 (1:1 EtOAc/isohexanes).

Mp: 150 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.66 (s, 1H, NH), 9.04 (s, 1H, 7-H), 4.29 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 1.29 (t, J = 7.1 Hz, 3H, OCH_2CH_3) ppm.

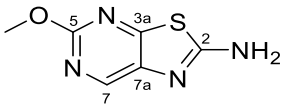
^{13}C NMR (101 MHz, DMSO-d_6): δ = 165.7 (C-3a), 160.3 (C-2), 154.1 (CONH), 152.4 (C-5), 149.0 (C-7), 140.8 (C-7a), 62.7 (OCH_2CH_3), 14.2 (OCH_2CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 2920, 1727, 1588, 1361, 1288, 1219, 1170, 1057, 778, 759, 719 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_8H_6ClN_4O_2S$ $[M-H]^-$: 256.9905; found: 256.9905.

Purity (HPLC): ND.

5-Methoxythiazolo[5,4-*d*]pyrimidin-2-amine (124)



$C_6H_6N_4OS$

M_w = 182.21 g/mol

5-Methoxythiazolopyrimidin-2-amine **124** was prepared as described in literature[124] from carbamate **125** (259 mg, 1.00 mmol, 1.0 eq), using sodium methoxide (10.2 mL, 25% in MeOH, 46.0 mmol, 46 eq). Colourless solid (155 mg, 0.851 mmol, 85%).

R_f: 0.45 (95:5 DCM/MeOH).

Mp: 265 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.41 (s, 1H, 7-H), 7.82 (s, 2H, NH_2), 3.88 (s, 3H, OCH_3) ppm.

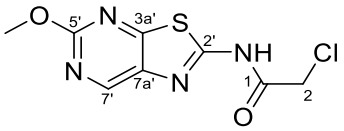
^{13}C NMR (101 MHz, DMSO-d_6): δ = 166.0 (C-3a), 163.3 (C-2), 159.6 (C-5), 143.7 (C-7), 141.0 (C-7a), 54.7 (OCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3293, 3111, 1651, 1529, 1466, 1377, 1335, 1271, 1038, 913, 776, 667 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_6H_7N_4OS$ $[M+H]^+$: 183.0336; found: 183.0336.

Purity (HPLC): >83% (λ = 210 nm), >90% (λ = 254 nm), Method 1e.

2-Chloro-*N*-(5-methoxythiazolo[5,4-*d*]pyrimidin-2-yl)acetamide (128)



$C_8H_7N_4O_2S$

M_w = 258.68 g/mol

Chloroacetamide **128** was prepared following General procedure E, using thiazolopyrimidin-2-amine **124** (149 mg, 0.820 mmol, 1.0 eq) and 2-chloroacetyl chloride in THF for 2 h. After extraction, the residue was purified by flash column chromatography (1:1 EtOAc/isohexanes) to give product **128** (210 mg, 0.812 mmol, 99%) as a beige solid.

R_f: 0.49 (1:1 EtOAc/isohexanes).

Mp: 250 °C (decomposition).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.93 (s, 1H, NH), 9.00 (s, 1H, 7'-H), 4.49 (s, 2H, 2-H), 3.98 (s, 3H, OCH_3) ppm.

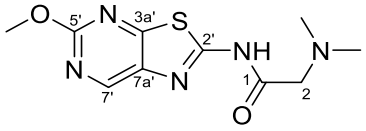
^{13}C NMR (126 MHz, DMSO-d_6): δ = 166.5 (C-1), 165.0 (C-3a'), 161.5 (C-5), 154.7 (C-2'), 149.9 (C-7'), 136.2 (C-7a'), 55.2 (OCH_3), 42.5 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 1703, 1598, 1563, 1470, 1390, 1378, 1333, 1255, 1026, 785, 744, 679 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_8H_7N_4O_2S$ $[M]^{\cdot+}$: 257.9973; found: 257.9974.

Purity (HPLC): ND.

2-(Dimethylamino)-*N*-(5-methoxythiazolo[5,4-*d*]pyrimidin-2-yl)acetamide (108)



$C_{10}H_{13}N_5O_2S$

M_w = 267.31 g/mol

2-(Dimethylamino)acetamide **108** was prepared following General procedure F, using chloroacetamide **128** (199 mg, 0.770 mmol, 1.0 eq) and dimethylamine (0.85 mL, 2 M in THF, 1.7 mmol, 2.2 eq). The mixture was stirred for 1 h, and the extraction was conducted with $CHCl_3$/isopropanol (3:1, 5 x 15 mL). The crude product was purified by flash column chromatography (94:5:1 DCM/MeOH/triethylamine), yielding product **108** (80 mg, 0.30 mmol, 39%) as a colourless solid.

R_f: 0.39 (98:1:1 DCM/MeOH/triethylamine).

Mp: 220 – 223 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.90 (s, 1H, CONH), 8.89 (s, 1H, 7'-H), 3.96 (s, 3H, OCH_3), 3.39 (s, 2H, 2-H), 2.36 (s, 6H, $N(CH_3)_2$) ppm.

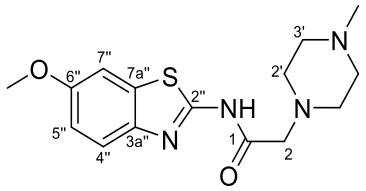
^{13}C NMR (126 MHz, DMSO-d_6): δ = 170.3 (C-1), 165.3 (C-3a'), 161.2 (C-5'), 156.1 (C-2'), 148.9 (C-7'), 136.4 (C-7a'), 61.2 (C-2), 55.0 (OCH_3), 44.8 ($N(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1712, 1595, 1468, 1386, 1376, 1330, 1261, 1154, 1030, 824, 722, 687 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{10}H_{13}N_5O_2S$ $[M]^{\cdot+}$: 267.0790; found: 267.0784.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3b.

***N*-(6-Methoxybenzo[*d*]thiazol-2-yl)-2-(4-methylpiperazin-1-yl)acetamide (132)**



$C_{15}H_{20}N_4O_2S$

M_w = 320.41 g/mol

Amine **132** was prepared following General procedure F, using chloroacetamide **67** (100 mg, 0.390 mmol, 1.0 eq) and 1-methylpiperazine (95 µL, 0.86 mmol, 2.2 eq). The mixture was stirred for 18 h, and the extraction was conducted with EtOAc. The residue was recrystallised from DCM to give **132** (98 mg, 0.31 mmol, 79%) as a dark yellow solid.

Mp: 104 – 105 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.31 (s br, 1H, NH), 7.68 (d, *J* = 9.0 Hz, 1H, 4''-H), 7.29 (d, *J* = 2.5 Hz, 1H, 7''-H), 7.07 – 7.01 (m, 1H, 4''-H), 3.87 (s, 3H, OCH_3), 3.29 (s, 2H, 2-H), 2.71 (d, *J* = 5.0 Hz, 4H, 3'-H), 2.67 – 2.54 (m, 4H, 2'-H), 2.37 (s, 3H, NCH_3) ppm.

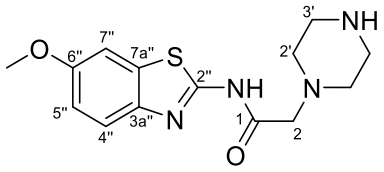
^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.9 (C-1), 157.1 (C-6''), 155.2 (C-2''), 142.8 (C-3a''), 133.6 (C-7a''), 121.7 (C-4''), 115.5 (C-5''), 104.4 (C-7''), 61.1 (C-2), 56.0 (OCH_3), 54.9 (C-2'), 53.4 (C-3'), 45.9 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 2819, 1688, 1604, 1543, 1472, 1260, 1222, 1137, 1057, 829, 818, 695 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{15}H_{20}N_4O_2S$ $[M]^{\cdot+}$: 320.1302; found: 320.1299.

Purity (HPLC): >97% (λ = 210 nm), >98% (λ = 254 nm), Method 1a.

N-(6-Methoxybenzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (133)



$C_{14}H_{18}N_4O_2S$

M_w = 306.39 g/mol

Amine **133** was prepared following General procedure F, using chloroacetamide **67** (257 mg, 1.00 mmol, 1.0 eq), piperazine (96 mg, 1.1 mmol, 1.1 eq) and triethylamine (0.153 mL, 1.10 mmol, 1.1 eq). The mixture was stirred for 2 h, and the extraction was conducted with $CHCl_3$/isopropanol. The residue was purified by flash column chromatography (88:10:2 DCM/MeOH/triethylamine), yielding product **133** (70 mg, 0.23 mmol, 23%) as a colourless solid.

R_f: 0.56 (89:10:1 DCM/MeOH/triethylamine).

Mp: 243 – 245 °C (decomposition and melting).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.63 (d, *J* = 8.8 Hz, 1H, 4''-H), 7.57 (d, *J* = 2.6 Hz, 1H, 7''-H), 7.03 (dd, *J* = 8.8, 2.6 Hz, 1H, 5''-H), 3.80 (s, 3H, OCH_3), 3.27 (s, 2H, 2-H), 2.72 (t, *J* = 4.8 Hz, 4H, 3'-H), 2.45 (t, *J* = 4.5 Hz, 4H, 2'-H) ppm.

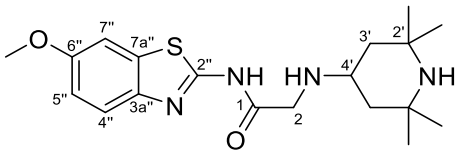
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.2 (C-1), 156.1 (C-6''), 155.5 (C-2''), 142.5 (C-3a''), 132.8 (C-7a''), 121.1 (C-4''), 114.9 (C-5''), 104.7 (C-7''), 61.0 (C-2), 55.6 (OCH_3), 53.8 (C-2'), 45.5 (C-3') ppm.

IR (ATR): $\tilde{\nu}$ = 2256, 1686, 1604, 1546, 1523, 1467, 1263, 1022, 977, 833, 813, 752 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{14}H_{19}N_4O_2S$ $[M+H]^+$: 307.1224; found: 307.1229.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

N-(6-Methoxybenzo[*d*]thiazol-2-yl)-2-((2,2,6,6-tetramethylpiperidin-4-yl)amino)acetamide (136)



$C_{19}H_{28}N_4O_2S$

M_w = 376.52 g/mol

Amine **136** was prepared following General procedure F, using chloroacetamide **67** (257 mg, 1.00 mmol, 1.0 eq), 4-amino-2,2,6,6-tetramethylpiperidine (172 mg, 1.10 mmol, 1.1 eq), and triethylamine (0.153 mL, 1.10 mmol, 1.1 eq). The mixture was stirred for 2 h, and the extraction was conducted with $CHCl_3$/isopropanol. The residue was purified by flash column chromatography (93:6:1 DCM/MeOH/triethylamine), yielding product **136** (176 mg, 0.467 mmol, 47%) as a colourless solid.

R_f: 0.44 (93:6:1 DCM/MeOH/triethylamine).

Mp: 170 – 171 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.61 (d, *J* = 8.8 Hz, 1H, 4''-H), 7.55 (d, *J* = 2.6 Hz, 1H, 7''-H), 7.01 (dd, *J* = 8.8, 2.6 Hz, 1H, 5''-H), 3.80 (s, 3H, OCH_3), 3.49 (s, 2H, 2-H), 2.87 (ddt, *J* = 11.6, 7.1, 3.5 Hz, 1H, 4'-H), 1.72 (dd, *J* = 12.4, 3.5 Hz, 2H, 3'-H), 1.09 (s, 6H, $C(CH_3)_2$), 1.02 (s, 6H, $C(CH_3)_2$), 0.84 (t, *J* = 11.9 Hz, 2H, 3'-H) ppm.

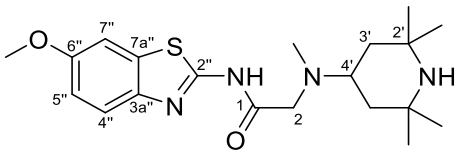
^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.7 (C-1), 156.1 (C-2'' or C-6''), 156.0 (C-2'' or C-6''), 142.7 (C-3a''), 132.9 (7a''), 121.0 (C-4''), 114.8 (C-5''), 104.8 (C-7''), 55.6 (OCH_3), 50.6 (C-2'), 49.4 (C-4'), 49.1 (C-2), 45.0 (C-3'), 34.6 ($C(\underline{C}H_3)_2$), 28.8 ($C(\underline{C}H_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1675, 1603, 1543, 1469, 1257, 1225, 1122, 1030, 859, 825, 738, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{19}H_{29}N_4O_2S$ $[M+H]^+$: 377.2006; found: 377.2008.

Purity (HPLC): >97% (λ = 210 nm), >95% (λ = 254 nm), Method 1a.

_N_-(6-Methoxybenzo[_d_]thiazol-2-yl)-2-(methyl(2,2,6,6-tetramethylpiperidin-4-yl)amino)acetamide (137)



$C_{20}H_{30}N_4O_2S$

M_w = 390.55 g/mol

Amine **137** was prepared following General procedure F, using chloroacetamide **67** (257 mg, 1.00 mmol, 1.0 eq), *N*,2,2,6,6-pentamethylpiperidin-4-amine (0.213 mL, 1.10 mmol, 1.1 eq), and triethylamine (0.153 mL, 1.10 mmol, 1.1 eq). The mixture was stirred for 4 h, and the extraction was conducted with $CHCl_3$/isopropanol. The residue was purified by flash column chromatography (96:3:1 DCM/MeOH/triethylamine), yielding product **137** (187 mg, 0.479 mmol, 48%) as a pale yellow solid.

R_f: 0.16 (96:3:1 DCM/MeOH/triethylamine).

Mp: 47 – 48 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.62 (d, *J* = 8.8 Hz, 1H, 4''-H), 7.56 (d, *J* = 2.6 Hz, 1H, 7''-H), 7.03 (dd, *J* = 8.8, 2.6 Hz, 1H, 5''-H), 3.80 (s, 3H, OCH_3), 3.36 (s, 2H, 2-H), 3.04 – 2.93 (m, 1H, 4'-H), 2.30 (s, 3H, NCH_3), 1.62 (dd, *J* = 12.3, 3.1 Hz, 2H, 3'-H), 1.11 (s, 6H, $C(CH_3)_2$), 1.06 (s, 1H, $C(CH_3)_2$), 1.03 (s, 8H, $C(CH_3)_2$), 1.06 - 0.99 (m, 3'-H) ppm.

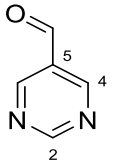
^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.7 (C-1), 156.1 (C-2'' or C-6''), 155.5 (C-2'' or C-6''), 142.6 (C-3a''), 132.8 (7a''-H), 121.1 (C-4''), 114.9 (C-5''), 104.8 (C-7''), 56.6 (C-2), 55.6 (OCH_3), 55.0 (C-4'), 50.7 (C-2'), 40.3 (C-3'), 38.2 (NCH_3), 34.8 ($C(\underline{C}H_3)_2$), 28.7 ($C(\underline{C}H_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 2829, 1692, 1604, 1536, 1467, 1435, 1256, 1220, 1059, 1028, 822, 698 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{20}H_{31}N_4O_2S$ $[M+H]^+$: 391.2163; found: 391.2177.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

Pyrimidine-5-carbaldehyde (147)



$C_5H_4N_2O_2$

M_w = 108.10 g/mol

Aldehyde **147** was prepared as described in literature[131], treating pyrimidine-5-methanol (**146**, 899 mg, 8.00 mmol, 1.0 eq) with MnO_2 (6.95 g, 80.0 mmol, 10 eq). Yellow oil (35 mg, 2.8 mmol, 35%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.17 (s, 1H, CHO), 9.44 (s, 1H, 2-H), 9.19 (s, 2H, 4-H) ppm.

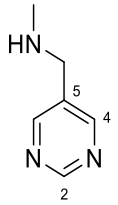
^{13}C NMR (101 MHz, $CDCl_3$): δ = 190.3 (CHO), 163.1 (C-2), 158.5 (C-4), 129.2 (C-5) ppm.

IR (ATR): $\tilde{\nu}$ = 3216, 1672, 1590, 1517, 1410, 1318, 1216, 723 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_5H_5N_2O$ $[M+H]^+$: 109.0397; found: 109.0399.

Purity (HPLC): ND.

N-Methyl-1-(pyrimidin-5-yl)methanamine (145)



$C_6H_9N_3$

M_w = 123.16 g/mol

Aldehyde **147** (270 mg, 2.50 mmol, 1.0 eq) was dissolved in methylamine solution (4.05 mL, 33% in EtOH, 32.5 mmol, 13 eq). AcOH (0.05 mL) was added, and the mixture was stirred at room temperature for 2 h. After cooling with an ice-bath, $NaBH_4$ (189 mg, 5.00 mmol, 2.0 eq) was added, and the mixture was stirred for 1 h. Aq. sat. $NaHCO_3$ solution (20 mL) was added, and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 4 x 20 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure to give amine **145** (216 mg, 1.75 mmol, 70%) as a yellow oil. The crude product was used without further purification for the next step.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.12 (s, 1H, 2-H), 8.70 (d, J = 0.8 Hz, 2H, 4-H), 3.78 – 3.75 (m, 2H, CH_2), 2.46 (s, 3H, CH_3) ppm.

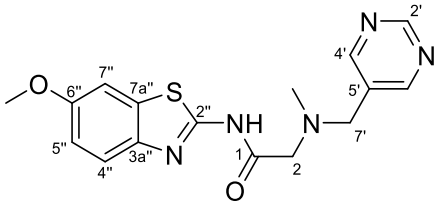
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.9 (C-2), 156.9 (C-4), 133.3 (C-5), 51.1 (CH_2), 36.2 (CH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3287, 2943, 1661, 1563, 1440, 1405, 1029, 727, 680 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_6H_9N_3$ $[M]^{\cdot+}$: 123.0791; found: 123.0789.

Purity (HPLC): ND.

_N_-(6-Methoxybenzo[_d_]thiazol-2-yl)-2-(methyl(pyrimidin-5-ylmethyl)amino)acetamide (138)



$C_{16}H_{17}N_5O_2S$

M_w = 343.41 g/mol

Amine **138** was prepared following General procedure F, using chloroacetamide **67** (282 mg, 1.10 mmol, 1.0 eq), amine **145** (149 mg, 1.21 mmol, 1.1 eq) and triethylamine (0.169 mL, 1.21 mmol, 1.1 eq). The mixture was stirred for 1 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (97:3 DCM/MeOH), yielding product **138** (134 mg, 0.390 mmol, 36%) as an off-white solid.

Mp: 155 – 156 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.06 (s, 1H, NH), 9.10 (s, 1H, 2'-H), 8.81 (s, 2H, 4'-H), 7.64 (d, J = 8.8 Hz, 1H, 5''-H), 7.57 (d, J = 2.6 Hz, 1H, 7''-H), 7.03 (dd, J = 8.8, 2.6 Hz, 1H, 4''-H), 3.81 (s, 3H, OCH_3), 3.75 (s, 2H, 7'-H), 3.47 (s, 2H, 2-H), 2.29 (s, 3H, NCH_3) ppm.

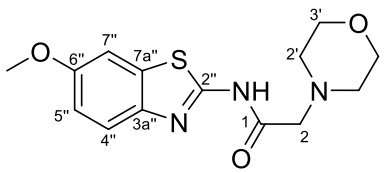
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.6 (C-1), 157.4 (C-2'), 157.3 (C-4'), 156.1 (C-6''), 155.5 (C-2''), 142.6 (C-3a''), 132.8 (C-7a''), 131.9 (C-5'), 121.1 (C-4''), 114.9 (C-5''), 104.7 (C-7''), 59.1 (C-2), 55.6 (OCH_3), 55.4 (C-7'), 41.7 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1694, 1606, 1567, 1468, 1286, 1258, 1223, 1177, 1056, 959, 824, 812 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{16}H_{16}N_5O_2S$ $[M-H]^-$: 342.1030; found: 342.1030.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

_N_-(6-Methoxybenzo[_d_]thiazol-2-yl)-2-morpholinoacetamide (139)



$C_{14}H_{17}N_3O_3S$

M_w = 307.37 g/mol

Amide **139** was prepared as described in literature[130] from chloroacetamide **67** (250 mg, 0.974 mmol, 1.0 eq) and morpholine (0.187 mL, 2.14 mmol, 2.2 eq). Bright beige solid (200 mg, 0.650 mmol, 67%).

Mp: 132 – 133 °C. [Ref.[167]: 129 – 130 °C.]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.96 (s, 1H, NH), 7.63 (d, J = 8.8 Hz, 1H, 4''-H), 7.57 (d, J = 2.5 Hz, 1H, 7''-H), 7.03 (dd, J = 8.8, 2.6 Hz, 1H, 5''-H), 3.80 (s, 3H, OCH_3), 3.66 – 3.56 (m, 4H, 3'-H), 3.32 (s, 2H, 2-H), 2.58 – 2.52 (m, 4H, 2'-H) ppm.

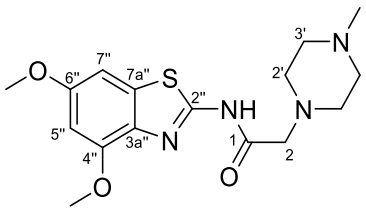
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.0 (C-1), 156.2 (C-6''), 155.4 (C-2''), 142.5 (C-3a''), 132.8 (C-7a''), 121.1 (C-4''), 115.0 (C-5''), 104.8 (C-7''), 66.1 (C-3'), 60.5 (C-2), 55.6 (OCH_3), 53.0 (C-2'') ppm.

IR (ATR): $\tilde{\nu}$ = 1682, 1606, 1543, 1293, 1263, 1226, 1112, 1013, 907, 863, 807, 744 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{14}H_{18}N_3O_3S$ $[M+H]^+$: 308.1064; found: 308.1064.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 2a.

N-(4,6-Dimethoxybenzo[*d*]thiazol-2-yl)-2-(4-methylpiperazin-1-yl)acetamide (134)



$C_{16}H_{22}N_4O_3S$

M_w = 350.44 g/mol

Amine **134** was prepared following General procedure F, using chloroacetamide **82** (143 mg, 0.500 mmol, 1.0 eq) and 1-methylpiperazine (0.122 mL, 1.10 mmol, 2.2 eq). The mixture was stirred for 2 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (93:6:1 DCM/MeOH/triethylamine), yielding product **134** (160 mg, 0.457 mmol, 91%) as a colourless solid.

R_f: 0.18 (94:5:1 DCM/MeOH/triethylamine).

Mp: 162 – 164 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.11 (d, J = 2.3 Hz, 1H, 7''-H), 6.59 (d, J = 2.2 Hz, 1H, 5''-H), 3.88 (s, 3H, (C-4'')OCH_3), 3.80 (s, 3H, (C-6'')OCH_3), 3.28 (s, 2H, 2-H), 2.54 – 2.50 (m, 4H, 2'-H), 2.34 (s, 4H, 3'-H), 2.16 (s, 3H, NCH_3) ppm.

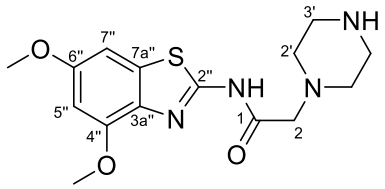
^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.9 (C-1), 157.3 (C-6''), 153.7 (C-2''), 152.2 (C-4''), 133.5 (C-3a'' or C-7a''), 132.7 (C-3a'' or C-7a''), 98.0 (C-5''), 95.7 (C-7''), 60.2 (C-2), 55.8 ((C-4'')O<u>C</u>H$_3$ or (C-6'')O<u>C</u>H$_3$), 55.7 ((C-4'')O<u>C</u>H$_3$ or (C-6'')O<u>C</u>H$_3$), 54.6 (C-3'), 52.5 (C-2'), 45.8 (NCH$_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2809, 1686, 1603, 1541, 1457, 1281, 1261, 1150, 1043, 820, 751, 659 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{16}H_{21}N_4O_3S$ [M-H]$^-$: 349.1339; found: 349.1335.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

N-(4,6-Dimethoxybenzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (135)



$C_{15}H_{20}N_4O_3S$

M_w = 336.41 g/mol

Amine **135** was prepared following General procedure F, using chloroacetamide **82** (143 mg, 0.500 mmol, 1.0 eq), piperazine (48 mg, 0.55 mmol, 1.1 eq) and triethylamine (77 µL, 0.55 mmol, 1.1 eq). The mixture was stirred for 2 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (93:6:1 DCM/MeOH/triethylamine), yielding product **135** (75 mg, 0.22 mmol, 44%) as a colourless solid.

R_f: 0.24 (94:5:1 DCM/MeOH/triethylamine).

Mp: 185 – 187 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.11 (d, *J* = 2.3 Hz, 1H, 7''-H), 6.59 (d, *J* = 2.3 Hz, 1H, 5''-H), 3.88 (s, 3H, (C-4'')OCH$_3$), 3.80 (s, 3H, (C-6'')OCH$_3$), 3.25 (s, 2H, 2-H), 2.73 (t, *J* = 4.8 Hz, 4H, 3'-H), 2.45 (t, *J* = 4.8 Hz, 4H, 2'-H) ppm.

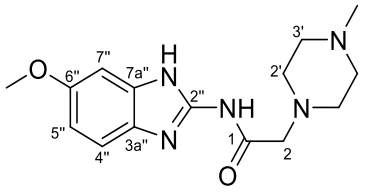
^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.0 (C-1), 157.3 (C-6''), 153.8 (C-2''), 152.2 (C-4''), 133.5(C-3a'' or C-7a''), 132.7 (C-3a'' or C-7a''), 98.0 (C-5''), 95.7 (C-7''), 61.0 (C-2), 55.8 ((C-4'')O<u>C</u>H$_3$ or (C-6'')O<u>C</u>H$_3$), 55.7 ((C-4'')O<u>C</u>H$_3$ or (C-6'')O<u>C</u>H$_3$), 53.7 (C-3'), 45.4 (C-2') ppm.

IR (ATR): $\tilde{\nu}$ = 2851, 1681, 1601, 1534, 1459, 1259, 1212, 1148, 1042, 806, 777, 749 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{15}H_{20}N_4O_3S$ [M]$^{\cdot+}$: 336.1251; found: 336.1254.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

_N_-(6-Methoxy-1_H_-benzo[_d_]imidazol-2-yl)-2-(4-methylpiperazin-1-yl)acetamide (140)



$C_{15}H_{21}N_5O_2$

M_w = 303.37 g/mol

Amine **140** was prepared following General procedure F, using chloroacetamide **110** (204 mg, 1.40 mmol, 1.0 eq) and 1-methylpiperazine (0.342, 3.08 mmol, 2.2 eq). The mixture was stirred for 1 h, and the extraction was conducted with $CHCl_3$/isopropanol. The residue was purified by flash column chromatography (88:10:2 DCM/MeOH/triethylamine), yielding product **140** (204 mg, 0.672 mmol, 48%) as a colourless solid.

R_f: 0.44 (96:3:1 DCM/MeOH/triethylamine).

Mp: 115 – 117 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.48 (s, 1H, CONH), 7.31 (d, J = 8.7 Hz, 1H, 4''-H), 6.99 (d, J = 2.4 Hz, 1H, 7''-H), 6.71 (dd, J = 8.7, 2.5 Hz, 1H, 5''-H), 3.74 (s, 3H, OCH_3), 3.25 (s, 2H, 2-H), 2.55 (s br, 4H, 2'-H or 3'-H), 2.35 (s br, 4H, 2'-H or 3'-H), 2.16 (s, 3H, NCH_3) ppm.

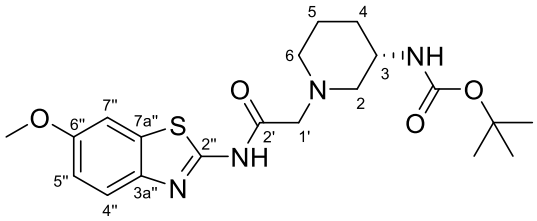
^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.3 (C-1), 155.0 (C-6''), 109.7 (C-5''), 60.5 (C-2), 55.4 (OCH_3), 54.7 (C-3'), 52.5 (C-2'), 45.8 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 2799, 1684, 1575, 1436, 1282, 1155, 1133, 1016, 836, 785, 719 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{15}H_{22}N_5O_2$ $[M+H]^+$: 304.1768; found: 304.1775.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

***tert*-Butyl (*S*)-(1-(2-((6-methoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)carbamate (141)**



$C_{20}H_{28}N_4O_4S$

M_w = 420.53 g/mol

Piperidinecarbamate **141** was prepared following General procedure F, using chloroacetamide **67** (257 mg, 1.00 mmol, 1.0 eq), (*S*)-3-(*tert*-butoxycarbonylamino)piperidine (220 mg, 1.10 mmol, 1.1 eq) and triethylamine (0.153 mL, 1.10 mmol, 1.1 eq). The mixture was stirred for 1 h, and the extraction was conducted with EtOAc (3:1, 5 x 30 mL). The crude product was purified by flash column chromatography (97:3 DCM/MeOH), yielding product **141** (380 mg, 0.904 mmol, 90%) as a pale yellow solid.

R_f: 0.49 (97:3 DCM/MeOH).

Mp: 81 – 84 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.28 (s, 1H, OCONH), 7.70 (d, *J* = 8.8 Hz, 1H, 4''-H), 7.30 (d, *J* = 2.6 Hz, 1H, 7''-H), 7.06 (dd, *J* = 8.9, 2.6 Hz, 1H, 5''-H), 4.82 (s, 1H, OCONH), 3.89 (s, 3H, OCH_3), 3.83 (s, 1H, 3-H), 3.30 (s, 2H, 1'-H), 2.94 (m, 1H, 6-H), 2.65 (s, 1H, 2-H), 2.45 (m, 2H, 2-H, 6-H), 1.84 (s br, 3H, 4-H, 5-H), 1.45 (s, 10H, 4-H, $OC(CH_3)_3$) ppm.

^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.0 (C-6''), 155.2 (C-2' or C-2''), 155.1 (C-2' or C-2''), 142.8 (C-3a''), 133.6 (C-7a''), 121.8 (C-4''), 115.4 (C-5''), 104.4 (C-7''), 79.7 ($O\underline{C}(CH_3)_3$), 61.5 (C-1'), 59.6 (C-6), 56.0 (OCH_3), 54.2 (C-2), 46.8 (C-3), 29.5 (C-4), 28.5 ($C(\underline{C}H_3)_3$), 22.9 (C-5) ppm.

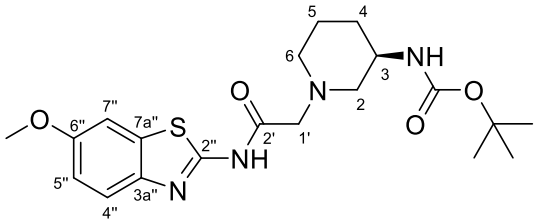
IR (ATR): $\tilde{\nu}$ = 3300, 2973, 1687, 1538, 1469, 1258, 1222, 1162, 1059, 1027, 809, 699 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{20}H_{29}N_4O_4S$ $[M+H]^+$: 421.1905; found: 421.1910.

Specific rotation: $[\alpha]_D^{20}$ = -5.5 (c = 0.25).

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

tert-butyl (*R*)-(1-(2-((6-methoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)carbamate (142)



$C_{20}H_{28}N_4O_4S$

M_w = 420.53 g/mol

Piperidinecarbamate **142** was prepared following General procedure F, using chloroacetamide **67** (257 mg, 1.00 mmol, 1.0 eq), (*R*)-3-(*tert*-butoxycarbonylamino)piperidine (220 mg, 1.10 mmol, 1.1 eq) and triethylamine (0.153 mL, 1.10 mmol, 1.1 eq). The mixture was stirred for 1 h, and the extraction was conducted with EtOAc (3:1, 5 x 30 mL). The crude product was

purified by flash column chromatography (97:3 DCM/MeOH), yielding product **142** (401 mg, 0.954 mmol, 95%) as a pale yellow solid.

R_f: 0.49 (97:3 DCM/MeOH).

Mp: 75 – 76 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.28 (s, 1H, OCONH), 7.70 (d, *J* = 8.8 Hz, 1H, 4''-H), 7.30 (d, *J* = 2.6 Hz, 1H, 7''-H), 7.06 (dd, *J* = 8.9, 2.6 Hz, 1H, 5''-H), 4.82 (s, 1H, OCONH), 3.89 (s, 3H, OCH_3), 3.83 (s, 1H, 3-H), 3.30 (s, 2H, 1'-H), 2.94 (m, 1H, 6-H), 2.65 (s, 1H, 2-H), 2.45 (m, 2H, 2-H, 6-H), 1.84 (s br, 3H, 4-H, 5-H), 1.45 (s, 10H, 4-H, $OC(CH_3)_3$) ppm.

^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.0 (C-6''), 155.2 (C-2' or C-2''), 155.1 (C-2' or C-2''), 142.8 (C-3a''), 133.6 (C-7a''), 121.8 (C-4''), 115.4 (C-5''), 104.4 (C-7''), 79.7 ($OC(CH_3)_3$), 61.5 (C-1'), 59.6 (C-6), 56.0 (OCH_3), 54.2 (C-2), 46.8 (C-3), 29.5 (C-4), 28.5 ($C(CH_3)_3$), 22.9 (C-5) ppm.

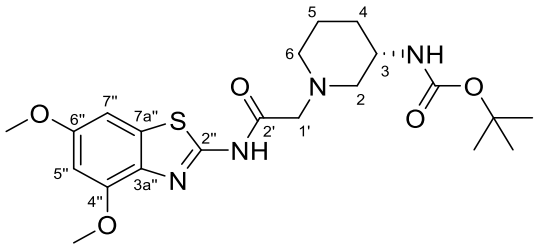
IR (ATR): $\tilde{\nu}$ = 3298, 2936, 1688, 1539, 1469, 1258, 1162, 1059, 1027, 809, 699 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{20}H_{29}N_4O_4S$ $[M+H]^+$: 421.1905; found: 421.1910.

Specific rotation: $[\alpha]_D^{20}$ = +4.5 (c = 0.20).

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

tert-Butyl (*S*)-(1-(2-((4,6-dimethoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)carbamate (143)



$C_{21}H_{30}N_4O_5S$

M_w = 450.56 g/mol

Piperidinecarbamate **143** was prepared following General procedure F, using chloroacetamide **82** (143 mg, 0.500 mmol, 1.0 eq), (*S*)-3-(*tert*-butoxycarbonylamino)piperidine (110 mg, 0.550 mmol, 1.1 eq) and triethylamine (78 µL, 0.55 mmol, 1.1 eq). The mixture was stirred for 2 h, and the extraction was conducted with EtOAc (3:1, 5 x 30 mL). The crude product was purified by flash column chromatography (97:3 DCM/MeOH), yielding product **143** (191 mg, 0.424 mmol, 85%) as a pale yellow solid.

R_f: 0.50 (97:3 DCM/MeOH).

Mp: 145 – 146 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.20 (s, 1H, NH), 6.86 (d, *J* = 2.2 Hz, 1H, 7''-H), 6.53 (d, *J* = 2.2 Hz, 1H, 5''-H), 4.73 (s, 1H, OCONH), 3.99 (s, 3H, (C-4'')OCH_3), 3.86 (s, 3H, (C-6'')OCH_3), 3.76 (s, 1H, 3-H), 3.23 (s, 2H, 1'-H), 2.89 (s br, 1H, 6-H), 2.53 (s, 1H, 2-H), 2.42 (s, 1H, 2-H), 2.34 (s, 1H, 6-H), 1.71 (s br, 3H, 4-H, 5-H), 1.43 (s, 10H, 4-H, $OC(CH_3)_3$) ppm.

^{13}C NMR (101 MHz, $CDCl_3$): δ = 167.0 (OCONH), 158.1 (C-1), 155.2 (C-6''), 153.9 (C-2''), 152.7 (C-4''), 134.4 (C-3a'' or C-7a''), 133.1 (C-3a'' or C-7a''), 97.9 (C-5''), 95.2 (C-7''), 79.6 ($O\underline{C}(CH_3)_3$), 61.8 (C-1'), 59.8 (C-6), 56.1 ((C-4'')$O\underline{C}H_3$ or (C-6'')$O\underline{C}H_3$), 56.0 ((C-4'')$O\underline{C}H_3$ or (C-6'')$O\underline{C}H_3$), 54.4 (C-2), 46.7 (C-3), 29.6 (C-4), 28.5 ($C(\underline{C}H_3)_3$), 23.0 (C-5) ppm.

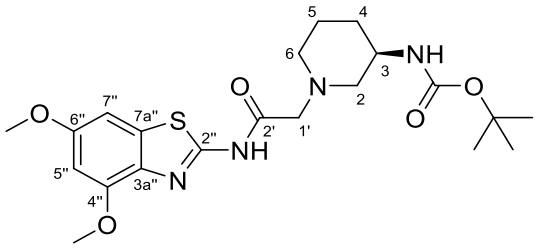
IR (ATR): $\tilde{\nu}$ = 2932, 1681, 1601, 1540, 1454, 1289, 1252, 1215, 1151, 1047, 822 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{21}H_{31}N_4O_5S$ $[M+H]^+$: 451.2010; found: 451.2004.

Specific rotation: $[\alpha]_D^{20}$ = -10.0 (c = 0.21).

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3f.

tert-Butyl (*R*)-(1-(2-((4,6-dimethoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)carbamate (144)



$C_{21}H_{30}N_4O_5S$

M_w = 450.56 g/mol

Piperidinecarbamate **144** was prepared following General procedure F, using chloroacetamide **82** (100 mg, 0.350 mmol, 1.0 eq), (*R*)-3-(*tert*-butoxycarbonylamino)piperidine (77 mg, 0.35 mmol, 1.1 eq) and triethylamine (54 µL, 0.35 mmol, 1.1 eq). The mixture was stirred for 2 h, and the extraction was conducted with EtOAc (3:1, 5 x 30 mL). The crude product was purified by flash column chromatography (97:3 DCM/MeOH), yielding product **144** (131 mg, 0.291 mmol, 83%) as a pale yellow solid.

R_f: 0.39 (97:3 DCM/MeOH).

Mp: 135 – 137 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.29 (s, 1H, NH), 6.84 (d, *J* = 2.2 Hz, 1H, 7''-H), 6.50 (d, *J* = 2.2 Hz, 1H, 5''-H), 4.85 (s, 1H, OCONH), 3.97 (s, 3H, (C-4'')OCH_3), 3.85 (s, 3H, (C-6'')OCH_3), 3.81 (s, 1H, 3-H), 3.31 (s, 2H, 1'-H), 2.89 (s br, 1H, 6-H), 2.74 – 2.33 (m, 3H, 2-H, 6-H), 1.74 (s br, 3H, 4-H, 5-H), 1.43 (s, 10H, 4-H, $OC(CH_3)_3$) ppm.

^{13}C NMR (126 MHz, $CDCl_3$): δ = 166.7 (OCONH), 158.1 (C-1), 155.2 (C-6''), 154.0 (C-2''), 152.6 (C-4''), 134.3 (C-3a'' or C-7a''), 132.9 (C-3a'' or C-7a''), 97.9 (C-5''), 95.2 (C-7''), 79.7 ($O\underline{C}(CH_3)_3$), 61.5 (C-1'), 59.5 (C-6), 56.0 ((C-4'')$O\underline{C}H_3$ or (C-6'')$O\underline{C}H_3$), 55.9 ((C-4'')$O\underline{C}H_3$ or (C-6'')$O\underline{C}H_3$), 54.2 (C-2), 46.5 (C-3), 29.4 (C-4), 28.5 ($C(\underline{C}H_3)_3$), 22.8 (C-5) ppm.

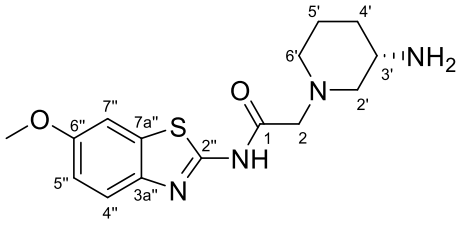
IR (ATR): $\tilde{\nu}$ = 3350, 2934, 1680, 1601, 1528, 1453, 1251, 1214, 1150, 1047, 819 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{21}H_{31}N_4O_5S$ $[M+H]^+$: 451.2010; found: 451.2004.

Specific rotation: $[\alpha]_D^{20}$ = +9.9 (c = 0.18).

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3d.

(*S*)-2-(3-Aminopiperidin-1-yl)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (148)



$C_{15}H_{20}N_4O_2S$

M_w = 320.41 g/mol

3-Aminopiperidine **148** was prepared following General procedure H from Boc-derivative **141** (336 mg, 0.800 mmol, 1.0 eq). The mixture was extracted with $CHCl_3$/isopropanol (3:1, 5 x 10 mL), and the residue purified by flash column chromatography (94:5:1 DCM/MeOH/triethylamine), yielding product **148** (205 mg, 0.640 mmol, 80%) as an off-white solid.

R_f: 0.49 (94:5:1 DCM/MeOH/triethylamine).

Mp: 128 – 130 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.62 (d, *J* = 8.8 Hz, 1H, 4''-H), 7.55 (d, *J* = 2.6 Hz, 1H, 7''-H), 7.02 (dd, *J* = 8.8, 2.6 Hz, 1H, 5''-H), 4.71 (s, 2H, NH_2), 3.80 (s, 3H, OCH_3), 3.34 – 3.21 (m, 2H, 2-H), 2.80 – 2.69 (m, 2H, 3'-H, 6'-H), 2.69 – 2.60 (m, 1H, 2'-H), 2.18 (dd, *J* = 11.7, 8.9 Hz, 1H, 2'-H), 1.96 (t, *J* = 9.9 Hz, 1H, 6'-H), 1.74 – 1.60 (m, 2H, 4'-H, 5'-H), 1.48 (td, *J* = 10.0, 5.1 Hz, 1H, 5'-H), 1.02 (q, *J* = 10.1 Hz, 1H, 4'-H) ppm.

^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.7 (C-1), 156.1 (C-6''), 155.9 (C-2''), 142.6 (C-3a''), 132.8 (C-7a''), 121.0 (C-4''), 114.8 (C-5''), 104.7 (C-7''), 61.9 (C-6'), 60.8 (C-2), 55.6 (OCH_3), 53.1 (C-2'), 47.6 (C-3'), 32.8 (C-4'), 23.4 (C-5') ppm.

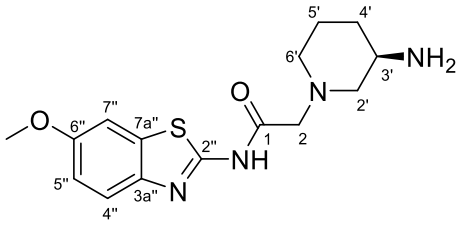
IR (ATR): $\tilde{\nu}$ = 1688, 1609, 1537, 1467, 1264, 1211, 1060, 1026, 844, 802, 763, 706 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{15}H_{21}N_4O_2S$ $[M+H]^+$: 321.1380; found: 321.1384.

Specific rotation: $[\alpha]_D^{20}$ = +14.1 (c = 0.26).

Purity (HPLC): >95% (λ = 210 nm), >92% (λ = 254 nm), Method 1a.

(*R*)-2-(3-Aminopiperidin-1-yl)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (149)



$C_{15}H_{20}N_4O_2S$

M_w = 320.41 g/mol

3-Aminopiperidine **149** was prepared following General procedure H from Boc-derivative **142** (421 mg, 1.00 mmol, 1.0 eq). The mixture was extracted with $CHCl_3$/isopropanol (3:1, 5 x 10 mL), and the residue purified by flash column chromatography (94:5:1 DCM/MeOH/triethylamine), yielding product **149** (277 mg, 0.865 mmol, 87%) as an off-white solid.

R_f: 0.49 (94:5:1 DCM/MeOH/triethylamine).

Mp: 125 – 127 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.62 (d, *J* = 8.8 Hz, 1H, 4''-H), 7.55 (d, *J* = 2.6 Hz, 1H, 7''-H), 7.02 (dd, *J* = 8.8, 2.6 Hz, 1H, 5''-H), 3.80 (s, 3H, OCH_3), 3.33 – 3.21 (m, 2H, 2-H), 2.75 (ddd, *J* = 12.1, 9.7, 4.1 Hz, 2H, 3'-H, 6'-H), 2.68 – 2.61 (m, 1H, 2'-H), 2.22 – 2.14 (m, 1H, 2'-H), 1.99 – 1.92 (m, 1H, 6'-H), 1.73 – 1.60 (m, 2H, 4'-H, 5'-H), 1.52 – 1.40 (m, 1H, 5'-H), 1.02 (q, *J* = 11.0, 9.7 Hz, 1H, 4'-H) ppm.

^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.7 (C-1), 156.1 (C-6''), 155.9 (C-2''), 142.6 (C-3a''), 132.8 (C-7a''), 121.0 (C-4''), 114.8 (C-5''), 104.7 (C-7''), 61.9 (C-6'), 60.8 (C-2), 55.6 (OCH_3), 53.1 (C-2'), 47.6 (C-3'), 32.9 (C-4'), 23.4 (C-5') ppm.

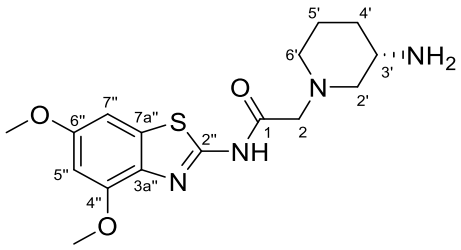
IR (ATR): $\tilde{\nu}$ = 1688, 1609, 1537, 1468, 1264, 1211, 1060, 1027, 844, 802, 762, 706 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{15}H_{21}N_4O_2S$ $[M+H]^+$: 321.1380; found: 321.1383.

Specific rotation: $[\alpha]_D^{20}$ = -13.3 (c = 0.23).

Purity (HPLC): >95% (λ = 210 nm), >91% (λ = 254 nm), Method 1a.

(*S*)-2-(3-Aminopiperidin-1-yl)-*N*-(4,6-dimethoxybenzo[*d*]thiazol-2-yl)acetamide (150)



$C_{16}H_{22}N_4O_3S$

M_w = 350.44 g/mol

3-Aminopiperidine **150** was prepared following General procedure H from Boc-derivative **143** (162 mg, 0.360 mmol, 1.0 eq). The mixture was extracted with $CHCl_3$/isopropanol (3:1, 5 x 10 mL), and the residue purified by flash column chromatography (94:5:1 DCM/MeOH/triethylamine), yielding product **150** (125 mg, 0.357 mmol, 99%) as a colourless solid.

R_f: 0.13 (96:3:1 DCM/MeOH/triethylamine).

Mp: 166 – 168 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.10 (d, *J* = 2.2 Hz, 1H, 7''-H), 6.58 (d, *J* = 2.3 Hz, 1H, 5''-H), 4.43 (s, 2H, NH_2), 3.88 (s, 3H, (C-4'')OCH_3), 3.80 (s, 3H, (C-6'')OCH_3), 3.32 – 3.18 (m, 2H, 2-H), 2.79 – 2.67 (m, 2H, 3'-H, 6'-H), 2.67 – 2.59 (m, 1H, 2'-H), 2.16 (s br, 1H, 2'-H), 1.93 (t, *J* = 9.4 Hz, 1H, 6'-H), 1.73 – 1.58 (m, 2H, 4'-H, 5'-H), 1.47 (s br, 1H, 5'-H), 1.03 – 0.93 (m, 1H, 4'-H) ppm.

^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.4 (C-1), 157.2 (C-6''), 154.1 (C-2''), 152.1 (C-4''), 133.5 (C-3a'' or C-7a''), 132.8 (C-3a'' or C-7a''), 98.0 (C-5''), 95.7 (C-7''), 62.1 (C-6'), 60.8 (C-2), 55.8 ((C-4'')O<u>C</u>H_3 or (C-6'')O<u>C</u>H_3), 55.6 ((C-4'')O<u>C</u>H_3 or (C-6'')O<u>C</u>H_3), 53.1 (C-2'), 47.7 (C-3'), 33.1 (C-3'), 23.4 (C-5') ppm.

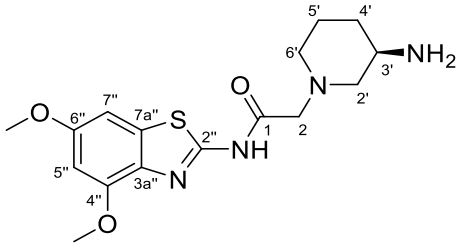
IR (ATR): $\tilde{\nu}$ = 1677, 1601, 1539, 1467, 1284, 1253, 1215, 1152, 1042, 826, 750, 737 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{16}H_{21}N_4O_2S$ $[M-H]^-$: 349.1339; found: 349.1337.

Specific rotation: $[\alpha]_D^{20}$ = +13.7 (c = 0.22).

Purity (HPLC): >99% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

(*R*)-2-(3-Aminopiperidin-1-yl)-*N*-(4,6-dimethoxybenzo[*d*]thiazol-2-yl)acetamide (151)



$C_{16}H_{22}N_4O_3S$

M_w = 350.44 g/mol

3-Aminopiperidine **151** was prepared following General procedure H from Boc-derivative **144** (113 mg, 0.250 mmol, 1.0 eq). The mixture was extracted with $CHCl_3$/isopropanol (3:1, 5 x 10 mL), and the residue purified by flash column chromatography (94:5:1 DCM/MeOH/triethylamine), yielding product **151** (97 mg, 0.24 mmol, 99%) as a colourless solid.

R_f: 0.10 (96:3:1 DCM/MeOH/triethylamine).

Mp: 169 – 170 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.10 (d, *J* = 2.2 Hz, 1H, 7''-H), 6.58 (d, *J* = 2.3 Hz, 1H, 5''-H), 4.60 (s, 2H, NH_2), 3.88 (s, 3H, (C-4'')OCH_3), 3.80 (s, 3H, (C-6'')OCH_3), 3.33 – 3.18 (m, 2H, 2-H), 2.78 – 2.67 (m, 2H, 3'-H, 6'-H), 2.67 – 2.60 (m, 1H, 2'-H), 2.16 (t, *J* = 10.1 Hz, 1H, 2'-H), 1.93 (t, *J* = 9.2 Hz, 1H, 6'-H), 1.73 – 1.59 (m, 2H, 4'-H, 5'-H), 1.54 – 1.41 (m, 1H, 5'-H), 1.03 – 0.91 (m, 1H, 4'-H) ppm.

^{13}C NMR (101 MHz, DMSO-d_6): δ = 169.5 (C-1), 157.2 (C-6''), 154.1 (C-2''), 152.1 (C-4''), 133.5 (C-3a'' or C-7a''), 132.8 (C-3a'' or C-7a''), 98.0 (C-5''), 95.7 (C-7''), 62.2 (C-6'), 60.9 (C-2), 55.8 ((C-4'')O<u>C</u>H_3 or (C-6'')O<u>C</u>H_3), 55.6 ((C-4'')O<u>C</u>H_3 or (C-6'')O<u>C</u>H_3), 53.1 (C-2'), 47.7 (C-3'), 33.1 (C-3'), 23.4 (C-5') ppm.

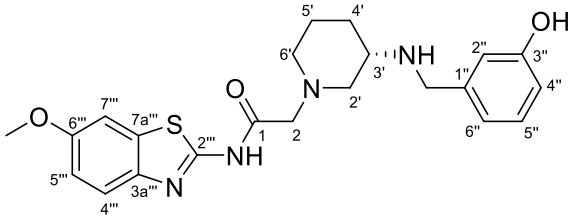
IR (ATR): $\tilde{\nu}$ = 1675, 1602, 1578, 1540, 1283, 1253, 1215, 1153, 1041, 829, 750, 738 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{16}H_{21}N_4O_2S$ $[M-H]^-$: 349.1339; found: 349.1338.

Specific rotation: $[\alpha]_D^{20}$ = -15.0 (c = 0.21).

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1a.

(*S*)-2-(3-((3-Hydroxybenzyl)amino)piperidin-1-yl)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (159)



$C_{22}H_{26}N_4O_3S$

M_w = 426.54 g/mol

N-Substituted 3-aminopiperidine **159** was prepared following General procedure I from 3-aminopiperidine **148**. Colourless solid (127 mg, 0.298 mmol, 60%).

R_f: 0.57 (94:5:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 68 – 70 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.22 (s, 1H, CONH), 7.63 (d, *J* = 8.9 Hz, 1H, 4'''-H), 7.57 (d, *J* = 2.6 Hz, 1H, 7'''-H), 7.07 – 7.00 (m, 2H, 5''-H, 5'''-H), 6.76 (t, *J* = 2.0 Hz, 1H, 2''-H), 6.72 (dt, *J* = 7.4, 1.2 Hz, 1H, 6''-H), 6.58 (ddd, *J* = 8.1, 2.6, 1.1 Hz, 1H, 4''-H), 4.05 (s, 1H, N<u>H</u>CH_2), 3.80 (s, 3H, OCH_3), 3.64 (d, *J* = 2.1 Hz, 2H, NHC<u>H</u>$_2$), 3.29 (s, 2H, 2-H), 2.88 – 2.82 (m, 1H, 2'-H), 2.62 (dd, *J* = 15.3, 11.1 Hz, 1H, 6'-H), 2.56 (dq, *J* = 8.3, 4.2 Hz, 1H, 3'-H), 2.23 (t, *J* = 10.1 Hz, 1H, 6'-H), 2.10 (t, *J* = 9.3 Hz, 1H, 2'-H), 1.76 (dd, *J* = 13.0, 5.1 Hz, 1H, 4'-H), 1.65 (dt, *J* = 13.2, 4.4 Hz, 1H, 5'-H), 1.44 (qt, *J* = 10.0, 3.7 Hz, 1H, 5'-H), 1.15 – 1.02 (m, 1H, 4-H) ppm.

^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.6 (C-1), 157.2 (C-3''), 156.1 (C-6'''), 155.5 (C-2'''), 142.9 (C-1'' or C-3a''), 142.6 (C-1'' or C-3a''), 132.8 (C-7a'''), 129.0 (C-5''), 121.1 (C-4'''), 118.5 (C-6''), 114.9 (C-5'''), 114.7 (C-2''), 113.3 (C-4''), 104.7 (C-7'''), 60.8 (C-2), 59.0 (C-2'), 55.6 (OCH_3), 53.4 (C-6'), 52.8 (C-3'), 49.9 ($NHCH_2$), 30.0 (C-4''), 23.3 (C-5'') ppm.

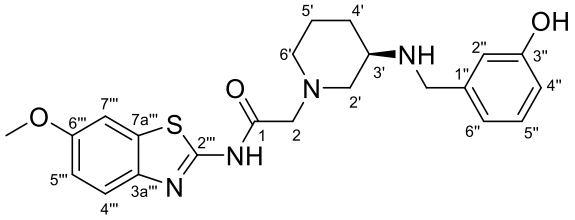
IR (ATR): $\tilde{\nu}$ = 2931, 1692, 1602, 1537, 1469, 1259, 1221, 1160, 1059, 827, 783, 697 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{22}H_{26}N_4O_3S$ $[M]^{\cdot+}$: 426.1712; found: 426.1714.

Specific rotation: $[\alpha]_D^{20}$ = +7.2 (c = 0.25).

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

(*R*)-2-(3-((3-Hydroxybenzyl)amino)piperidin-1-yl)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (160)



$C_{22}H_{26}N_4O_3S$

M_w = 426.54 g/mol

N-Substituted 3-aminopiperidine **160** was prepared following General procedure I from 3-aminopiperidine **149**. Colourless solid (149 mg, 0.349 mmol, 70%).

R_f: 0.51 (94:5:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 69 – 72 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.22 (s, 1H, CONH), 7.63 (d, *J* = 8.8 Hz, 1H, 4'''-H), 7.57 (d, *J* = 2.6 Hz, 1H, 7'''-H), 7.07 – 7.00 (m, 2H, 5''-H, 5'''-H), 6.76 (t, *J* = 2.1 Hz, 1H, 2''-H), 6.72 (dt, *J* = 7.5, 1.3 Hz, 1H, 6''-H), 6.58 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 1H, 4''-H), 4.02 (s, 1H, N<u>H</u>CH_2), 3.80 (s, 3H, OCH_3), 3.64 (d, *J* = 2.0 Hz, 2H, NHC<u>H</u>$_2$), 3.29 (s, 2H, 2-H), 2.89 – 2.82 (m, 1H, 2'-H), 2.63 (dd, *J* = 9.4, 5.8 Hz, 1H, 6'-H), 2.56 (tt, *J* = 8.1, 3.6 Hz, 1H, 3'-H), 2.27 – 2.19 (m, 1H, 6'-H), 2.10 (t, *J* = 9.4 Hz, 1H, 2'-H), 1.79 – 1.72 (m, 1H, 4'-H), 1.65 (dt, *J* = 13.1, 4.3 Hz, 1H, 5'-H), 1.44 (tdd, *J* = 13.8, 7.8, 3.8 Hz, 1H, 5'-H), 1.15 – 1.06 (m, 1H, 4'-H) ppm.

^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.5 (C-1), 157.2 (C-3''), 156.1 (C-6'''), 155.5 (C-2'''), 142.9 (C-1'' or C-3a''), 142.6 (C-1'' or C-3a''), 132.8 (C-7a'''), 129.0 (C-5''), 121.1 (C-4'''), 118.5 (C-6''), 114.9 (C-5'''), 114.7 (C-2''), 113.3 (C-4''), 104.7 (C-7'''), 60.8 (C-2), 59.0 (C-2'), 55.6 (OCH_3), 53.3 (C-6'), 52.8 (C-3'), 49.9 ($NHCH_2$), 30.0 (C-4''), 23.3 (C-5'') ppm.

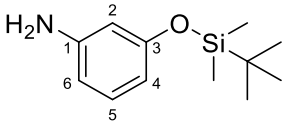
IR (ATR): $\tilde{\nu}$ = 2934, 1690, 1602, 1537, 1469, 1259, 1221, 1160, 1059, 827, 783, 698 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{22}H_{26}N_4O_3S$ $[M]^{\cdot+}$: 426.1712; found: 426.1708.

Specific rotation: $[\alpha]_D^{20}$ = -8.7 (c = 0.23).

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1a.

3-((*tert*-Butyldimethylsilyl)oxy)aniline (169)



$C_{12}H_{21}NOSi$

M_w = 223.39 g/mol

Silyl ether **169** was prepared as described in literature[133] from 3-aminophenol (**167**, 546 mg, 5.00 mmol, 1.0 eq), using *tert*-butyldimethylchlorosilane (904 mg, 6.00 mmol, 1.2 eq) and imidazole (681 mL, 10.0 mmol, 2.0 eq). Colourless oil (1.09 g, 4.86 mmol, 97%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.00 (td, *J* = 8.0, 0.9 Hz, 1H, 5-H), 6.32 (ddd, *J* = 7.9, 2.2, 0.9 Hz, 1H, 6-H), 6.27 (ddd, *J* = 8.0, 2.3, 0.9 Hz, 1H, 4-H), 6.22 (t, *J* = 2.2 Hz, 1H, 2-H), 0.97 (d, *J* = 0.8 Hz, 9H, $SiC(CH_3)_3$), 0.19 (d, *J* = 1.0 Hz, 6H, $Si(CH_3)_2$) ppm.

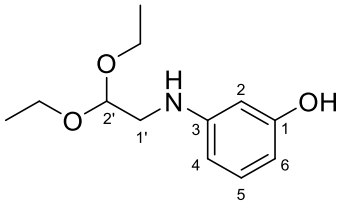
^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.9 (C-3), 147.2 (C-1), 130.1 (C-5), 111.0 (C-4), 108.9 (C-6), 107.6 (C-2), 25.8 ($SiC(\underline{C}H_3)_3$), 18.3 ($Si\underline{C}(CH_3)_3$), -4.3 ($Si(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 2857, 1597, 1491, 1461, 1284, 1252, 1191, 1153, 977, 835, 778, 686 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{22}NOSi$ $[M+H]^+$: 224.1466; found: 224.1463.

Purity (HPLC): ND.

3-((2,2-Diethoxyethyl)amino)phenol (168)



$C_{12}H_{19}NO_3$

M_w = 225.29 g/mol

N-Substituted aromatic amine **168** was prepared following General procedure P, using 3-aminophenol (**167**, 1.64 g, 15.0 mmol, 1.0 eq), bromoacetaldehyde diethyl acetal (2.56 mL, 16.5 mmol, 1.1 eq), and $NaHCO_3$ (1.38 g, 16.5 mmol 1.1 eq). The reaction mixture was stirred at 120 °C for 16 h. The mixture was extracted with diethyl ether, and the crude purified by flash column chromatography (1:4 → 1:3 EtOAc/isohexanes), yielding product **168** (2.29 g, 10.2 mmol, 68%) as a light yellow oil.

R_f: 0.30 (1:4 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.02 (t, *J* = 8.0 Hz, 1H, 5-H), 6.23 (ddd, *J* = 8.1, 2.2, 0.9 Hz, 1H, 4-H), 6.20 (ddd, *J* = 8.0, 2.4, 0.8 Hz, 1H, 6-H), 6.15 (t, *J* = 2.3 Hz, 1H, 2-H), 4.68 (t, *J* = 5.5 Hz, 1H, 2'-H), 3.73 (dq, *J* = 9.5, 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 3.57 (dq, *J* = 9.4, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.23 (d, *J* = 5.5 Hz, 2H, 1'-H), 1.24 (t, *J* = 7.1 Hz, 6H, 2 $OCH_2C\underline{H}_3$) ppm.

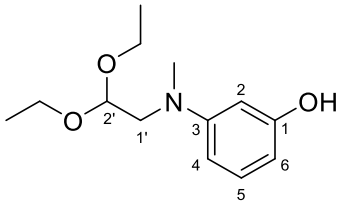
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.0 (C-1), 149.4 (C-3), 130.4 (C-5), 106.4 (C-4), 105.1 (C-6), 100.9 (C-2'), 100.3 (C-2), 62.6 ($O\underline{C}H_2CH_3$), 46.6 (C-1'), 15.5 ($OCH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3388, 2975, 1593, 1496, 1182, 1158, 1045, 826, 759, 687 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{18}NO_3$ $[M\text{-}H]^-$: 224.1292; found: 224.1490.

Purity (HPLC): >98% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

3-((2,2-Diethoxyethyl)(methyl)amino)phenol (171)



$C_{13}H_{21}NO_3$

M_w = 239.31 g/mol

N-Methylaminophenol **171** was prepared following General procedure J, using aminophenol **168** (901 mg, 4.00 mmol, 1.0 eq) and iodomethane. Pink oil (638 mg, 2.67 mmol, 67%).

R_f: 0.48 (1:3 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.07 (t, *J* = 8.1 Hz, 1H, 5-H), 6.32 (dd, *J* = 8.3, 2.4 Hz, 1H, 4-H), 6.25 (s, 1H, 2-H), 6.20 (dd, *J* = 7.9, 2.2 Hz, 1H, 6-H), 5.03 (s, 1H, OH), 4.65 (t, *J* = 5.3 Hz, 1H, 2'-H), 3.77 – 3.66 (m, 2H, $OC\underline{H}_2CH_3$), 3.59 – 3.47 (m, 2H, $OC\underline{H}_2CH_3$), 3.43 (d, *J* = 5.3 Hz, 2H, 1'-H), 2.99 (s, 3H, NCH_3), 1.20 (td, J = 7.1, 0.6 Hz, 6H, 2 $OCH_2C\underline{H}_3$) ppm.

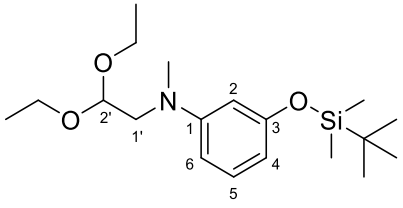
^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.9 (C-1), 150.8 (C-3), 130.2 (C-5), 104.9 (C-4), 103.6 (C-6), 101.4 (C-2'), 99.3 (C-2), 63.5 ($O\underline{C}H_2CH_3$), 56.4 (C-1'), 39.8 (NCH_3), 15.6 ($OCH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 1613, 1577, 1503, 1373, 1166, 1110, 1046, 1000, 980, 818, 753, 686 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{13}H_{20}NO_3$ $[M\text{-}H]^-$: 238.1448; found: 238.1446.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

3-((*tert*-Butyldimethylsilyl)oxy)-*N*-(2,2-diethoxyethyl)-*N*-methylaniline (172)



$C_{19}H_{35}NO_3Si$

M_w = 353.58 g/mol

tert-Butyldimethylchlorosilane (127 mg, 0.840 mmol, 1.2 eq) was added to a solution of phenol **171** (168 mg, 0.700 mmol, 1.0 eq) and imidazole (95 mg, 1.4 mmol, 2 eq) in 2 mL anhydrous THF. The reaction mixture was stirred at room temperature for 18 h. Water (10 mL) was added, and the mixture was extracted with EtOAc (3 x 10 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (95:5 EtOAc/isohexanes), yielding silyl ether **172** (181 mg, 0.512 mmol, 73%) as a colourless oil.

R_f: 0.46 (95:5 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.07 (t, *J* = 8.2 Hz, 1H, 5-H), 6.39 (s br, 1H, 6-H), 6.25 (m, 2H, 2-H, 4-H), 4.66 (s, 1H, 2'-H), 3.71 (dq, *J* = 9.3, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.52 (dq, *J* = 9.2, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.42 (d, *J* = 5.3 Hz, 2H, 1'-H), 2.99 (s, 3H, NCH_3), 1.20 (t, *J* = 7.0 Hz, 6H, 2 $OCH_2C\underline{H}_3$), 0.98 (s, 9H, $SiC(CH_3)_3$), 0.20 (s, 6H, $Si(CH_3)_2$) ppm.

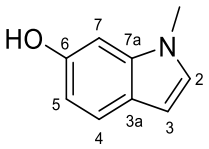
^{13}C NMR (126 MHz, $CDCl_3$): δ = 156.9 (C-3), 150.4 (C-1), 129.9 (C-5), 108.5 (C-6), 105.8 (C-2 or C-4), 104.2 (C-2 or C-4), 101.3 (C-2'), 63.6 ($O\underline{C}H_2CH_3$), 56.6 (C-1'), 39.8 (NCH_3), 25.9 ($SiC(\underline{C}H_3)_3$), 18.4 ($Si\underline{C}(CH_3)_3$), 15.6 ($OCH_2\underline{C}H_3$), -4.2 ($Si(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 2929, 1603, 1573, 1497, 1249, 1123, 1061, 1004, 940, 831, 778, 687 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{19}H_{36}NO_3Si$ $[M+H]^+$: 354.2459; found: 354.2460.

Purity (HPLC): >98% (λ = 210 nm), 100% (λ = 254 nm), Method 1d.

1-Methyl-1*H*-indol-6-ol (173)



C_9H_9NO

M_w = 147.18 g/mol

1-Methyl-1*H*-indol-6-ol (**173**) was a by-product of acetal deprotection of compound **172**. Diethyl acetal **172** (145 mg, 0.410 mmol, 1.0 eq) was dissolved in 2 mL ACN, and HCl (37 µL, 37% aq. solution, 0.45 mmol, 1.1 eq) was added at 0 °C. The mixture was stirred at 0 °C for 1 h. Aq. sat. $NaHCO_3$ solution was added to neutralise the mixture, that was extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were removed under reduced pressure. The crude product was purified by flash column

chromatography (1:4 EtOAc/isohexanes), yielding indole **173** (20 mg, 0.37 mmol, 33%) as a colourless solid.

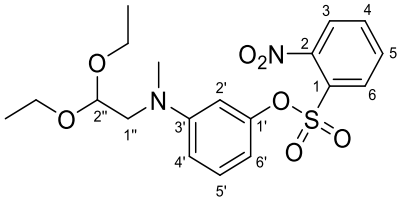
^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 8.5 Hz, 1H, 4-H), 6.93 (d, *J* = 3.1 Hz, 1H, 2-H), 6.78 (d, *J* = 2.2 Hz, 1H, 7-H), 6.68 (dd, *J* = 8.5, 2.2 Hz, 1H, 5-H), 6.40 (dd, *J* = 3.0, 1.0 Hz, 1H, 3-H), 4.93 (s, 1H, OH), 3.70 (s, 3H, CH_3) ppm.

^{13}C NMR (126 MHz, $CDCl_3$): δ = 151.8 (C-6), 137.7 (C-7a), 128.0 (C-2), 123.1 (C-3a), 121.6 (C-4), 109.5 (C-5), 101.0 (C-3), 95.2 (C-7), 32.9 (CH_3) ppm.

HRMS (EI): m/z = calculated for C_9H_9NO $[M]^{\cdot+}$: 147.0679; found: 147.0672.

Purity (HPLC): ND.

3-((2,2-Diethoxyethyl)(methyl)amino)phenyl 2-nitrobenzenesulfonate (176)



$C_{19}H_{24}N_2O_7S$

M_w = 424.47 g/mol

Nosyl derivative **176** was prepared following General procedure K from *N*-methylaminophenol **171** (362 mg, 2.64 mmol, 1.0 eq). The crude product was purified by flash column chromatography (1:3 EtOAc/isohexanes), yielding product **176** (1.08 g, 2.54 mmol, 96%) as bright orange oil.

R_f: 0.30 (1:2 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 – 7.97 (m, 1H, 3-H), 7.87 – 7.75 (m, 2H, 5-H, 6-H), 7.67 (ddd, *J* = 7.9, 6.2, 2.7 Hz, 1H, 4-H), 7.13 (t, *J* = 8.3 Hz, 1H, 5'-H), 6.69 – 6.64 (m, 1H, 4'-H), 6.57 (t, *J* = 2.4 Hz, 1H, 2'-H), 6.48 (ddd, *J* = 8.1, 2.3, 0.8 Hz, 1H, 6'-H), 4.58 (t, *J* = 5.2 Hz, 1H, 2''-H), 3.70 (dq, *J* = 9.3, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.49 (dq, *J* = 9.3, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.38 (d, *J* = 5.2 Hz, 2H, 1''-H), 2.95 (s, 3H, NCH_3), 1.18 (t, *J* = 7.0 Hz, 6H, 2 $OCH_2C\underline{H}_3$) ppm.

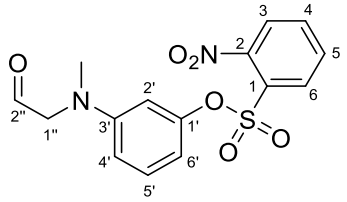
^{13}C NMR (101 MHz, $CDCl_3$): δ = 150.5 (C-1'), 150.4 (C-3'), 148.9 (C-2), 135.3 (C-5), 132.3 (C-3), 132.0 (C-4), 130.2 (C-5'), 128.9 (C-1), 124.9 (C-6), 111.5 (C-4'), 109.7 (C-6'), 106.0 (C-2'), 101.0 (C-2''), 63.6 ($O\underline{C}H_2CH_3$), 56.3 (C-1''), 39.9 (NCH_3), 15.6 ($OCH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 1609, 1543, 1502, 1367, 1194, 1119, 1057, 997, 910, 799, 780, 739 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{19}H_{24}N_2O_7S$ $[M]^{\cdot+}$: 424.1299; found: 424.1302.

Purity (HPLC): >79% (λ = 210 nm), >95% (λ = 254 nm), Method 3d.

3-(Methyl(2-oxoethyl)amino)phenyl 2-nitrobenzenesulfonate (175)



$C_{15}H_{14}N_2O_6S$

M_w = 350.35 g/mol

Aldehyde **175** was prepared following General procedure L from diethyl acetal **176** (1.06 g, 2.50 mmol, 1.0 eq). Brown waxy solid (419 mg, 1.20 mmol, 48%).

R_f: 0.33 (2:1 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.65 (t, *J* = 1.0 Hz, 1H, 2''-H), 7.97 – 7.92 (m, 1H, 3-H), 7.82 – 7.80 (m, 2H, 5-H, 6-H), 7.70 – 7.64 (m, 1H, 4-H), 7.14 (t, *J* = 8.3 Hz, 1H, 5'-H), 6.58 – 6.51 (m, 2H, 4'-H, 6'-H), 6.45 (t, *J* = 2.4 Hz, 1H, 2'-H), 4.03 (d, *J* = 1.0 Hz, 2H, 1''-H), 3.02 (s, 3H, NCH_3) ppm.

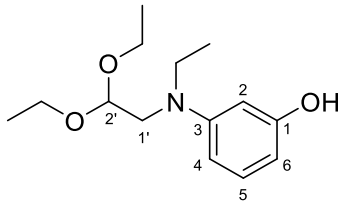
^{13}C NMR (101 MHz, $CDCl_3$): δ = 200.2 (C-2''), 150.6 (C-1'), 150.4 (C-3'), 148.8 (C-2), 135.4 (C-5), 132.4 (C-3), 132.1 (C-4), 130.5 (C-5'), 128.6 (C-1), 124.9 (C-6), 111.4 (C-4'), 110.6 (C-6'), 106.1 (C-2'), 62.7 (C-1''), 39.9 (NCH_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1607, 1540, 1499, 1362, 1191, 1118, 998, 851, 799, 776, 737, 681 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{15}H_{15}N_2O_6S$ $[M+H]^+$: 351.9646; found: 351.0644.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

3-((2,2-Diethoxyethyl)(ethyl)amino)phenol (177)



$C_{14}H_{23}NO_3$

M_w = 253.34 g/mol

N-Ethylaminophenol **177** was prepared following General procedure J, using aminophenol **168** (951 mg, 4.22 mmol, 1.0 eq) and iodoethane. Pale pink oil (850 mg, 3.36 mmol, 80%).

R_f: 0.36 (1:4 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.06 (t, *J* = 8.1 Hz, 1H, 5-H), 6.30 (d, *J* = 8.4 Hz, 1H, 4-H), 6.24 (s br, 1H, 2-H), 6.18 (s br, 1H, 6-H), 5.21 (s, 1H, OH), 4.67 (d, J = 5.4 Hz, 1H, 2'-H), 3.73 (dq, *J* = 9.3, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.54 (dq, *J* = 9.3, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.45 (d, *J* = 7.1 Hz, 1H, $NC\underline{H}_2CH_3$), 3.4 (d, *J* = 5.2 Hz, 2H, 1-'H), 1.21 (t, *J* = 7.1 Hz, 6H, 2 $OCH_2C\underline{H}_3$), 1.14 (t, *J* = 7.0 Hz, 3H, $NCH_2C\underline{H}_3$) ppm.

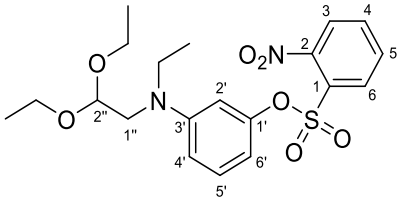
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.1 (C-1), 149.2 (C-3), 130.3 (C-5), 104.7 (C-4), 103.0 (C-6), 101.6 (C-2'), 98.9 (C-2), 63.5 ($O\underline{C}H_2CH_3$), 54.3 (C-1'), 45.9 ($N\underline{C}H_2CH_3$), 15.6 ($OCH_2\underline{C}H_3$), 11.8 ($NCH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2974, 1614, 1577, 1502, 1212, 1166, 1114, 1048, 1012, 818, 752, 687 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{14}H_{22}NO_3$ $[M-H]^-$: 252.1605; found: 252.1602.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3f.

3-((2,2-Diethoxyethyl)(ethyl)amino)phenyl 2-nitrobenzenesulfonate (179)



$C_{20}H_{26}N_2O_7S$

M_w = 438.50 g/mol

Nosyl derivative **179** was prepared following General procedure K from *N*-ethylaminophenol **177** (798 mg, 3.15 mmol, 1.0 eq). The crude product was purified by flash column chromatography (1:3 EtOAc/isohexanes), yielding product **179** (1.24 g, 2.79 mmol, 89%) as bright orange oil.

R_f: 0.33 (1:3 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99 (ddd, *J* = 7.9, 1.2, 0.5 Hz, 1H, 3-H), 7.84 – 7.76 (m, 2H, 5-H, 6-H), 7.67 (ddd, *J* = 7.9, 6.5, 2.4 Hz, 1H, 4-H), 7.10 (t, *J* = 8.3 Hz, 1H, 5'-H), 6.62 (dd, *J* = 8.4, 2.5 Hz, 1H, 4'-H), 6.52 (t, *J* = 2.4 Hz, 1H, 2'-H), 6.47 – 6.39 (m, 1H, 6'-H), 4.56 (t, *J* = 5.1 Hz, 1H, 2''-H), 3.70 (dq, *J* = 9.3, 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 3.50 (dq, *J* = 9.3, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.38 (q, *J* = 7.1 Hz, 2H, $NC\underline{H}_2CH_3$), 3.33 (d, *J* = 5.1 Hz, 2H, 1''-H), 1.19 (t, *J* = 7.0 Hz, 6H, 2 $OCH_2C\underline{H}_3$), 1.06 (t, *J* = 7.0 Hz, 3H, $NCH_2C\underline{H}_3$) ppm.

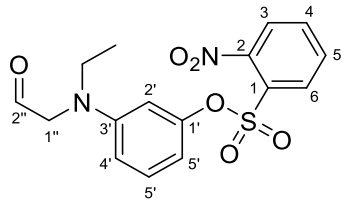
^{13}C NMR (101 MHz, $CDCl_3$): δ = 150.7 (C-1'), 149.3 (C-3'), 148.9 (C-2), 135.2 (C-5), 132.3 (C-3), 132.0 (C-4), 130.2 (C-5'), 129.0 (C-1), 124.8 (C-6), 111.1 (C-4'), 108.9 (C-6'), 105.6 (C-2'), 101.2 (C-2''), 63.6 ($O\underline{C}H_2CH_3$), 54.3 (C-1''), 46.1 ($N\underline{C}H_2CH_3$), 15.5 ($OCH_2\underline{C}H_3$), 11.5 ($NCH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 1609, 1544, 1500, 1372, 1194, 1119, 1057, 875, 793, 780, 739, 685 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{20}H_{26}N_2O_7S$ $[M]^{\cdot+}$: 438.1456; found: 438.1454.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3f.

3-(Ethyl(2-oxoethyl)amino)phenyl 2-nitrobenzenesulfonate (178)



$C_{16}H_{16}N_2O_6S$

M_w = 364.38 g/mol

Aldehyde **178** was prepared following General procedure L from diethyl acetal **179** (570 mg, 1.30 mmol, 1.0 eq). Brown sticky oil (360 mg, 0.988 mmol, 76%).

R_f: 0.22 (1:2 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.60 (t, *J* = 1.2 Hz, 1H, 2''-H), 7.97 – 7.94 (m, 1H, 3-H), 7.83 – 7.80 (m, 2H, 5-H, 6-H), 7.70 – 7.65 (m, 1H, 4-H), 7.13 (t, *J* = 8.3 Hz, 1H, 5'-H), 6.53 (ddd, *J* = 8.1, 2.2, 0.8 Hz, 1H, 4'-H), 6.48 (ddd, *J* = 8.4, 2.6, 0.8 Hz, 1H, 6'-H), 6.42 (t, *J* = 2.4 Hz, 1H, 2'-H), 3.96 (d, *J* = 1.3 Hz, 2H, 1''-H), 3.42 (q, *J* = 7.1 Hz, 2H, $NC\underline{H}_2CH_3$), 1.14 (t, *J* = 7.1 Hz, 3H, $NCH_2C\underline{H}_3$) ppm.

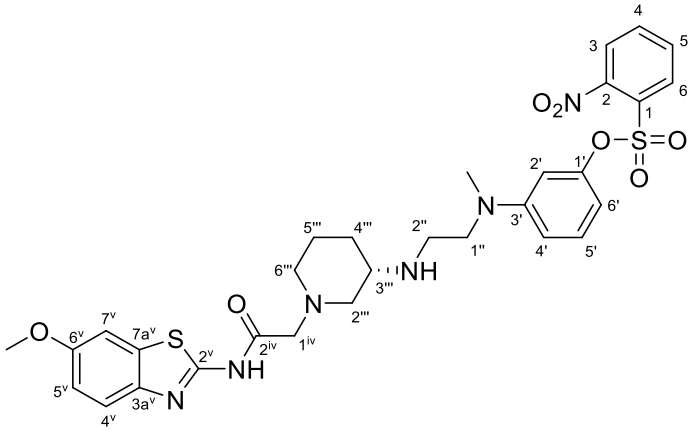
^{13}C NMR (101 MHz, $CDCl_3$): δ = 201.2 (C-2''), 150.7 (C-1'), 149.1 (C-3'), 148.8 (C-2), 135.4 (C-5), 132.3 (C-3), 132.1 (C-4), 130.6 (C-5'), 128.7 (C-1), 124.9 (C-6), 111.2 (C-6'), 110.3 (C-4'), 106.0 (C-2'), 60.6 (C-1''), 46.7 ($N\underline{C}H_2CH_3$), 12.3 ($NCH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2974, 1729, 1607, 1541, 1499, 1365, 1191, 1118, 851, 778, 738 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{16}H_{17}N_2O_6S$ $[M+H]^+$: 365.0802; found: 365.0800.

Purity (HPLC): >98% (λ = 210 nm), >98% (λ = 254 nm), Method 3f.

(*S*)-3-((2-((1-(2-((6-Methoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)amino)ethyl)(methyl)amino)phenyl 2-nitrobenzenesulfonate (180)



$C_{30}H_{34}N_6O_7S_2$

M_w = 654.77 g/mol

Secondary amine **180** was prepared following General procedure M, using 3-aminopiperidine **148** (123 mg, 0.350 mmol, 1.0 eq) and aldehyde **175**. Light brown solid (112 mg, 0.171 mmol, 49%).

R_f: 0.42 (96:3:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 62 – 65 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.18 (dd, *J* = 8.0, 1.1 Hz, 1H, 6-H), 8.04 (td, *J* = 7.7, 1.4 Hz, 1H, 5-H), 7.97 (dd, *J* = 8.1, 1.4 Hz, 1H, 3-H), 7.86 (td, *J* = 7.8, 1.3 Hz, 1H, 4-H), 7.62 (d, *J* = 8.8 Hz, 1H, 4^v -H), 7.56 (d, *J* = 2.6 Hz, 1H, 7^v -H), 7.12 (t, *J* = 8.2 Hz, 1H, 5'-H), 7.03 (dd, *J* = 8.8, 2.6 Hz, 1H, 5^v -H), 6.63 (dd, *J* = 8.5, 2.4 Hz, 1H, 4'-H), 6.31 (t, *J* = 2.3 Hz, 1H, 2'-H), 6.29 (dd, *J* = 7.9, 2.2 Hz, 1H, 6'-H), 3.81 (s, 3H, OCH_3), 3.30 (s, 2H, 1^{iv} -H), 3.27 (d, *J* = 7.0 Hz, 2H, 1''-H), 2.81 (d, *J* = 8.2 Hz, 1H, 6'''-H), 2.79 (s, 3H, NCH_3), 2.68 – 2.62 (m, 1H, 2'''-H), 2.59 (t, *J* = 7.1 Hz, 2H, 2''-H), 2.55 (m, 1H, 3'''-H), 2.27 – 2.20 (m, 1H, 2'''-H), 2.03 (t, *J* = 9.6 Hz, 1H, 6'''-H), 1.77 – 1.67 (m, 1H, 4'''-H), 1.64 (t, *J* = 7.7 Hz, 1H, 5'''-H), 1.52 – 1.40 (m, 1H, 5'''-H), 1.04 (d, *J* = 9.4 Hz, 1H, 4'''-H) ppm.

^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.6 (C-2^{iv}), 156.2 (C-6^v), 155.4 (C-2^v), 150.4 (C-3'), 150.1 (C-1'), 148.0 (C-2), 142.5 (C-3a^v), 136.8 (C-5), 132.9 (C-4), 132.8 (C-7a^v), 131.8 (C-3), 130.2 (C-5'), 126.4 (C-1), 125.2 (C-6), 121.1 (C-4^v), 114.9 (C-5^v), 110.9 (C-4'), 107.6 (C-6'), 104.7 (C-7^v), 104.3 (C-2'), 60.7 (C-1^{iv}), 59.0 (C-6'''), 55.6 (OCH_3), 53.7 (C-3'''), 53.3 (C-2'''), 52.3 (C-1''), 43.0 (C-2''), 38.2 (NCH_3), 30.1 (C-4'''), 23.2 (C-5''') ppm.

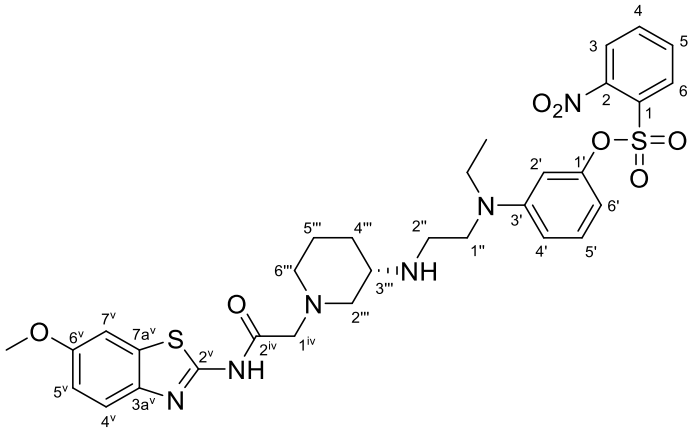
IR (ATR): $\tilde{\nu}$ = 2923, 1606, 1538, 1377, 1259, 1192, 1119, 1059, 997, 781, 739, 683 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{30}H_{35}N_6O_7S_2$ $[M+H]^+$: 655.2003; found: 655.1992.

Specific rotation: $[\alpha]_D^{20}$ = +4.1 (c = 0.25).

Purity (HPLC): >70% (λ = 210 nm), >82% (λ = 254 nm), Method 3f.

(*S*)-3-(Ethyl(2-((1-(2-((6-methoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)amino)ethyl)amino)phenyl 2-nitrobenzenesulfonate (181)



$C_{31}H_{36}N_6O_7S_2$

M_w = 668.80 g/mol

Secondary amine **181** was prepared following General procedure M, using 3-aminopiperidine **148** (182 mg, 0.500 mmol, 1.0 eq) and aldehyde **178**. Dark yellow solid (167 mg, 0.251 mmol, 50%).

R_f: 0.31 (96:3:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 57 – 60 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.18 (dd, *J* = 8.0, 1.2 Hz, 1H, 6-H), 8.04 (td, *J* = 7.8, 1.4 Hz, 1H, 5-H), 7.98 (dd, *J* = 8.0, 1.4 Hz, 1H, 3-H), 7.86 (td, *J* = 7.8, 1.2 Hz, 1H, 4-H), 7.62 (d, *J* = 8.8 Hz, 1H, 4^v-H), 7.56 (d, *J* = 2.6 Hz, 1H, 7^v-H), 7.10 (t, *J* = 8.5 Hz, 1H, 5'-H), 7.03 (dd, *J* = 8.8, 2.6 Hz, 1H, 5^v-H), 6.64 – 6.56 (m, 1H, 4'-H), 6.28 – 2.24 (m, 2H, 2'-H, 6'-H), 3.81 (s, 3H, OCH_3), 3.30 (s, 2H, 1^{iv}-H), 3.24 – 3.18 (m, 4H, 1''-H, C<u>H</u>$_2$CH$_3$), 2.81 (d, *J* = 10.1 Hz, 1H, 6'''-H), 2.65 (d, *J* = 7.0 Hz, 1H, 2'''-H), 2.60 – 2.51 (m, 3H, 2''-H, 3-H), 2.27 – 2.20 (m, 1H, 2'''-H), 2.04 (t, *J* = 9.7 Hz, 1H, 6'''-H), 1.72 (d, *J* = 9.6 Hz, 1H, 4'''-H), 1.69 – 1.59 (m, 1H, 5'''-H), 1.52 – 1.41 (m, 1H, 5'''-H), 1.05 (d, *J* = 11.1 Hz, 1H, 4'''-H), 0.91 (t, *J* = 7.0 Hz, 3H, CH$_2$C<u>H</u>$_3$) ppm.

^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.6 (C-2^{iv}), 156.2 (C-6^v), 155.5 (C-2^v), 150.2 (C-3'), 149.0 (C-1'), 147.9 (C-2), 142.5 (C-3a^v), 136.8 (C-5), 132.9 (C-4), 132.8 (C-7a^v), 131.7 (C-3), 130.4 (C-5'), 126.5 (C-1), 125.2 (C-6), 121.1 (C-4^v), 114.9 (C-5^v), 110.6 (C-4'), 107.2 (C-6'), 104.7 (C-7^v), 104.0 (C-2'), 60.7 (C-1^{iv}), 59.0 (C-6'''), 55.6 (OCH_3), 53.7 (C-3'''), 53.4 (C-2'''),

50.4 (C-1'' or $\underline{C}H_2CH_3$), 44.5 (C-1'' or $\underline{C}H_2CH_3$), 43.7 (C-2''), 30.2 (C-4'''), 23.3 (C-5'''), 11.6 ($CH_2\underline{C}H_3$) ppm.

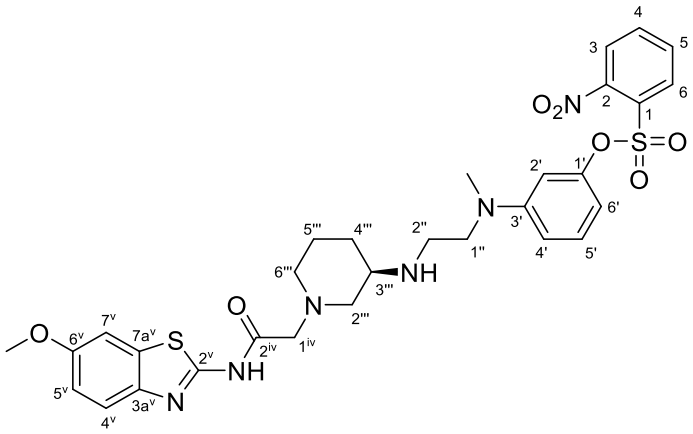
IR (ATR): $\tilde{\nu}$ = 1729, 1607, 1541, 1499, 1265, 1191, 1118, 949, 851, 778, 738, 683 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{31}H_{37}N_6O_7S_2$ $[M+H]^+$: 669.2160; found: 669.2148.

Specific rotation: $[\alpha]_D^{20}$ = +2.6 (c = 0.23).

Purity (HPLC): 100% (λ = 210 nm), >97% (λ = 254 nm), Method 3f.

(*R*)-3-((2-((1-(2-((6-Methoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)amino)ethyl)(methyl)amino)phenyl 2-nitrobenzenesulfonate (182)



$C_{30}H_{34}N_6O_7S_2$

M_w = 654.77 g/mol

Secondary amine **182** was prepared following General procedure M, using 3-aminopiperidine **149** (175 mg, 0.500 mmol, 1.0 eq) and aldehyde **175**. Light brown solid (164 mg, 0.250 mmol, 50%).

R_f: 0.42 (96:3:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 60 – 63 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.18 (dd, *J* = 8.0, 1.1 Hz, 1H, 6-H), 8.04 (td, *J* = 7.7, 1.4 Hz, 1H, 5-H), 7.97 (dd, *J* = 8.1, 1.4 Hz, 1H, 3-H), 7.86 (td, *J* = 7.8, 1.3 Hz, 1H, 4-H), 7.62 (d, *J* = 8.8 Hz, 1H, 4^v-H), 7.56 (d, *J* = 2.6 Hz, 1H, 7^v-H), 7.12 (t, *J* = 8.2 Hz, 1H, 5'-H), 7.02 (dd, *J* = 8.8, 2.6 Hz, 1H, 5^v-H), 6.62 (dd, *J* = 8.5, 2.4 Hz, 1H, 4'-H), 6.37 – 6.27 (m, 2H, 2'-H, 6'-H), 3.80 (s, 3H, OCH_3), 3.30 (s, 2H, 1^{iv}-H), 3.26 (d, *J* = 7.0 Hz, 2H, 1''-H), 2.79 (d, *J* = 8.2 Hz, 1H, 6'''-H), 2.79 (s, 3H, NCH_3), 2.69 – 2.61 (m, 1H, 2'''-H), 2.60 – 2.54 (m, 3H, 2''-H, 3-H), 2.26 – 2.21 (m, 1H, 2'''-H), 2.03 (t, *J* = 9.6 Hz, 1H, 6'''-H), 1.77 – 1.59 (m, 2H, 4'''-H, 5'''-H), 1.54 – 1.38 (m, 1H, 5'''-H), 1.05 – 1.02 (m, 1H, 4'''-H) ppm.

^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.6 (C-2^{iv}), 156.2 (C-6^{v}), 155.4 (C-2^{v}), 150.4 (C-3'), 150.1 (C-1'), 148.0 (C-2), 142.5 (C-3a^{v}), 136.8 (C-5), 132.9 (C-4), 132.8 (C-7a^{v}), 131.8 (C-3), 130.2 (C-5'), 126.4 (C-1), 125.2 (C-6), 121.1 (C-4^{v}), 114.9 (C-5^{v}), 110.9 (C-4'), 107.6 (C-6'), 104.7 (C-7^{v}), 104.3 (C-2'), 60.7 (C-1^{iv}), 59.0 (C-6'''), 55.6 (OCH_3), 53.7 (C-3'''), 53.3 (C-2'''), 52.3 (C-1''), 43.0 (C-2''), 38.2 (NCH_3), 30.2 (C-4'''), 23.3 (C-5''') ppm.

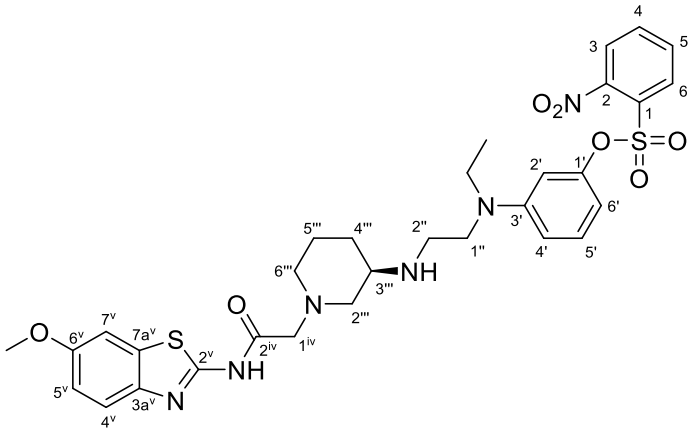
IR (ATR): $\tilde{\nu}$ = 2935, 1690, 1606, 1539, 1468, 1377, 1259, 1192, 1119, 781, 739, 683 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{30}H_{35}N_6O_7S_2$ $[M+H]^+$: 655.2003; found: 655.1998.

Specific rotation: $[\alpha]_D^{20}$ = -3.4 (c = 0.21).

Purity (HPLC): >98% (λ = 210 nm), >97% (λ = 254 nm), Method 3f.

(*R*)-3-(Ethyl(2-((1-(2-((6-methoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)amino)ethyl)amino)phenyl 2-nitrobenzenesulfonate (183)



$C_{31}H_{36}N_6O_7S_2$

M_w = 668.80 g/mol

Secondary amine **183** was prepared following General procedure M, using 3-aminopiperidine **149** (273 mg, 0.750 mmol, 1.0 eq) and aldehyde **178**. Dark yellow solid (265 mg, 0.396 mmol, 53%).

R_f: 0.38 (94:5:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 65 – 68 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.18 (dd, *J* = 8.0, 1.2 Hz, 1H, 6-H), 8.04 (td, *J* = 7.8, 1.4 Hz, 1H, 5-H), 7.98 (dd, *J* = 8.0, 1.4 Hz, 1H, 3-H), 7.86 (td, *J* = 7.8, 1.2 Hz, 1H, 4-H), 7.62 (d, *J* = 8.8 Hz, 1H, 4^{v}-H), 7.56 (d, *J* = 2.5 Hz, 1H, 7^{v}-H), 7.10 (t, *J* = 8.5 Hz, 1H, 5'-H), 7.03 (dd, *J* = 8.8, 2.7 Hz, 1H, 5^{v}-H), 6.63 – 6.57 (m, 1H, 4'-H), 6.26 (dd, *J* = 7.5, 1.9 Hz, 2H, 2'-H, 6'-H), 3.81 (s, 3H, OCH_3), 3.30 (s, 2H, 1^{iv}-H), 3.24 – 3.18 (m, 4H, 1''-H, $C\underline{H}_2CH_3$), 2.84 – 2.78 (m, 1H,

6’’’-H), 2.65 (d, *J* = 11.2 Hz, 1H, 2’’’-H), 2.61 – 2.51 (m, 3H, 2’’-H, 3-H), 2.26 – 2.22 (m, 1H, 2’’’-H), 2.04 (t, *J* = 9.5 Hz, 1H, 6’’’-H), 1.72 (d, *J* = 11.8 Hz, 1H, 4’’’-H), 1.68 – 1.62 (m, 1H, 5’’’-H), 1.50 – 1.44 (m, 1H, 5’’’-H), 1.05 (d, *J* = 11.3 Hz, 1H, 4’’’-H), 0.91 (t, *J* = 6.9 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

^{13}C NMR (126 MHz, DMSO-*d*$_6$): *δ* = 169.6 (C-2^{iv}), 156.2 (C-6^{v}), 155.5 (C-2^{v}), 150.2 (C-3’), 149.0 (C-1’), 147.9 (C-2), 142.5 (C-3a^{v}), 136.8 (C-5), 132.9 (C-4), 132.8 (C-7a^{v}), 131.7 (C-3), 130.4 (C-5’), 126.5 (C-1), 125.2 (C-6), 121.1 (C-4^{v}), 114.9 (C-5^{v}), 110.6 (C-4’), 107.2 (C-6’), 104.7 (C-7^{v}), 104.0 (C-2’), 60.7 (C-1^{iv}), 59.0 (C-6’’’), 55.6 (OCH_3), 53.7 (C-3’’’), 53.4 (C-2’’’), 50.5 (C-1’’ or $\underline{C}H_2CH_3$), 44.5 (C-1’’ or $\underline{C}H_2CH_3$), 43.7 (C-2’’), 30.2 (C-4’’’), 23.3 (C-5’’’), 11.6 ($CH_2\underline{C}H_3$) ppm.

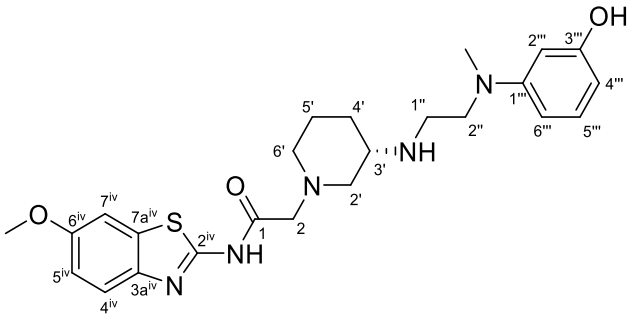
IR (ATR): $\tilde{\nu}$ = 1690, 1605, 1538, 1468, 1377, 1259, 1193, 1119, 1059, 830, 779, 739 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{31}H_{37}N_6O_7S_2$ $[M+H]^+$: 669.2160; found: 669.2147.

Specific rotation: $[\alpha]_D^{20}$ = -1.9 (c = 0.21).

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3f.

(*S*)-2-(3-((2-((3-Hydroxyphenyl)(methyl)amino)ethyl)amino)piperidin-1-yl)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (155)



$C_{24}H_{31}N_5O_3S$

M_w = 469.61 g/mol

Phenol **155** was prepared following General procedure N from nosyl derivative **180** (78 mg, 0.12 mmol, 1.0 eq). Off-white solid (43 mg, 0.091 mmol, 76%).

R_f: 0.38 (92:7:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 76 – 78 °C.

^{1}H NMR (500 MHz, DMSO-*d*$_6$): *δ* = 8.94 (s, 1H, CONH), 7.63 (d, *J* = 8.8 Hz, 1H, 4^{iv}-H), 7.56 (d, *J* = 2.6 Hz, 1H, 7^{iv}-H), 7.03 (dd, *J* = 8.8, 2.6 Hz, 1H, 5^{iv}-H), 6.88 (t, *J* = 8.1 Hz, 1H, 5’’’-H), 6.12 (dd, *J* = 8.3, 2.4 Hz, 1H, 6’’’-H), 6.07 (t, *J* = 2.3 Hz, 1H, 2’’’-H), 6.01 (dd, *J* = 7.9, 2.1 Hz,

1H, 4'''-H), 3.81 (s, 3H, OCH_3), 3.33 – 3.21 (m, 4H, 2-H, 2''-H), 2.89 – 2.82 (m, 1H, 6'-H), 2.81 (s, 3H, NCH_3), 2.67 (t, *J* = 7.2 Hz, 3H, 2'-H, 1''-H), 2.58 (tt, *J* = 8.3, 3.7 Hz, 1H, 3'-H), 2.30 – 2.17 (m, 1H, 2'-H), 2.04 (t, *J* = 9.6 Hz, 1H, 6'-H), 1.74 (d, *J* = 12.4 Hz, 1H, 4'-H), 1.64 (dt, *J* = 12.9, 4.0 Hz, 1H, 5'-H), 1.47 (qd, *J* = 10.2, 6.0 Hz, 1H, 5'-H), 1.05 (d, *J* = 11.0 Hz, 1H, 4'-H) ppm.

^{13}C NMR (126 MHz, DMSO-*d*$_6$): *δ* = 169.6 (C-1), 158.2 (C-3'''), 156.1 (C-6^{iv}), 155.5 (C-2^{iv}), 150.6 (C-1'''), 142.6 (C-3a^{iv}), 132.8 (C-7a^{iv}), 129.5 (C-5'''), 121.1 (C-4^{iv}), 114.9 (C-5^{iv}), 104.7 (C-7^{iv}), 103.2 (C-6'''), 103.1 (C-4'''), 98.9 (C-2'''), 60.7 (C-2), 59.1 (C-6'), 55.6 (OCH_3), 53.8 (C-3'), 53.3 (C-2'), 52.6 (C-2''), 43.3 (C-1''), 38.2 (NCH_3), 30.3 (C-4'), 23.3 (C-5') ppm.

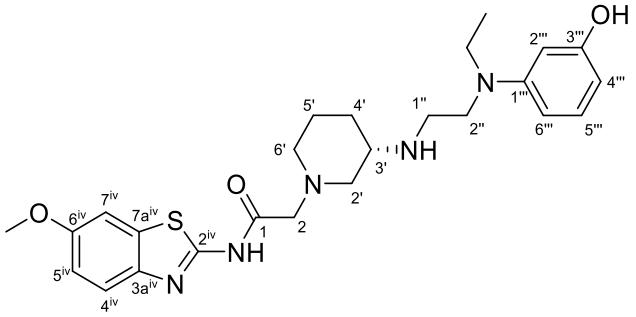
IR (ATR): $\tilde{\nu}$ = 2920, 1690, 1604, 1537, 1469, 1259, 1221, 1167, 1059, 823, 752, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{32}N_5O_3S$ $[M+H]^+$: 470.2221; found: 470.2213.

Specific rotation: $[\alpha]_D^{20}$ = +8.8 (c = 0.19).

Purity (HPLC): >97% (λ = 210 nm), >97% (λ = 254 nm), Method 1c.

(*S*)-2-(3-((2-(Ethyl(3-hydroxyphenyl)amino)ethyl)amino)piperidin-1-yl)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (156)



$C_{25}H_{33}N_5O_3S$

M_w = 483.63 g/mol

Phenol **156** was prepared following General procedure N from nosyl derivative **181** (140 mg, 0.210 mmol, 1.0 eq). Off-white solid (86 mg, 0.18 mmol, 85%).

R_f: 0.24 (94:5:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 87 – 88 °C.

^{1}H NMR (500 MHz, DMSO-*d*$_6$): *δ* = 7.63 (d, *J* = 8.7 Hz, 1H, 4^{iv}-H), 7.56 (d, *J* = 2.6 Hz, 1H, 7^{iv}-H), 7.03 (dd, *J* = 8.8, 2.6 Hz, 1H, 5^{iv}-H), 6.87 (t, *J* = 8.0 Hz, 1H, 5'''-H), 6.09 (dd, *J* = 8.3, 2.4 Hz, 1H, 6'''-H), 6.05 (t, *J* = 2.3 Hz, 1H, 2'''-H), 5.97 (dd, *J* = 7.9, 2.1 Hz, 1H, 4'''-H), 3.80 (s, 3H, OCH_3), 3.30 (d, *J* = 2.5 Hz, 2H, 2-H), 3.28 – 3.18 (m, 5H, 2''-H, $C\underline{H}_2CH_3$), 2.84 (d, *J* = 10.7 Hz,

1H, 6'-H), 2.67 (t, *J* = 7.3 Hz, 3H, 2'-H, 1''-H), 2.59 (tt, *J* = 8.1, 3.4 Hz, 1H, 3'-H), 2.24 (t, *J* = 10.1 Hz, 1H, 2'-H), 2.05 (t, *J* = 9.4 Hz, 1H, 6'-H), 1.79 – 1.69 (m, 1H, 4'-H), 1.65 (dt, *J* = 13.6, 4.3 Hz, 1H, 5'-H), 1.47 (qd, *J* = 11.7, 6.7 Hz, 1H, 5'-H), 1.10 – 1.04 (m, 1H, 4'-H), 1.01 (t, *J* = 6.9 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.6 (C-1), 158.3 (C-3'''), 156.2 (C-6^{iv}), 155.5 (C-2^{iv}), 149.1 (C-1'''), 142.6 (C-3a^{iv}), 132.8 (C-7a^{iv}), 129.7 (C-5'''), 121.1 (C-4^{iv}), 114.9 (C-5^{iv}), 104.7 (C-7^{iv}), 102.9 (C-6'''), 102.6 (C-4'''), 98.5 (C-2'''), 60.7 (C-2), 59.1 (C-6'), 55.6 (OCH_3), 53.8 (C-3'), 53.3 (C-2'), 50.5 (C-2''), 44.4 ($\underline{C}H_2CH_3$), 44.1 (C-1''), 30.3 (C-4'), 23.3 (C-5'), 12.2 ($CH_2\underline{C}H_3$) ppm.

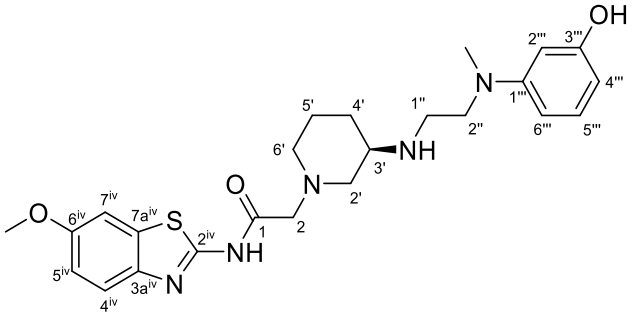
IR (ATR): $\tilde{\nu}$ = 2920, 1690, 1604, 1537, 1469, 1259, 1221, 1167, 1059, 823, 752, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{25}H_{34}N_5O_3S$ $[M+H]^+$: 484.2377; found: 484.23768.

Specific rotation: $[\alpha]_D^{20}$ = +5.0 (c = 0.18).

Purity (HPLC): >98% (λ = 210 nm), >98% (λ = 254 nm), Method 1c.

(*R*)-2-(3-((2-((3-Hydroxyphenyl)(methyl)amino)ethyl)amino)piperidin-1-yl)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (157)



$C_{24}H_{31}N_5O_3S$

M_w = 469.61 g/mol

Phenol **157** was prepared following General procedure N from nosyl derivative **182** (157 mg, 0.240 mmol, 1.0 eq). Off-white solid (95 mg, 0.20 mmol, 84%).

R_f: 0.23 (94:5:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 85 – 87 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.94 (s, 1H, CONH), 7.63 (d, *J* = 8.8 Hz, 1H, 4^{iv}-H), 7.56 (d, *J* = 2.6 Hz, 1H, 7^{iv}-H), 7.03 (dd, *J* = 8.8, 2.6 Hz, 1H, 5^{iv}-H), 6.88 (t, *J* = 8.1 Hz, 1H, 5'''-H), 6.12 (ddd, *J* = 8.4, 2.5, 0.8 Hz, 1H, 6'''-H), 6.07 (t, *J* = 2.3 Hz, 1H, 2'''-H), 6.01 (ddd, *J* = 8.0, 2.2, 0.8 Hz, 1H, 4'''-H), 3.80 (s, 3H, OCH_3), 3.32 – 3.25 (m, 4H, 2-H, 2''-H), 2.85 (s br, 1H, 6'-

H), 2.81 (s, 3H, NCH_3), 2.67 (t, *J* = 7.1 Hz, 3H, 2'-H, 1''-H), 2.58 (p, *J* = 4.7 Hz, 1H, 3'-H), 2.23 (t, *J* = 9.7 Hz, 1H, 2'-H), 2.04 (t, *J* = 9.5 Hz, 1H, 6'-H), 1.79 – 1.68 (m, 1H, 4'-H), 1.69 – 1.56 (m, 1H, 5'-H), 1.47 (dt, *J* = 13.5, 10.0 Hz, 1H, 5'-H), 1.05 (q, *J* = 10.3 Hz, 1H, 4'-H) ppm.

^{13}C NMR (126 MHz, DMSO-*d*$_6$): *δ* = 169.6 (C-1), 158.2 (C-3'''), 156.1 (C-6^{iv}), 155.5 (C-2^{iv}), 150.6 (C-1'''), 142.6 (C-3a^{iv}), 132.8 (C-7a^{iv}), 129.5 (C-5'''), 121.1 (C-4^{iv}), 114.9 (C-5^{iv}), 104.7 (C-7^{iv}), 103.2 (C-6'''), 103.1 (C-4'''), 98.9 (C-2'''), 60.7 (C-2), 59.1 (C-6'), 55.6 (OCH_3), 53.8 (C-3'), 53.3 (C-2'), 52.6 (C-2''), 43.3 (C-1''), 38.2 (NCH_3), 30.3 (C-4'), 23.3 (C-5') ppm.

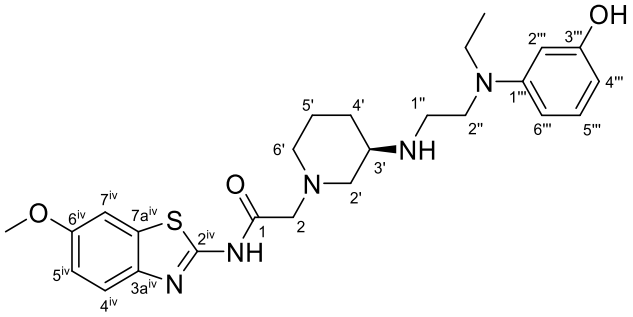
IR (ATR): $\tilde{\nu}$ = 2926, 1690, 1604, 1537, 1469, 1259, 1221, 1167, 1059, 823, 751, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{24}H_{32}N_5O_3S$ $[M+H]^+$: 470.2221; found: 470.2214.

Specific rotation: $[\alpha]_D^{20}$ = -7.4 (c = 0.26).

Purity (HPLC): >98% (λ = 210 nm), >98% (λ = 254 nm), Method 1c.

(*R*)-2-(3-((2-(Ethyl(3-hydroxyphenyl)amino)ethyl)amino)piperidin-1-yl)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (158)



$C_{25}H_{33}N_5O_3S$

M_w = 483.63 g/mol

Phenol **158** was prepared following General procedure N from nosyl derivative **183** (201 mg, 0.300 mmol, 1.0 eq). Off-white solid (88 mg, 0.27 mmol, 84%).

R_f: 0.38 (92:7:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 72 – 74 °C.

^{1}H NMR (500 MHz, DMSO-*d*$_6$): *δ* = 7.63 (d, *J* = 8.8 Hz, 1H, 4^{iv}-H), 7.56 (d, *J* = 2.6 Hz, 1H, 7^{iv}-H), 7.03 (dd, *J* = 8.8, 2.6 Hz, 1H, 5^{iv}-H), 6.87 (t, *J* = 8.1 Hz, 1H, 5'''-H), 6.09 (ddd, *J* = 8.4, 2.5, 0.8 Hz, 1H, 6'''-H), 6.05 (t, *J* = 2.3 Hz, 1H, 2'''-H), 5.97 (ddd, *J* = 7.9, 2.1, 0.8 Hz, 1H, 4'''-H), 3.80 (s, 3H, OCH_3), 3.30 (d, *J* = 1.6 Hz, 2H, 2-H), 3.28 – 3.19 (m, 5H, 2''-H, $C\underline{H}_2CH_3$), 2.84 (d, *J* = 10.7 Hz, 1H, 6'-H), 2.71 – 2.62 (m, 3H, 2'-H, 1''-H), 2.59 (dq, *J* = 8.2, 4.2 Hz, 1H, 3'-H), 2.24 (t, *J* = 9.9 Hz, 1H, 2'-H), 2.05 (t, *J* = 9.5 Hz, 1H, 6'-H), 1.82 – 1.70 (m, 1H, 4'-H), 1.65 (dd,

J = 9.1, 4.6 Hz, 1H, 5'-H), 1.47 (dd, *J* = 10.0, 3.8 Hz, 1H, 5'-H), 1.08 (t, *J* = 10.8 Hz, 1H, 4'-H), 1.01 (t, *J* = 6.9 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

^{13}C NMR (126 MHz, DMSO-*d*$_6$): *δ* = 169.6 (C-1), 158.3 (C-3'''), 156.2 (C-6^{iv}), 155.5 (C-2^{iv}), 149.1 (C-1'''), 142.6 (C-3a^{iv}), 132.8 (C-7a^{iv}), 129.7 (C-5'''), 121.1 (C-4^{iv}), 114.9 (C-5^{iv}), 104.7 (C-7^{iv}), 102.9 (C-6'''), 102.6 (C-4'''), 98.5 (C-2'''), 60.7 (C-2), 59.1 (C-6'), 55.6 (OCH_3), 53.8 (C-3'), 53.3 (C-2'), 50.5 (C-2''), 44.4 ($\underline{C}H_2CH_3$), 44.1 (C-1''), 30.3 (C-4'), 23.3 (C-5'), 12.1 ($CH_2\underline{C}H_3$) ppm.

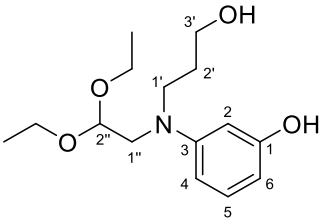
IR (ATR): $\tilde{\nu}$ = 1692, 1604, 1537, 1468, 1259, 121, 1167, 1059, 1025, 823, 731, 699 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{25}H_{34}N_5O_3S$ $[M+H]^+$: 484.2377; found: 484.2367.

Specific rotation: $[\alpha]_D^{20}$ = -4.7 (c = 0.26).

Purity (HPLC): >98% (λ = 210 nm), >99% (λ = 254 nm), Method 1c.

3-((2,2-Diethoxyethyl)(3-hydroxypropyl)amino)phenol (184)



$C_{15}H_{25}NO_4$

M_w = 283.37 g/mol

N-Substituted aromatic amine **184** was prepared following General procedure P, using secondary aromatic amine **168** (1.83 g, 8.10 mmol, 1.0 eq), 3-bromo-1-propanol (1.41 mL, 16.2 mmol, 2.0 eq), and $NaHCO_3$ (1.36 g, 16.2 mmol 2.0 eq). The reaction was stirred at 100 °C for 5 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:1 EtOAc/isohexanes), yielding product **184** (1.60 g, 5.65 mmol, 70%) as a colourless oil.

R_f: 0.45 (1:1 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 7.06 (t, *J* = 8.1 Hz, 1H, 5-H), 6.32 (d, *J* = 8.1 Hz, 2H, 2-H, 4-H or 6-H), 6.20 (d, *J* = 7.9 Hz, 1H, 4-H or 6-H), 5.76 (s, 1H), 4.66 (t, *J* = 5.1 Hz, 1H, 2''-H), 3.82 – 3.64 (m, 4H, 3'-H, $OC\underline{H}_2CH_3$), 3.59 – 3.47 (m, 4H, 1'-H, $OC\underline{H}_2CH_3$), 3.42 (d, *J* = 5.1 Hz, 2H, 1''-H), 1.85 (dq, *J* = 7.7, 6.0 Hz, 2H, 2'-H), 1.21 (t, *J* = 7.0 Hz, 6H, 2 $OCH_2C\underline{H}_3$) ppm.

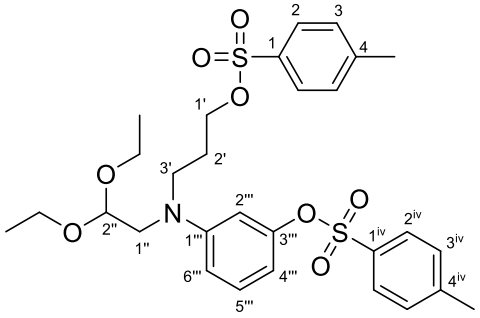
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.2 (C-1), 150.0 (C-3), 130.4 (C-5), 105.2 (C-2, C-4, or C-6), 103.5 (C-4 or C-6), 101.3 (C-2''), 99.9 (C-2, C-4, or C-6), 63.4 ($O\underline{C}H_2CH_3$), 60.7 (C-3'), 55.4 (C-1''), 48.9 (C-1'), 29.7 (C-2'), 15.6 ($OCH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3364, 2882, 1613, 1577, 1503, 1373, 1166, 1122, 1044, 1008, 753, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{15}H_{24}NO_4$ $[M-H]^-$: 282.1710; found: 282.1707.

Purity (HPLC): >95% (λ = 210 nm), >92% (λ = 254 nm), Method 3f.

3-((2,2-Diethoxyethyl)(3-(tosyloxy)phenyl)amino)propyl 4-methylbenzenesulfonate (187)



$C_{29}H_{37}NO_8S_2$

M_w = 591.75 g/mol

Bis-tosylate **187** was prepared following General procedure O from phenol **184** (1.56 g, 5.50 mmol, 1.0 eq). The crude was purified by flash column chromatography (1:5 EtOAc/isohexanes) to give product **187** (2.16 g, 3.65 mmol, 66%) as a light-pink oil.

R_f:0.24 (1:4 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.83 – 7.76 (m, 2H, 3-H or 3^{iv}-H), 7.76 – 7.66 (m, 2H, 3-H or 3^{iv}-H), 7.40 – 7.33 (m, 2H, 2-H or 2^{iv}-H), 7.33 – 7.28 (m, 2H, 2-H or 2^{iv}-H), 7.05 (t, *J* = 8.3 Hz, 1H, 5'''-H), 6.52 (dd, *J* = 8.4, 2.5 Hz, 1H, 6'''-H), 6.26 (dd, *J* = 7.9, 2.1 Hz, 1H, 4'''-H), 6.23 (t, *J* = 2.4 Hz, 1H, 2'''-H), 4.49 (t, *J* = 5.1 Hz, 1H, 2''-H), 4.02 (t, *J* = 5.9 Hz, 2H, 1'-H), 3.65 (dq, *J* = 9.2, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.45 (dq, *J* = 9.2, 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 3.37 – 3.30 (m, 2H, 3'-H), 3.25 (d, *J* = 5.1 Hz, 2H, 1''-H), 2.43 (s, 3H, (C-4)CH_3 or (C-4^{iv})CH_3), 2.45 (s, 3H, (C-4)CH_3 or (C-4^{iv})CH_3), 1.86 – 1.77 (m, 2H, 2'-H), 1.16 (t, *J* = 7.0 Hz, 6H, 2 $OCH_2C\underline{H}_3$) ppm.

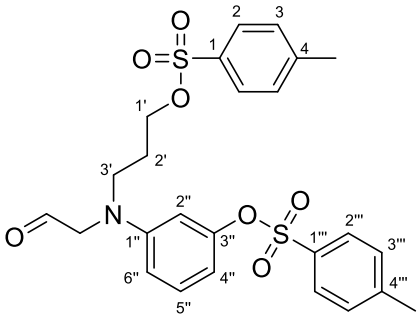
^{13}C NMR (126 MHz, $CDCl_3$): δ = 151.1 (C-3), 148.8 (C-1), 145.3 (C-4 or C-4^{iv}), 145.1 (C-4 or C-4^{iv}), 133.0 (C-1 or C-1^{iv}), 132.9 (C-1 or C-1^{iv}), 130.1 (C-5'''), 130.0 (C-2 or C-2^{iv}), 129.8 (C-2 or C-2^{iv}), 128.7 (C-3 or C-3^{iv}), 128.0 (C-3 or C-3^{iv}), 110.8 (C-6'''), 109.9 (C-4'''), 106.2 (C-2'''), 100.7 (C-2''), 68.2 (C-1'), 63.4 ($O\underline{C}H_2CH_3$), 54.8 (C-1''), 48.1 (C-3'), 26.2 (C-2'), 21.9 ((C-4)$\underline{C}H_3$ or (C-4^{iv})$\underline{C}H_3$), 21.8 ((C-4)$\underline{C}H_3$ or (C-4^{iv})$\underline{C}H_3$), 15.6 ($OCH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 1608, 1499, 1359, 1190, 1174, 1092, 1057, 921, 813, 780, 686, 660 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{29}H_{38}NO_8S_2$ $[M+H]^+$: 592.2034; found: 592.2034.

Purity (HPLC): ND.

3-((2-Oxoethyl)(3-(tosyloxy)phenyl)amino)propyl 4-methylbenzenesulfonate (188)



$C_{25}H_{27}NO_7S_2$

M_w = 517.62 g/mol

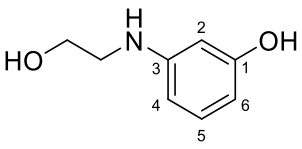
Aldehyde **175** was prepared following General procedure L from diethyl acetal **187** (166 mg, 0.28 mmol, 1.0 eq). The crude was purified by flash column chromatography (1:1 EtOAc/isohexanes) to give product **187** (90 mg, 0.17 mmol, 62%) as a light-brown oil. ^{13}C NMR, IR, and purity by HPLC were not determined.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.55 (d, *J* = 0.9 Hz, 1H, CHO), 7.81 – 7.76 (m, 2H, 3-H or 3'''-H), 7.72 – 7.68 (m, 2H, 3-H or 3'''-H), 7.38 – 7.33 (m, 2H, 2-H or 2'''-H), 7.33 – 7.29 (m, 2H, 2-H or 2'''-H), 7.07 (t, *J* = 8.3 Hz, 1H, 5''-H), 6.38 (ddd, *J* = 8.4, 2.7, 0.8 Hz, 1H, 4''-H or 6''-H), 6.33 (ddd, *J* = 8.1, 2.1, 0.7 Hz, 1H, 4''-H or 6''-H), 6.14 (t, *J* = 2.4 Hz, 1H, 2''-H), 4.05 (t, *J* = 5.7 Hz, 2H, 3'-H), 3.94 (d, *J* = 0.9 Hz, 2H, C$\underline{H_2}$CHO), 3.40 (t, *J* = 6.9 Hz, 2H, 1'-H), 2.46 (s, 3H, (C-4)CH_3 or (C-4''')CH_3), 2.44 (s, 3H, (C-4)CH_3 or (C-4''')CH_3), 1.92 – 1.80 (m, 2H, 2'-H) ppm.

HRMS (ESI): *m/z* = calculated for $C_{25}H_{28}NO_7S_2$ $[M+H]^+$: 518.1302; found: 518.1297.

Purity (HPLC): ND.

3-((2-Hydroxyethyl)amino)phenol (191)



$C_8H_{11}NO_2$

M_w = 153.18 g/mol

N-Substituted aromatic amine **191** was prepared following General procedure P, using 3-aminophenol (**167**, 1.09 g, 10.0 mmol, 1.1 eq), 2-bromo-1-ethanol (0.428 mL, 11.0 mmol, 1.1 eq), and $NaHCO_3$ (294 mg, 11.0 mmol 1.1 eq). The reaction mixture was stirred at 100 °C for 18 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (2:1 → 4:1 EtOAc/isohexanes), yielding product **191** (1.44 g, 9.43 mmol, 94%) as a dark orange oil.

R_f: 0.25 (2:1 EtOAc/isohexanes).

^{1}H NMR (500 MHz, CD_3OD): δ = 6.91 (t, J = 8.0 Hz, 1H, 5-H), 6.20 – 6.15 (m, 1H, 4-H), 6.13 (t, J = 2.2 Hz, 1H, 2-H), 6.11 (ddd, J = 7.9, 2.3, 0.9 Hz, 1H, 6-H), 3.70 (t, J = 5.8 Hz, 2H, $NCH_2C\underline{H}_2OH$), 3.18 (t, J = 5.8 Hz, 2H, $NC\underline{H}_2CH_2OH$) ppm.

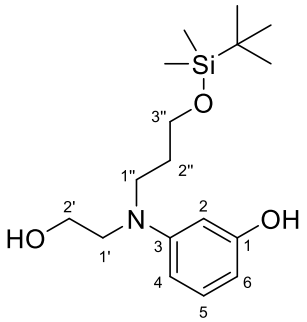
^{13}C NMR (126 MHz, CD_3OD): δ = 159.3 (C-1), 151.5 (C-3), 130.8 (C-5), 106.3 (C-4), 105.5 (C-2), 101.1 (C-6), 61.5 ($NCH_2\underline{C}H_2OH$), 47.2 ($N\underline{C}H_2CH_2OH$) ppm.

IR (ATR): $\tilde{\nu}$ = 1651, 1590, 1496, 1386, 1184, 1157, 1098, 1058, 762, 690, 661 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_8H_{11}NO_2$ $[M]^{\cdot+}$: 153.0785; found: 153.0784.

Purity (HPLC): >95% (λ = 210 nm), >97% (λ = 254 nm), Method 3f.

3-((3-((*tert*-Butyldimethylsilyl)oxy)propyl)(2-hydroxyethyl)amino)phenol (192)



$C_{17}H_{31}NO_3Si$

M_w = 325.52 g/mol

N-Substituted aromatic amine **192** was prepared following General procedure P, using secondary amine **191** (429 mg, 2.80 mmol, 1.0 eq), (3-bromopropoxy)(*tert*-butyl)dimethylsilane (1.42 g, 5.60 mmol, 2.0 eq), and $NaHCO_3$ (470 mg, 5.60 mmol 2.0 eq). The reaction was stirred at 100 °C for 16 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:1 EtOAc/isohexanes), yielding product **192** (552 mg, 1.69 mmol, 60%) as a dark yellow oil.

R_f: 0.54 (1:1 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.05 (t, *J* = 8.1 Hz, 1H, 5-H), 6.36 (ddd, *J* = 8.4, 2.4, 0.8 Hz, 1H, 4-H), 6.26 (t, *J* = 2.4 Hz, 1H, 2-H), 6.18 (ddd, *J* = 8.0, 2.3, 0.8 Hz, 1H, 6-H), 3.79 (t, *J* = 5.8 Hz, 2H, 2'-H), 3.67 (t, *J* = 5.7 Hz, 2H, 3''-H), 3.50 – 3.37 (m, 4H, 1'-H, 1''-H), 1.84 – 1.72 (m, 2H, 2''-H), 0.91 (s, 9H, $SiC(CH_3)_3$), 0.07 (s, 6H, $Si(CH_3)_2$) ppm.

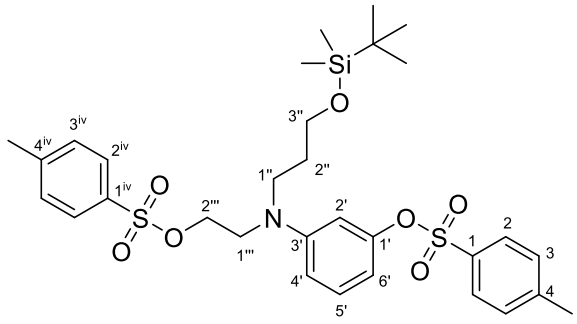
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.0 (C-1), 150.2 (C-3), 130.3 (C-5), 105.8 (C-4), 103.8 (C-6), 100.12 (C-2), 60.7 (C-3''), 60.5 (C-2'), 53.8 (C-1'), 48.4 (C-1''), 30.0 (C-2''), 26.1 ($SiC(\underline{C}H_3)_3$), 18.4 ($Si\underline{C}(CH_3)_3$), -5.2 ($Si(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3318, 2928, 1614, 1578, 1503, 1471, 1251, 1168, 1093, 831, 773, 687 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{17}H_{31}NO_3Si$ $[M]^{\cdot+}$: 325.2065; found: 325.2065.

Purity (HPLC): ND.

3-((3-((*tert*-Butyldimethylsilyl)oxy)propyl)(2-(tosyloxy)ethyl)amino)phenyl 4-methylbenzenesulfonate (193)



$C_{31}H_{43}NO_7S_2Si$

M_w = 633.90 g/mol

Bis-tosylate **193** was prepared following General procedure O from phenol **192** (488 mg, 1.50 mmol, 1.0 eq). The crude was purified by flash column chromatography (1:4 EtOAc/isohexanes) to give product **193** (514 mg, 0.811 mmol, 54%) as a dark-orange oil.

R_f: 0.54 (1:4 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 – 7.68 (m, 4H, 3-H, 3^{iv}-H), 7.33 – 7.27 (m, 4H, 2-H, 2^{iv}-H), 7.00 (t, *J* = 8.2 Hz, 1H, 5'-H), 6.47 – 6.41 (m, 1H, 6'-H), 6.22 (ddd, *J* = 8.0, 2.2, 0.7 Hz, 1H, 4'-H), 6.13 (t, *J* = 2.4 Hz, 1H, 2'-H), 4.05 (t, *J* = 6.1 Hz, 2H, 2'''-H), 3.56 (t, *J* = 5.7 Hz, 2H, 3''-H), 3.50 (t, *J* = 6.1 Hz, 2H, 1'''-H), 3.30 – 3.21 (m, 2H, 1''-H), 2.44 (s, 3H, (C-4)CH_3 or (C-4^{iv})CH_3), 2.42 (s, 3H, (C-4)CH_3 or (C-4^{iv})CH_3), 1.60 (dt, *J* = 12.9, 6.0 Hz, 2H, 2''-H), 0.90 (s, 9H, $SiC(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_2$) ppm.

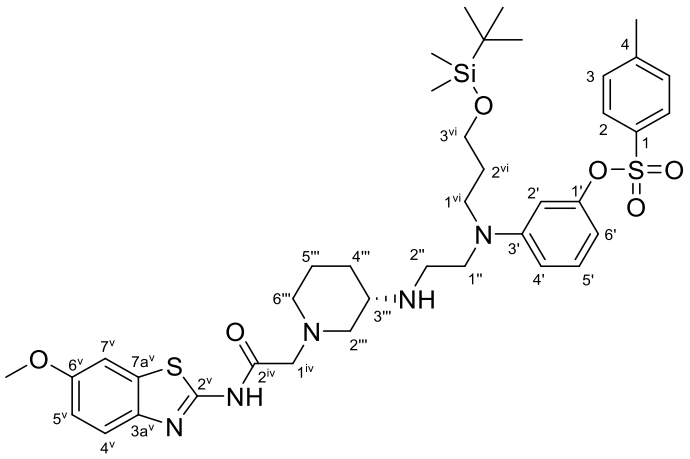
^{13}C NMR (101 MHz, $CDCl_3$): δ = 151.1 (C-1'), 148.3 (C-3'), 145.3 (C-4 or C-4^{iv}), 145.1 (C-4 or C-4^{iv}), 132.9 (C-1 or C-1^{iv}), 132.8 (C-1 or C-1^{iv}), 130.1 (C-5'), 130.0 (C-2 or C-2^{iv}), 129.8 (C-2 or C-2^{iv}), 128.7 (C-3 or C-3^{iv}), 128.0 (C-3 or C-3^{iv}), 110.6 (C-4'), 109.7 (C-6'), 105.9 (C-2'), 66.5 (C-2'''), 60.2 (C-3''), 49.6 (C-1'''), 48.2 (C-1''), 29.9 (C-2''), 26.1 ($SiC(\underline{C}H_3)_3$), 21.8 ((C-4)CH_3 or (C-4''')CH_3), 21.8 ((C-4)CH_3 or (C-4''')CH_3), 18.4 ($Si\underline{C}(CH_3)_3$), -5.2 ($Si(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 2928, 2856, 1609, 1499, 1360, 1190, 1174, 1093, 833, 813, 772, 660 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{31}H_{44}NO_7S_2Si$ $[M+H]^+$: 634.2323; found: 634.2332.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3f.

(*S*)-3-((3-((*tert*-Butyldimethylsilyl)oxy)propyl)(2-((1-(2-((6-methoxybenzo[*d*]thiazol-2-yl)amino)-2-oxoethyl)piperidin-3-yl)amino)ethyl)amino)phenyl 4-methylbenzenesulfonate (195)



$C_{39}H_{55}N_5O_6S_2Si$

M_w = 782.11 g/mol

Tosylate **193** (133 mg, 0.210 mmol, 1.0 eq) and DIPEA (0.181 mL, 1.05 mmol, 5.0 eq) were dissolved in 0.7 mL MeOH in a pressure tube. A solution of amine **149** (67 mg, 0.21 mmol, 1.0 eq) in 2 mL MeOH was added, the pressure tube capped, and the reaction mixture was stirred at 140 °C for 3 h. After cooling down to room temperature, water was added (20 mL), and the mixture was extracted with $CHCl_3$/isopropanol (3:1, 3 x 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (97:2:1 DCM/MeOH/25% NH_3 aq. solution), yielding secondary amine **195** (14 mg, 0.018 mmol, 9%) as a beige solid. Mp, ^{13}C NMR, IR, and purity by HPLC were not determined.

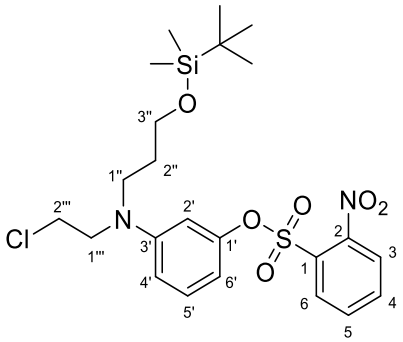
^{1}H NMR (500 MHz, CD_3OD): δ = 7.63 (d, *J* = 8.1 Hz, 2H, 2-H or 3-H), 7.61 (d, *J* = 8.8 Hz, 1H, 4^v-H), 7.40 (d, *J* = 2.5 Hz, 1H, 7^v-H), 7.32 (d, *J* = 8.0 Hz, 2H, 2-H or 3-H), 7.03 (dd, *J* = 8.9,

2.6 Hz, 1H, 5ᵛ-H), 6.98 (t, *J* = 8.2 Hz, 1H, 5'-H), 6.63 (dd, *J* = 8.6, 2.5 Hz, 1H, 4'-H or 6'-H), 6.30 (t, *J* = 2.3 Hz, 1H, 2'-H), 6.13 (dd, *J* = 8.0, 2.1 Hz, 1H, 4'-H or 6'-H), 3.85 (s, 3H, OCH_3), 3.54 (t, *J* = 5.7 Hz, 2H), 3.38 (t, *J* = 7.6 Hz, 2H), 3.31 – 3.26 (m, 2H), 2.92 – 2.61 (m, 5H), 2.44 (s, 1H), 2.40 (s, 3H), 2.28 (d, *J* = 11.0 Hz, 1H), 1.82 (s, 2H), 1.72 – 1.62 (m, 1H), 1.58 (dq, *J* = 11.9, 6.3 Hz, 2H), 1.26 (d, *J* = 23.2 Hz, 3H), 0.90 (s, 9H, $OSiC(CH_3)_3$), 0.04 (s, 6H, $OSi(CH_3)_2$) ppm.

HRMS (ESI): *m/z* = calculated for $C_{39}H_{56}N_5O_6S_2Si$ $[M+H]^+$: 782.3436; found: 782.3427.

Purity (HPLC): ND.

3-((3-((*tert*-Butyldimethylsilyl)oxy)propyl)(2-chloroethyl)amino)phenyl 4-nitrobenzenesulfonate (197)



$C_{23}H_{34}N_2O_7SSi$

M_w = 529.12 g/mol

Alkyl chloride **197** was obtained as by-product of nosylation of compound **192**. Phenol **192** (488 mg, 1.50 mmol, 1.0 eq) was dissolved in 2.25 mL ACN. 2-Nosyl chloride (997 mg, 4.50 mmol, 3.0 eq) and triethylamine (0.627 mL, 4.50 mmol, 3.0 eq) were added at 0 °C, and the mixture was stirred for 2 h. Ice-water was added, and the mixture was extracted with DCM (3 x 30 mL). The organic layers were combined and washed with 2 M HCl aq. solution (2 x 30). The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (1:4 EtOAc/isohexanes), yielding product **197** (459 mg, 0.867 mmol, 58%) as an orange oil.

R_f: 0.72 (2:3 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.00 – 7.97 (m, 1H, 6-H), 7.85 – 7.78 (m, 2H, 3-H, 5-H), 7.68 (ddd, *J* = 7.7, 6.3, 2.5 Hz, 1H, 4-H), 7.14 (t, *J* = 8.2 Hz, 1H, 5'-H), 6.68 – 6.63 (m, 1H, 6'-H), 6.53 – 6.45 (m, 2H, 2'-H, 4'-H), 3.62 (t, *J* = 5.7 Hz, 2H, 3''-H), 3.58 (t, *J* = 7.5 Hz, 2H, 1'''-H), 3.49 (dd, *J* = 7.6, 6.4 Hz, 2H, 2'''-H), 3.45 – 3.39 (m, 2H, 1''-H), 1.71 (dq, *J* = 7.9, 5.9 Hz, 2H, 2''-H), 0.91 (s, 9H, $SiC(CH_3)_3$), 0.06 (s, 6H, $Si(CH_3)_2$) ppm.

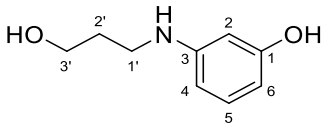
^{13}C NMR (126 MHz, $CDCl_3$): δ = 150.7 (C-1'), 148.9 (C-2), 148.4 (C-3'), 135.3 (C-3 or C-5), 132.3 (C-3), 132.0 (C-6), 130.6 (C-5'), 128.8 (C-1), 124.9 (C-3 or C-5), 111.6 (C-6'), 110.1 (C-2'), 106.0 (C-4'), 60.1 (C-3''), 53.2 (C-1'''), 48.5 (C-1''), 39.9 (C-2'''), 30.0 (C-2''), 26.0 ($SiC(\underline{C}H_3)_3$), 18.4 ($Si\underline{C}(CH_3)_3$), -5.3 ($Si(CH_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1610, 1544, 1500, 1383, 1362, 1192, 1123, 1094, 833, 774, 738 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{23}H_{35}N_2O_7SSi$ $[M+H]^+$: 529.1590; found: 529.1584.

Purity (HPLC): >94% (λ = 210 nm), >97% (λ = 254 nm), Method 3f.

3-((3-Hydroxypropyl)amino)phenol (202)



$C_9H_{13}NO_2$

M_w = 167.21 g/mol

N-Substituted aromatic amine **202** was prepared following General procedure P, using 3-aminophenol (**167**, 1.09 g, 10.0 mmol, 1.0 eq), 3-bromo-1-propanol (0.956 mL, 11.0 mmol, 1.1 eq), and $NaHCO_3$ (924 mg, 11.0 mmol, 1.1 eq). The reaction was stirred at 100 °C for 16 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (2:1 → 5:1 EtOAc/isohexanes), yielding product **202** (970 mg, 5.80 mmol, 58%) as a light orange oil.

R_f: 0.40 (1:3 EtOAc/isohexanes).

^{1}H NMR (400 MHz, CD_3OD): δ = 6.90 (t, *J* = 8.0 Hz, 1H, 5-H), 6.16 (ddd, *J* = 8.1, 2.2, 0.9 Hz, 1H, 4-H), 6.12 (t, *J* = 2.2 Hz, 1H, 2-H), 6.09 (ddd, *J* = 7.9, 2.3, 0.9 Hz, 1H, 6-H), 3.67 (t, *J* = 6.3 Hz, 2H, 3'-H), 3.14 (t, *J* = 7.0 Hz, 2H, 1'-H), 1.85 – 1.76 (m, 2H, 2'-H) ppm.

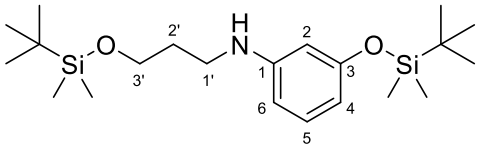
^{13}C NMR (101 MHz, CD_3OD): δ = 159.2 (C-1), 151.8 (C-3), 130.7 (C-5), 106.4 (C-4), 105.2 (C-6), 101.0 (C-2), 61.0 (C-3'), 42.0 (C-1'), 33.1 (C-2') ppm.

IR (ATR): $\tilde{\nu}$ = 3310, 2941, 1592, 1496, 1183, 1157, 1054, 827, 760, 687 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_9H_{13}NO_2$ $[M]^{\cdot+}$: 167.0941; found: 167.0940.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

3-((*tert*-Butyldimethylsilyl)oxy)-*N*-(3-((*tert*-butyldimethylsilyl)oxy)propyl)aniline (203)



$C_{21}H_{41}NO_2Si$

M_w = 395.73 g/mol

tert-Butyldimethylchlorosilane (1.08 g, 7.19 mmol, 2.2 eq) was added to a solution of phenol **202** (547 mg, 3.27 mmol, 1.0 eq) and imidazole (890 mg, 13.1 mmol, 2 eq) in 4.5 mL anhydrous THF. The reaction mixture was stirred at room temperature for 3 h. Water (30 mL) was added, and the mixture was extracted with DCM (3 x 30 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (1:9 EtOAc/isohexanes), yielding silyl ether **203** (975 mg, 2.46 mmol, 75%) as a light yellow oil.

R_f: 0.54 (1:9 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.01 (t, *J* = 8.0 Hz, 1H. 5-H), 6.35 – 6.26 (m, 1H, 4-H or 6-H), 6.23 (ddd, *J* = 8.0, 2.5, 0.9 Hz, 1H, 4-H or 6-H), 6.17 (d, *J* = 2.3 Hz, 1H, 2-H), 3.76 (t, *J* = 5.7 Hz, 2H, 3'-H), 3.21 (t, *J* = 6.5 Hz, 2H, 1-H), 1.90 – 1.78 (m, 2H, 2'-H), 0.97 (s, 9H, (C-3)OSiC$(CH_3)_3$), 0.91 (s, 9H, (C-3')OSiC$(CH_3)_3$), 0.19 (s, 6H, (C-3)OSi$(CH_3)_2$), 0.07 (s, 6H, (C-3')OSi$(CH_3)_2$) ppm.

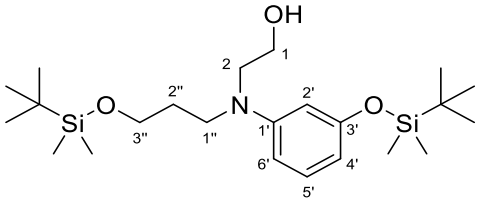
^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.9 (C-3), 150.0 (C-1), 130.0 (C-5), 110.0 (C-4 or C-6), 107.1 (C-4 or C-6), 105.4 (C-2), 62.2 (C-3'), 43.0 (C-1'), 31.7 (C-2'), 26.1 ((C-3)OSiC($\underline{C}H_3)_3$ or (C-3')OSiC($\underline{C}H_3)_3$), 25.9 ((C-3)OSiC($\underline{C}H_3)_3$ or (C-3')OSiC($\underline{C}H_3)_3$), 18.4 ((C-3)OSi$\underline{C}(CH_3)_3$ or (C-3')OSi$\underline{C}(CH_3)_3$), 18.3 ((C-3)OSi$\underline{C}(CH_3)_3$ or (C-3')OSi$\underline{C}(CH_3)_3$), -4.2 ((C-3)OSi($\underline{C}H_3)_2$), -5.3 ((C-3')OSi($\underline{C}H_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1598, 1581, 1505, 1257, 1204, 1159, 1071, 994, 834, 774, 759, 730, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{21}H_{42}NO_2Si$ $[M+H]^+$: 396.2749; found: 396.2742.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3f.

2-((3-((*tert*-Butyldimethylsilyl)oxy)phenyl)(3-((*tert*-butyldimethylsilyl)oxy)propyl)amino)ethan-1-ol (200)



$C_{23}H_{45}NO_3Si_2$

M_w = 439.79 g/mol

N-Substituted aromatic amine **200** was prepared following General procedure P, using secondary aromatic amine **203** (1.99 mg, 5.00 mmol, 1.0 eq), 2-bromo-1-ethanol (0.709 mL, 10.0 mmol, 2.0 eq), and $NaHCO_3$ (840 mg, 10.0 mmol 2.0 eq). The reaction was stirred at 100 °C for 18 h. The mixture was extracted with diethyl ether, and the crude purified by flash column chromatography (1:6 EtOAc/isohexanes), yielding product **200** (1.42 g, 3.24 mmol, 65%) as a light yellow oil.

R_f: 0.42 (5:95 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.04 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.50 – 6.36 (m, 1H, 6'-H), 6.25 (s, 1H, 2'-H), 6.21 (d, *J* = 7.9 Hz, 1H, 4'-H), 3.78 (q, *J* = 5.7 Hz, 2H, 1-H), 3.67 (t, *J* = 5.8 Hz, 2H, 3''-H), 3.55 – 3.36 (m, 4H, 2-H, 1''-H), 1.94 (t, *J* = 6.0 Hz, 1H, OH), 1.79 (p, *J* = 6.3 Hz, 2H, 2''-H), 0.98 (s, 9H, (C-3')OSiC(CH_3)$_3$), 0.91 (s, 9H, (C-3'')OSiC(CH_3)$_3$), 0.20 (s, 6H, (C-3')OSi(CH_3)$_2$), 0.06 (s, 6H, (C-3'')OSi(CH_3)$_2$) ppm.

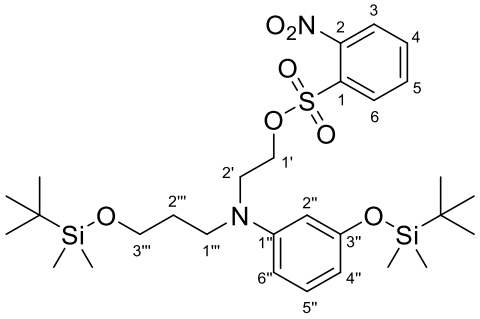
^{13}C NMR (126 MHz, $CDCl_3$): δ = 156.9 (C-3'), 150.1 (C-2'), 129.8 (C-5'), 108.7 (C-4'), 106.8 (C-6'), 105.3 (C-2'), 60.8 (C-3''), 60.5 (C-1), 54.1 (C-2 or C-1''), 48.6 (C-2 or C-1''), 30.0 (C-2''), 26.1 ((C-3')OSiC($\underline{C}H_3$)$_3$ or (C-3'')OSiC($\underline{C}H_3$)$_3$), 25.9 ((C-3')OSiC($\underline{C}H_3$)$_3$ or (C-3'')OSiC($\underline{C}H_3$)$_3$), 18.4 ((C-3')OSi$\underline{C}$(CH_3)$_3$ or (C-3'')OSi$\underline{C}$(CH_3)$_3$), 18.4 ((C-3')OSi$\underline{C}$(CH_3)$_3$ or (C-3'')OSi$\underline{C}$(CH_3)$_3$), -4.2 ((C-3')OSi($\underline{C}H_3$)$_2$), -5.2 ((C-3'')OSi($\underline{C}H_3$)$_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1598, 1582, 1505, 1470, 1252, 1203, 1071, 994, 833, 774, 730, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{23}H_{46}NO_3Si_2$ $[M+H]^+$: 440.3011; found: 440.3004.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 3f.

2-((3-((*tert*-Butyldimethylsilyl)oxy)phenyl)(3-((tert-butyldimethylsilyl)oxy)propyl)amino)ethyl 2-nitrobenzenesulfonate (201)



$C_{29}H_{48}N_2O_7SSi$

M_w = 624.24 g/mol

Nosyl derivative **201** was prepared following General procedure K from 2-aminoethanol derivative **200** (220 mg, 0.500 mmol, 1.0 eq). Triethylamine was added at first, followed by portion wise addition of 2-nosyl chloride. The crude product was purified by flash column chromatography (1:9 EtOAc/isohexanes), yielding product **201** (157 mg, 0.251 mmol, 50%) as light-red oil.

R_f: 0.25 (5:95 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.25 (dd, *J* = 7.8, 1.5 Hz, 1H, 3-H or 6-H), 8.21 (dd, *J* = 8.1, 1.4 Hz, 1H, 3-H or 6-H), 7.87 (td, *J* = 7.6, 1.3 Hz, 1H, 4-H or 5-H), 7.73 – 7.68 (m, 1H, 4-H or 5-H), 6.99 (t, *J* = 8.3 Hz, 1H, 5''-H), 6.22 (d, *J* = 38.2 Hz, 3H, 2''-H, 4''-H, 6''-H), 4.21 – 4.03 (m, 2H, 1'-H), 3.60 (t, *J* = 5.8 Hz, 2H, 3'''-H), 3.52 (ddt, *J* = 21.9, 14.7, 6.6 Hz, 2H, 2'-H), 3.33 (hept, *J* = 7.4 Hz, 2H, 1'''-H), 1.71 (s br, 2H, 2'''-H), 0.97 (d, *J* = 1.1 Hz, 9H, (C-3'')OSiC(CH_3)$_3$), 0.89 (d, *J* = 1.1 Hz, 9H, (C-3''')OSiC(CH_3)$_3$), 0.19 (d, *J* = 1.1 Hz, 6H, (C-3'')OSi(CH_3)$_2$), 0.04 (d, *J* = 1.1 Hz, 6H, (C-3''')OSi(CH_3)$_2$) ppm.

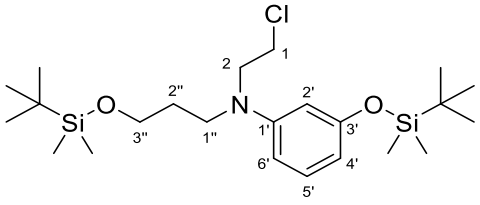
^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.0 (C-3''), 148.8 (C-1''), 145.9 (C-1 or C-2), 141.5 (C-1 or C-2), 134.7 (C-4 or C-5), 132.9 (C-4 or C-5), 130.0 (C-5'), 126.6 (C-3 or C-6), 125.4 (C-3 or C-6), 108.3 (C-2'', C-4'', or C-6''), 105.6 (C-2'', C-4'', or C-6''), 104.3 (C-2'', C-4'', or C-6''), 64.9 (C-1'), 60.0 (C-3'''), 50.5 (C-1'''), 48.4 (C-2'), 30.1 (C-2'''), 26.1 ((C-3'')OSiC($\underline{C}H_3$)$_3$ or (C-3''')OSiC($\underline{C}H_3$)$_3$), 25.9 ((C-3'')OSiC($\underline{C}H_3$)$_3$ or (C-3''')OSiC($\underline{C}H_3$)$_3$), 18.4 ((C-3'')OSi$\underline{C}$(CH_3)$_3$ or (C-3''')OSi$\underline{C}$(CH_3)$_3$), 18.4 ((C-3'')OSi$\underline{C}$(CH_3)$_3$ or (C-3''')OSi$\underline{C}$(CH_3)$_3$), -4.2 ((C-3'')OSi($\underline{C}H_3$)$_2$), -5.2 ((C-3''')OSi($\underline{C}H_3$)$_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 1598, 1532, 1496, 1345, 1251, 1135, 1097, 966, 831, 775, 688 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{29}H_{49}N_2O_7SSi$ $[M+H]^+$: 625.2794; found: 625.2789.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1e.

3-((*tert*-Butyldimethylsilyl)oxy)-*N*-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-*N*-(2-chloroethyl)aniline (204)



$C_{23}H_{44}ClNO_2Si$

M_w = 458.23 g/mol

Alkyl chloride **204** was prepared following General procedure K from 2-aminoethanol derivative **200** (44 mg, 0.10 mmol, 1.0 eq). 2-Nosyl chloride was added at first, followed by triethylamine. The crude product was purified by flash column chromatography (1:9 EtOAc/isohexanes), yielding product **204** (43 mg, 0.094 mmol, 94%) as light-red oil.

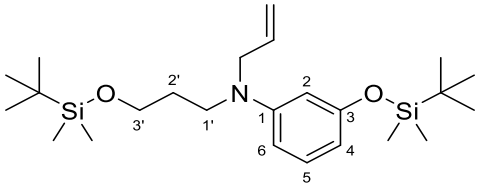
^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.05 (t, J = 8.1 Hz, 1H, 5'-H), 6.33 (d, J = 8.3 Hz, 1H, 6'-H), 6.24 – 6.09 (m, 2H, 2'-H, 4'-H), 3.66 (t, J = 5.8 Hz, 2H, 3''-H), 3.64 – 3.52 (m, 4H, 1-H, 2-H), 3.42 (t, J = 7.3 Hz, 2H, 1''-H), 1.83 – 1.73 (m, 2H, 2''-H), 0.98 (s, 9H, (C-3')OSiC$(CH_3)_3$), 0.92 (s, 9H, (C-3'')OSiC$(CH_3)_3$), 0.20 (s, 6H, (C-3')OSi$(CH_3)_2$), 0.07 (s, 6H, (C-3'')OSi$(CH_3)_2$) ppm.

^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.1 (C-3'), 148.6 (C-1'), 130.1 (C-5'), 108.5 (C-2' or C-4'), 105.6 (C-6'), 104.1 (C-2' or C-4'), 60.4 (C-3''), 53.3 (C-2), 48.2 (C-1''), 40.4 (C-1), 30.5 (C-2''), 26.1 ((C-3')OSiC($\underline{C}H_3)_3$ or (C-3'')OSiC($\underline{C}H_3)_3$), 25.9 ((C-3')OSiC($\underline{C}H_3)_3$ or (C-3'')OSiC($\underline{C}H_3)_3$), 18.4 ((C-3')OSi$\underline{C}(CH_3)_3$ or (C-3'')OSi$\underline{C}(CH_3)_3$), 18.4 ((C-3')OSi$\underline{C}(CH_3)_3$ or (C-3'')OSi$\underline{C}(CH_3)_3$), -4.2 ((C-3')OSi($\underline{C}H_3)_2$), -5.2 ((C-3'')OSi($\underline{C}H_3)_2$) ppm.

HRMS (ESI): *m/z* = calculated for $C_{23}H_{45}ClNO_2Si$ $[M+H]^+$: 458.2672; found: 458.2666.

Purity (HPLC): ND.

***N*-Allyl-3-((*tert*-butyldimethylsilyl)oxy)-*N*-(3-((*tert*-butyldimethylsilyl)oxy)propyl)aniline (206)**



$C_{24}H_{45}NO_2Si_2$

M_w = 435.80 g/mol

Secondary aromatic amine **203** (633 mg, 1.60 mmol, 1.0 eq) and $NaHCO_3$ (202 mg, 2.40 mmol, 1.5 eq) were suspended in 2 mL DMF. Allyl bromide (90 mg, 2.40 mmol, 1.5 eq) was added, and the reaction mixture was stirred at 60 °C for 2 h. After cooling down to room temperature, water was added, and the mixture extracted with diethyl ether (3 x 150 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (0.5:9.5 → 1:4 EtOAc/isohexanes), yielding tertiary aromatic amine **206** (203 mg, 0.466 mmol, 29%) as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.02 (s br, 1H, 5-H), 6.33 (d, J = 8.4 Hz, 1H, 6-H), 6.17 (s, 2H, 2-H, 4-H), 5.83 (s, 1H, $NCH_2C\underline{H}CH_2$), 5.24 – 5.05 (m, 2H, $NCH_2CHC\underline{H_2}$), 3.88 (d, J = 5.0 Hz, 2H, $NC\underline{H_2}CHCH_2$), 3.66 (t, J = 5.9 Hz, 2H, 3'-H), 3.38 (t, J = 7.3 Hz, 2H, 1'-H), 1.79 (s, 2H, 2'-H), 0.97 (s, 9H, (C-3)OSiC$(CH_3)_3$), 0.91 (s, 9H, (C-3')OSiC$(CH_3)_3$), 0.19 (s, 6H, (C-3)OSi$(CH_3)_2$), 0.06 (s, 6H, (C-3')OSi$(CH_3)_2$) ppm.

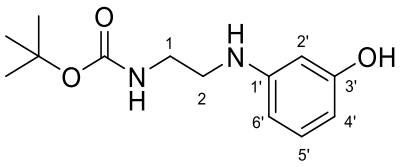
^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.8 (C-3), 150.0 (C-1), 134.5 ($NCH_2\underline{C}HCH_2$), 129.7 (C-5), 116.0 ($NCH_2CH\underline{C}H_2$), 107.8 (C-2 or C-4), 105.7 (C-6), 104.3 (C-2 or C-4), 60.8 (C-3'), 53.4 ($N\underline{C}H_2CHCH_2$), 47.7 (C-1'), 30.6 (C-2'), 26.1 ((C-3)OSiC($\underline{C}H_3)_3$ or (C-3')OSiC($\underline{C}H_3)_3$), 25.9 ((C-3)OSiC($\underline{C}H_3)_3$ or (C-3')OSiC($\underline{C}H_3)_3$), 18.4 ((C-3)OSi$\underline{C}(CH_3)_3$ or (C-3')OSi$\underline{C}(CH_3)_3$), 18.4 ((C-3)OSi$\underline{C}(CH_3)_3$ or (C-3')OSi$\underline{C}(CH_3)_3$), -4.2 ((C-3)OSi($\underline{C}H_3)_2$), -5.2 ((C-3')OSi($\underline{C}H_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 2954, 2928, 2857, 1602, 1497, 1252, 1192, 1168, 1096, 964, 830, 774 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{24}H_{46}NO_2Si_2$ $[M+H]^+$: 436.3062; found: 436.3055.

Purity (HPLC): ND.

tert-Butyl (2-((3-hydroxyphenyl)amino)ethyl)carbamate (207)



$C_{13}H_{20}N_2O_3$

M_w = 252.31 g/mol

N-Substituted aromatic amine **207** was prepared following General procedure P, using 3-aminophenol (**167**, 4.04 g, 37.0 mmol, 1.0 eq), *tert*-butyl *N*-(2-bromoethyl)carbamate (9.12 g, 40.7 mmol, 1.1 eq), and $NaHCO_3$ (3.42 g, 40.7 mmol, 1.1 eq). The reaction was stirred at 120 °C for 16 h. The mixture was extracted with EtOAc, and the crude purified by flash column

chromatography (1:1 EtOAc/isohexanes), yielding product **207** (2.18 g, 8.64 mmol, 23%) as a light brown oil.

R_f: 0.58 (1:1 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.01 (t, *J* = 8.0 Hz, 1H, 5'-H), 6.21 – 6.17 (m, 2H, 4'-H, 6'-H), 6.11 (t, *J* = 2.3 Hz, 1H, 2'-H), 3.36 (d, *J* = 6.5 Hz, 2H, 1-H), 3.22 (t, *J* = 5.8 Hz, 2H, 2-H), 1.45 (s, 9H, $C(CH_3)_3$) ppm.

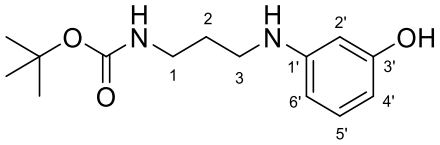
^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.0 (NCOO),149.7 (C-1'), 146.1 (C-3') 130.4 (C-5'), 105.9 (C-4' or C-6'), 104.7 (C-4' or C-6'), 99.6 (C-2'), 77.4 ($O\underline{C}(CH_3)_3$), 44.5 (C-2), 40.2 (C-1) 28.5 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 1682, 1528, 1450, 1361, 1299, 1192, 1158, 1143, 1043, 934, 862, 757 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{13}H_{19}N_2O_3$ $[M-H]^-$: 251.1401; found: 251.1399.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

tert-Butyl (3-((3-hydroxyphenyl)amino)propyl)carbamate (208)



$C_{14}H_{22}N_2O_3$

M_w = 266.34 g/mol

N-Substituted aromatic amine **208** was prepared following General procedure P, using 3-aminophenol (**167**, 2.00 g, 18.3 mmol, 1.0 eq), *tert*-butyl *N*-(3-bromopropyl)carbamate (4.79 g, 20.1 mmol, 1.1 eq), and $NaHCO_3$ (1.69 g, 20.1 mmol 1.1 eq). The reaction was stirred at 120 °C for 19 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:2 → 1:1 EtOAc/isohexanes), yielding secondary amine **208** (2.68 mg, 10.1 mmol, 55%) as a white solid.

R_f: 0.52 (1:1 EtOAc/isohexanes).

Mp: 103 – 107 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.00 (t, *J* = 8.0 Hz, 1H, 5'-H), 6.24 – 6.15 (m, 2H, 4'-H, 6'-H), 6.10 (t, *J* = 2.3 Hz, 1H, 2'-H), 3.24 (p, *J* = 7.0 Hz, 2H, 3-H), 3.14 (t, *J* = 6.6 Hz, 2H, 1-H), 1.76 (p, *J* = 6.7 Hz, 2H, 2-H), 1.45 (s, 9H, $C(CH_3)_3$) ppm.

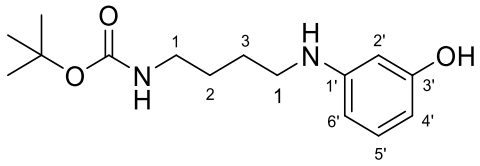
^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.1 (C-3'), 156.5 (NCOO), 149.8 (C-1'), 130.3 (C-5'), 106.0 (C-4' or C-6'), 104.6 (C-4'or C-6'), 99.9 (C-2'), 79.7 (O<u>C</u>$(CH_3)_3$), 41.1 (C-1), 38.3 (C-3), 29.8 (C-2), 28.6 (C(<u>C</u>$H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ **=** 3353, 2970, 2931, 1686, 1686, 1505, 1445, 1366, 1154, 1013, 842, 689 cm^{-1}.

HRMS (EI): *m/z* **=** calculated for $C_{14}H_{22}N_2O_3$ $[M]^{\cdot+}$: 266.1625; found: 266.1619.

Purity (HPLC): >88% (λ = 210 nm), >78% (λ = 254 nm), Method 1c.

tert-Butyl (4-((3-hydroxyphenyl)amino)butyl)carbamate (209)



$C_{15}H_{24}N_2O_3$

M_w = 280.37 g/mol

N-Substituted aromatic amine **209** was prepared following General procedure P, using 3-aminophenol (**167**, 1.53 g, 14.0 mmol, 1.0 eq), *tert*-butyl *N*-(4-bromobutyl)carbamate (3.88 g, 15.4 mmol, 1.1 eq), and $NaHCO_3$ (1.29 g, 15.4 mmol 1.1 eq). The reaction was stirred at 120 °C for 19 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:2 EtOAc/isohexanes), yielding secondary amine **209** (1.63 g, 5.81 mmol, 42%) as a yellow oil.

R_f: 0.41 (1:1 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.99 (t, *J* = 8.0 Hz, 1H, 5'-H), 6.17 (ddd, *J* = 17.5, 8.0, 2.2 Hz, 2H, 4'-H, 6'-H), 6.10 (t, *J* = 2.3 Hz, 1H, 2'-H), 3.18 (dq, *J* = 19.4, 6.9 Hz, 2H, 4-H), 3.07 (t, *J* = 6.6 Hz, 2H, 1-H), 1.59 (m, 4H, 2-H, 3-H), 1.47 (s, 9H, $C(CH_3)_3$) ppm.

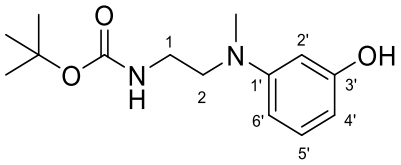
^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.3 (C-3'), 156.4 (NCOO), 150.0 (C-2'), 130.2 (C-5'), 105.8 (C-4' or C-6'), 104.5 (C-4' or C-6'), 99.7 (C-2'), 79.6 (O<u>C</u>$(CH_3)_3$), 43.6 (C-1), 40.4 (C-4), 28.6 (C(<u>C</u>$H_3)_3$), 27.8 (C-2), 27.8 (C-3) ppm.

IR (ATR): $\tilde{\nu}$ = 3331, 2959, 1673, 1474,1390, 1366, 1288, 1161, 859 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{15}H_{24}N_2O_3$ $[M]^{\cdot+}$: 280.1789; found: 280.1782.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

tert-Butyl (2-((3-hydroxyphenyl)(methyl)amino)ethyl)carbamate (210)



$C_{14}H_{22}N_2O_3$

M_w = 266.34 g/mol

N-Substituted aromatic amine **210** was prepared following General procedure P, using secondary amine **207** (757 mg, 3.00 mmol, 1.0 eq), methyl iodide (852 mg, 6.00 mmol, 2.0 eq), and $NaHCO_3$ (504 mg, 6.00 mmol 2.0 eq). The reaction was stirred at 100 °C for 4 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:1 EtOAc/isohexanes), yielding tertiary amine **210** (562 mg, 2.11 mmol, 70%) as a pink oil.

R_f: 0.66 (1:1 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.09 (t, *J* = 8.1 Hz, 5'-H), 6.34 (ddd, *J* = 8.3, 2.5, 0.8 Hz, 4'-H or 6'-H), 6.25 (s, 2'-H), 6.22 (ddd, *J* = 7.9, 2.3, 0.8 Hz, 4'-H or 6'-H), 3.45 (t, *J* = 6.5 Hz, 1-H), 3.36 – 3.29 (m, 2H, 1-H), 2.96 (s, NCH_3), 1.28 (t, *J* = 7.1 Hz, 9H, $C(CH_3)_3$) ppm.

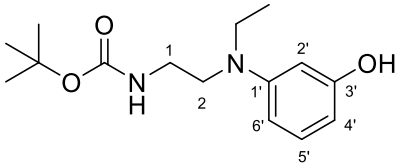
^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.9 (NCOO), 156.2 (C-1'), 155.2 (C-3'), 130.3 (C-1'), 105.2 (C-4' or C-6'), 104.7 (C-4' or C-6'), 99.4 (C-2'), 79.6 ($O\underline{C}(CH_3)_3$), 52.2 (C-2), 38.4 (NCH_3), 38.3 (C-1), 28.5 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3322, 3005, 2982, 2930, 1674, 1454, 1457, 1391, 1265, 1189, 780 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{14}H_{21}N_2O_3$ $[M-H]^-$: 265.1550; found: 265.1555.

Purity (HPLC): >99% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

tert-Butyl (2-(ethyl(3-hydroxyphenyl)amino)ethyl)carbamate (212)



$C_{15}H_{24}N_2O_3$

M_w = 280.37 g/mol

N-Substituted aromatic amine **212** was prepared following General procedure P, using secondary amine **207** (313 mg, 1.24 mmol, 1.0 eq), ethyl iodide (387 mg, 2.48 mmol, 2.0 eq), and $NaHCO_3$ (208 mg, 2.48 mmol, 2.0 eq). The reaction was stirred at 120 °C for 19 h. The

mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:1 EtOAc/isohexanes), yielding tertiary amine **212** (262 mg, 0.933 mmol, 75%) as a brown oil.

R_f: 0.57 (1:1 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.05 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.29 (ddd, *J* = 8.3, 2.5, 0.8 Hz, 1H, 4'-H), 6.23 (s, 1H, 2'-H), 6.16 (ddd, *J* = 7.9, 2.3, 0.8 Hz, 1H, 6'-H), 3.34 (tt, *J* = 16.3, 6.7 Hz, 6H, 1-H, 2-H, $C\underline{H}_2CH_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1.14 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

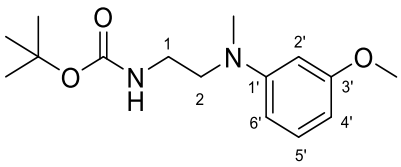
^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.1 (NCOO), 156.3 (C-3'), 149.6 (C-1'), 130.4 (C-5'), 105.0 (C-4'), 103.3 (C-6'), 99.3 (C-2'), 79.6 ($O\underline{C}(CH_3)_3$), 50.0 (C-1), 45.5 (C-2), 28.6 ($C(\underline{C}H_3)_3$), 21.2 ($\underline{C}H_2CH_3$),12.3 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3379, 2975, 2932, 1681, 1454, 1365, 1164, 754 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{15}H_{23}N_2O_3$ $[M-H]^-$: 279.1714; found: 279.1735.

Purity (HPLC): >94% (λ = 210 nm), >98% (λ = 254 nm), Method 1c.

tert-Butyl (2-((3-methoxyphenyl)(methyl)amino)ethyl)carbamate (211)



$C_{15}H_{24}N_2O_3$

M_w = 280.37 g/mol

N-Substituted aromatic amine **211** was prepared following General procedure P, using secondary amine **207** (550 mg, 2.18 mmol, 1.0 eq), methyl iodide (928 mg, 6.54 mmol, 3.0 eq), and $NaHCO_3$ (549 mg, 6.54 mmol, 3.0 eq). The reaction was stirred at 120 °C for 16 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:1 EtOAc/isohexanes), yielding tertiary amine **211** (224 mg, 0.799 mmol, 37%) as a purple oil.

R_f: 0.60 (1:1 EtOAc/isohexanes)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.13 (t, *J* = 8.2 Hz, 5'-H), 6.40 – 6.34 (m, 1H, 4'-H), 6.29 (dq, *J* = 5.1, 2.9 Hz, 2H, 2'-H, 6'-H), 3.79 (s, 3H, OCH_3), 3.44 (t, *J* = 6.5 Hz, 2H, 1-H), 3.31 (d, *J* = 6.6 Hz, 2H, 2-H), 2.94 (s, 3H, CH_3), 1.44 (s, 9H, $C(CH_3)_3$) ppm.

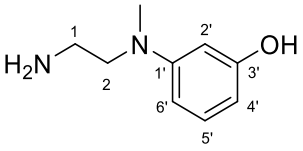
^{13}C NMR (126 MHz, $CDCl_3$): δ = 161.0 (NCOO), 156.1 (C-3'), 150.9 (C-1'), 130.1 (C-5'), 105.6 (C-4'), 103.8 (C-2' or C-6'), 99.1 (C-2' or C-6'), 79.5 ($O\underline{C}(CH_3)_3$), 55.3 (OCH_3), 52.4 (C-2), 38.7 (NCH_3), 38.7 (C-1), 28.5 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3380, 2979, 2932, 1682, 1445, 1375, 1327, 1165, 820 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{15}H_{24}N_2O_3$ $[M]^{\cdot+}$: 280.1782; found: 280.1783.

Purity (HPLC): >87% (λ = 210 nm), >92% (λ = 254 nm), Method 1c.

3-((2-Aminoethyl)(methyl)amino)phenol (214)



$C_9H_{14}N_2O$

M_w = 166.22 g/mol

Primary amine **214** was synthesised following General procedure Q from carbamate **210** (570 mg, 2.14 mmol, 1.0 eq). The reaction mixture was stirring for 4 h, yielding product **214** (267 mg, 1.61 mmol, 75%) as a dark brown oil.

R_f: 0.15 (100:10:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.00 – 6.95 (m, 1H, 5'-H), 6.30 – 6.26 (m, 1H, 4'-H), 6.22 – 6.20 (m, 1H, 2'-H), 6.13 (ddd, *J* = 8.0, 2.2, 0.7 Hz, 1H, 6'-H), 3.35 (t, *J* = 6.8 Hz, 2H, 2-H), 2.90 (s, 3H, NCH_3), 2.81 (t, *J* = 6.7 Hz, 2H, 1-H) ppm.

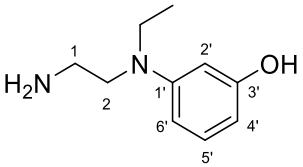
^{13}C NMR (101 MHz, CD_3OD): δ = 159.3 (C-3'), 152.6 (C-1'), 130.8 (C-5'), 105.7 (C-4'), 105.0 (C-6'), 100.8 (C-2'), 55.9 (C-2), 39.7 (NCH_3), 39.0 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3317, 2911, 2874, 1464, 1446, 1136, 1040, 749 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_9H_{14}N_2O$ $[M]^{\cdot+}$: 166.1101; found: 166.1100.

Purity (HPLC): >93% (λ = 210 nm), >93% (λ = 254 nm), Method 1c.

3-((2-Aminoethyl)(ethyl)amino)phenol (215)



$C_{10}H_{16}N_2O$

M_w = 180.25 g/mol

Primary amine **215** was synthesised following General procedure Q from carbamate **212** (561 mg, 2.00 mmol, 1.0 eq). The reaction mixture was stirring for 4 h, yielding product **215** (246 mg, 1.36 mmol, 68%) as a dark brown oil.

R_f: 0.46 (100:10:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 6.88 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.11 (dd, *J* = 8.3, 2.5 Hz, 1H, 4'-H), 6.07 (t, *J* = 2.4 Hz, 1H, 2'-H), 5.98 (dd, *J* = 7.8, 2.1 Hz, 1H, 6'-H), 3.28 (q, *J* = 7.0 Hz, 2H, $C\underline{H_2}CH_3$), 3.18 (d, *J* = 7.2 Hz, 2H, 2-H), 2.66 (t, *J* = 7.2 Hz, 2H, 1-H), 1.04 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H_3}$) ppm.

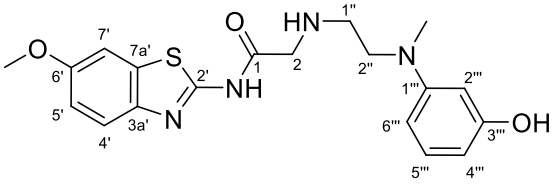
^{13}C NMR (126 MHz, DMSO-d_6): δ = 158.4 (C-3'), 149.4 (C-1'), 129.6 (C-5'), 102.9 (C-4'), 102.5 (C-6'), 98.6 (C-2'), 53.1 (C-2), 44.5 ($\underline{C}H_2CH_3$), 39.4 (C-1), 12.1 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3321, 2969, 2928, 1464, 1446, 1358, 1215, 1043, 749 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{10}H_{16}N_2O$ $[M]^{\cdot+}$: 180.1258; found: 180.1255.

Purity (HPLC): >97% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

2-((2-((3-Hydroxyphenyl)(methyl)amino)ethyl)amino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (161)



$C_{19}H_{22}N_4O_3S$

M_W = 386.47 g/mmol

Amine **161** was prepared following General procedure F, using chloroacetamide **67** (316 mg, 1.35 mmol, 1.0 eq), amine **214** (225 mg, 1.35 mmol, 1.1 eq), and triethylamine (0.189 mL, 1.35 mmol, 1.1 eq). The mixture was stirred for 4 h, and the extraction was conducted with $CHCl_3$/isopropanol (3:1). The residue was purified by flash column chromatography (100:3:1 → 100:5:1 DCM/MeOH/25% NH_3 aq. solution), yielding at first byproduct **216** (108 mg, 0.178 mmol, 15%) and then product **161** (21 mg, 0.056 mmol, 4%) as an off-white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.63 (d, *J* = 8.7 Hz, 1H, 4'-H), 7.56 (d, *J* = 2.5 Hz, 1H, 7'-H), 7.02 (dd, *J* = 8.8, 2.6 Hz, 1H, 5'-H), 6.90 (t, *J* = 8.0 Hz, 1H, 5'''-H), 6.17 (dd, *J* = 8.3, 2.3 Hz, 1H, 6'''-H), 6.11 (t, *J* = 2.3 Hz, 1H, 2'''-H), 6.03 (dd, *J* = 8.0, 2.1 Hz, 1H, 4'''-H), 3.80 (s, 3H, OCH_3), 3.49 (s, 2H, 2-H), 3.35 (m, 2H, 2''-H), 2.85 (s, 3H, NCH_3), 2.71 (t, *J* = 6.8 Hz, 2H, 1''-H) ppm.

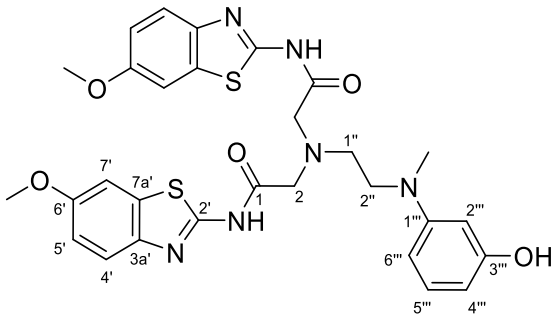
^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.4 (C-1), 158.3 (C-3'''), 156.2 (C-6'), 155.7 (C-2'), 150.7 (C-1'''), 142.6 (C-3a'), 132.8 (C-7a'), 129.6 (C-5'''), 121.2 (C-4'), 115.0 (C-5'), 104.8 (7'-H),

103.4 (C-4''' or C-6'''), 103.3 (C-4''' or C-6'''), 99.2 (C-2'''), 55.7 (C-2), 55.0 (OCH_3), 52.1 (C-1''), 51.9 (C-2''), 38.3 (NCH_3) ppm.

HRMS (ESI): *m/z* = calculated for $C_{19}H_{21}N_4O_3S$ $[M-H]^-$: 385.1339; found: 385.1333.

Purity (HPLC): ND.

2,2'-((2-((3-Hydroxyphenyl)(methyl)amino)ethyl)azanediyl)bis(*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide) (216)



$C_{28}H_{30}N_6O_5S_2$

M_w = 606.72 g/mmol

Tertiary amine **216** was obtained as by-product of the synthesis of amine **161**. Off-white waxy solid (108 mg, 0.178 mmol, 15%).

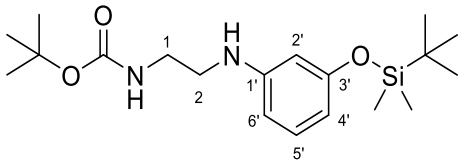
^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.67 (d, *J* = 8.8 Hz, 2H, 4'-H), 7.59 (d, *J* = 2.6 Hz, 2H, 7'-H), 7.05 (dd, *J* = 8.8, 2.6 Hz, 2H, 5'-H), 6.75 (t, *J* = 8.1 Hz, 1H, 5'''-H), 6.18 (dd, *J* = 8.3, 2.3 Hz, 1H, 6'''-H), 6.08 (t, *J* = 2.3 Hz, 1H, 2'''-H), 5.97 (dd, *J* = 8.0, 2.1 Hz, 1H, 4'''-H), 3.82 (s, 6H, 2 OCH_3), 3.71 (s, 4H, 2-H), 3.49 (t, *J* = 7.4 Hz, 2H, 2''-H), 2.84 (s, 3H, NCH_3), 2.81 (t, *J* = 7.4 Hz, 2H, 1''-H) ppm.

^{13}C NMR (126 MHz, DMSO-d_6): δ = 170.4 (C-1), 158.3 (C-3'''), 156.2 (C-6'), 155.6 (C-2'), 150.2 (C-1'''), 142.6 (C-3a'), 132.8 (C-7a'), 129.4 (C-5'''), 121.1 (C-4'), 115.0 (C-5'), 104.8 (7'-H), 103.4 (C-4''' or C-6'''), 103.3 (C-4''' or C-6'''), 99.1 (C-2'''), 57.9 (C-2), 55.6 (OCH_3), 51.2 (C-1''), 50.0 (C-2''), 38.2 (NCH_3) ppm.

HRMS (ESI): *m/z* = calculated for $C_{28}H_{31}N_6O_5S_2$ $[M+H]^+$: 607.1797; found: 607.1803.

Purity (HPLC): ND.

tert-Butyl (2-((3-((_tert_-butyldimethylsilyl)oxy)phenyl)amino)ethyl)carbamate (220)



$C_{19}H_{34}N_2O_3Si$

M_w = 366.58 g/mol

tert-Butyldimethylsilyl chloride (271 mg, 1.80 mmol, 1.2 eq) was added to a solution of phenol **207** (378 mg, 1.50 mmol, 1.0 eq) and imidazole (204 mg, 3.00 mmol, 2.0 eq) in THF (2.7 mL/mmol phenol) at room temperature for 18 h. After addition of water and EtOAc, the mixture was extracted with EtOAc (3 x 50 mL). Concentration of the organic layer under reduced pressure was followed by purification by flash column chromatography (1:3 EtOAc/isohexanes), yielding silyl ether **220** (353 mg, 0.962 mmol, 64%) as a light yellow oil.

R*f*: 0.71 (100:10:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.90 (t, *J* = 8.0 Hz, 1H, 5'-H), 6.12 (tdd, *J* = 7.8, 2.3, 0.9 Hz, 2H, 4'-H, 6' -H), 6.01 (t, *J* = 2.3 Hz, 1H, 2'-H), 3.26 (d, *J* = 6.3 Hz, 2H, 1-H), 3.12 (t, *J* = 5.7 Hz, 2H, 1-H), 1.35 (s, 9H, $OC(CH_3)_3$), 0.87 (s, 9H, $SiC(CH_3)_3$), 0.09 (s, 6H, $SiC(CH_3)_2$) ppm.

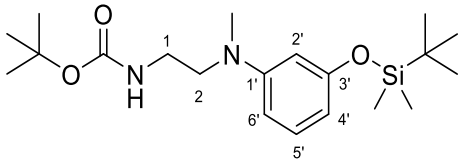
^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.9 (NCOO), 156.5 (C-1'), 149.5 (C-3'), 130.0 (C-5'), 109.6 (C-6'), 106.4 (C-4'), 104.8 (C-1'), 79.7 ($O\underline{C}(CH_3)_3$), 40.3 (C-1), 44.7 (C-2), 28.5 ($OC(\underline{C}H_3)_3$), 25.9 ($SiC(\underline{C}H_3)_3$), 18.3 ($Si\underline{C}(CH_3)_3$), -4.2 ($Si(\underline{C}H_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3385, 2956, 2886, 1692, 1510, 1463, 1391, 1365, 1158, 834, 778 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{19}H_{35}N_2O_3Si$ $[M+H]^+$: 367.2412; found: 367.2413.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

tert-Butyl (2-((3-((_tert_-butyldimethylsilyl)oxy)phenyl)amino)ethyl)carbamate (218)



$C_{20}H_{36}N_2O_3Si$

M_w = 380.6 g/mol

N-Substituted aromatic amine **218** was prepared following General procedure P, using secondary amine **220** (335 mg, 0.914 mmol, 1.0 eq), methyl iodide (195 mg, 1.37 mmol, 1.5 eq), and $NaHCO_3$ (115 mg, 1.37 mmol, 1.5 eq). The reaction was stirred at 100 °C for 4 h.

The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:3 EtOAc/isohexanes), yielding tertiary amine **218** (273 mg, 0.718 mmol, 79%) as a colourless oil.

R_f: 0.37 (1:1 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.05 (t, *J* = 8.3 Hz, 1H, 5'-H), 6.37 – 6.34 (m, 1H, 6'-H), 6.24 – 6.19 (m, 2H, 4'-H, 2'-H), 3.41 (t, *J* = 6.4 Hz, 2H, 1-H), 3.34 – 3.26 (m, 2H, 2-H), 2.92 (s, 3H, NCH_3), 1.44 (s, 9H, $OC(CH_3)_3$), 0.98 (d, *J* = 0.7 Hz, 10H, $SiC(CH_3)_3$), 0.20 (d, *J* = 0.8 Hz, 6H, $Si(CH_3)_2$) ppm.

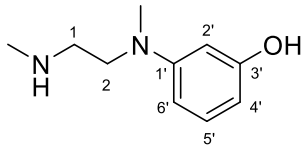
^{13}C NMR (126 MHz, $CDCl_3$): δ = 156.9 (C-1'), 156.9 (NCOO), 156.1 (C-3'), 129.9 (C-5'), 108.7 (C-6'), 106.0 (C-4'), 104.64 (C-2'), 79.5 ($O\underline{C}(CH_3)_3$), 52.4 (C-1), 38.5 (C-2), 28.5 ($OC(\underline{C}H_3)_3$), 25.9 ($SiC(\underline{C}H_3)_3$), 18.4 ($Si\underline{C}(CH_3)_3$), -4.2 ($Si(\underline{C}H_3)_2$) ppm.

IR (ATR): $\tilde{\nu}$ = 3362, 2956, 2930, 2858, 1693, 1462, 1451, 1390, 1364, 832, 778 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{20}H_{37}N_2O_3Si$ $[M+H]^+$: 381.2568; found: 381.2570.

Purity (HPLC): >95% (λ = 210 nm), >97% (λ = 254 nm), Method 1d.

3-(Methyl(2-(methylamino)ethyl)amino)phenol (219)



$C_{10}H_{16}N_2O$

M_w = 180.25 g/mol

Secondary amine **219** was synthesised following General procedure R from carbamate **218** (248 mg, 0.652 mmol, 1.0 eq). The filtrate was extracted with EtOAc (3 x 20 mL). The crude product was purified by flash column chromatography (100:5:1 DCM/MeOH/25% NH_3 aq. solution), yielding product **219** (68 mg, 0.38 mmol, 58%) as a dark brown oil.

R_f: 0.20 (100:20:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.05 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.30 (ddd, *J* = 8.3, 2.4, 0.8 Hz, 1H, 6'-H), 6.23 (t, *J* = 2.3 Hz, 1H, 2'-H), 6.18 (ddd, *J* = 7.9, 2.3, 0.8 Hz, 1H, 4'-H), 3.44 (t, *J* = 6.5 Hz, 2H, 2-H), 2.90 (s, 3H, NCH_3), 2.81 (t, *J* = 6.5 Hz, 2H, 1-H), 2.47 (s, 3H, $NHC\underline{H}_3$) ppm.

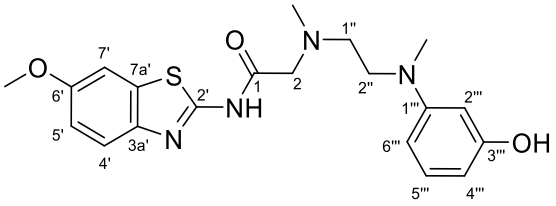
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.7 (C-3'), 151.4 (C-1'), 130.3 (C-5'), 104.8 (C-6'), 104.4 (C-4'), 100.1 (C-2'), 52.6 (C-2), 49.3 (C-1), 38.7 (NCH_3), 36.4 ($NHCH_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3273, 2945, 2865, 1573, 1450, 1352, 1154, 752 cm^{-1}.

HRMS (ESI): m/z = calculated for $C_{10}H_{17}N_2O$ $[M+H]^+$: 181.1336; found: 181.1335.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1c.

2-((2-((3-Hydroxyphenyl)(methyl)amino)ethyl)(methyl)amino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (217)



$C_{20}H_{24}N_4O_3S$

M_w = 400.50 g/mmol

Tertiary amine **217** was prepared following General procedure F, using secondary amine **219** (48 mg, 0.27 mmol, 1.0 eq), chloroacetamide **67** (75 mg, 0.20 mmol, 1.1 eq), and triethylamine (0.041 mL, 0.29 mmol, 1.1 eq). The mixture was stirred for 16 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding product **217** (78 mg, 0.19 mmol, 70%) as a colourless solid.

R_f: 0.43 (1:3 EtOAc/isohexanes).

Mp: 169 – 173 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.74 (d, *J* = 8.9 Hz, 1H, 4'-H), 7.27 (d, *J* = 2.6 Hz, 1H, 7'-H), 7.13 – 7.03 (m, 2H, 5'-H, 5'''-H), 6.51 (s, 1H, 2'''-H), 6.34 (dd, *J* = 8.2, 2.3 Hz, 1H, 4'''-H), 6.31 – 6.26 (dd, 1H, 6'''-H), 3.87 (s, 3H, OCH_3), 3.54 (t, *J* = 6.5 Hz, 2H, 2''-H), 3.37 (s, 2H, C-2), 2.92 (s, 3H, (C-1''')NCH_3), 2.78 (s, 2H, 1''-H), 2.39 (s, 3H, (C-2)NCH_3) ppm.

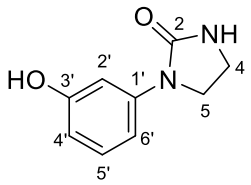
^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.4 (C-1), 157.7 (C-6'), 157.2 (C-3'''), 156.0 (C-2'), 151.1 (C-1'''), 142.0 (C-7a'), 133.3 (C-3a''), 130.7 (C-5'''), 121.6 (C-4'), 115.6 (C-5'), 106.1 (C-4'''), 105.8 (C-6'''),104.5 (C-7'), 100.9 (C-2'''), 62.2 (C-2), 56.0 (OCH_3),54.4 (C-1''), 52.0 (C-2''), 43.6 ((C-2)N<u>C</u>H_3), 38.4 ((C-1''')N<u>C</u>H_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3190, 2992, 2965, 1701, 1603, 1437, 1349, 1225, 814 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{20}H_{23}N_4O_3S$ $[M-H]^-$: 399.1496; found: 399.1496.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

1-(3-Hydroxyphenyl)imidazolidin-2-one (222)



$C_9H_{10}N_2O_2$

M_w = 178.19 g/mol

Imidazolidin-2-one **222** was obtained as only product isolated following General procedure R starting from carbamate **207** (169 mg, 0.670 mmol, 1.0 eq). The filtrate was extracted with $CHCl_3$/isopropanol (3:1), yielding product **222** (11 mg, 0.062 mmol, 9%) as a brown waxy solid.

R_f: 0.55 (98:2 EtOAc/acetone).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.12 (t, J = 8.2 Hz, 1H, 5'-H), 7.09 (t, J = 2.2 Hz, 1H, 2'-H), 6.91 (ddd, J = 8.2, 2.2, 0.9 Hz, 1H, 4'-H), 6.49 (ddd, J = 8.1, 2.4, 0.9 Hz, 1H, 6'-H), 3.94 – 3.89 (m, 2H, 5-H), 3.54 – 3.49 (m, 2H, 4-H) ppm.

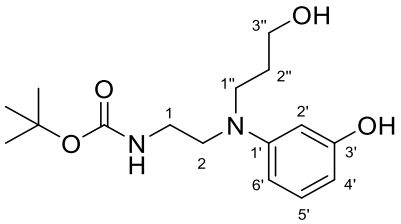
^{13}C NMR (101 MHz, CD_3OD): δ = 162.1 (C-2), 158.8 (C-3'), 142.6 (C-1'), 130.5 (C-5'), 111.0 (C-4'), 110.6 (C-6'), 106.8 (C-2'), 46.8 (C-5), 38.4 (C-4) ppm.

IR (ATR): $\tilde{\nu}$ = 2541, 1662, 1587, 1587, 1486, 1453, 1429, 1264, 1217, 1173, 873, 741 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_9H_{10}N_2O_2$ $[M]^{\cdot+}$: 178.0742; found: 178.0736.

Purity (HPLC): ND.

***tert*-Butyl (2-((3-hydroxyphenyl)(3-hydroxypropyl)amino)ethyl)carbamate (225)**



$C_{16}H_{26}N_2O_4$

M_w = 310.39 g/mol

N-Substituted aromatic amine **225** was prepared following General procedure P, using secondary amine **207** (275 mg, 1.09 mmol, 1.0 eq), 3-bromo-1-propanol (303 mg, 2.18 mmol, 2.0 eq), and $NaHCO_3$ (221 mg, 2.18 mmol, 2.0 eq). The reaction was stirred at 120 °C for 16 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (100:0.5 EtOAc/acetone), yielding tertiary amine **225** (76 mg, 0.25 mmol, 23%) as a brown oil.

R_f: 0.65 (95.5:0.5 EtOAc/acetone).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.06 (t, J = 8.1 Hz, 1H, 5'-H), 6.40 (s, 1H, 2'-H), 6.32 (d, J = 8.3 Hz, 1H, 4'-H), 6.26 (d, J = 8.4 Hz, 1H, 6'-H), 3.71 (t, J = 5.8 Hz, 2H, 3''-H), 3.40 (dt, J = 13.1, 6.9 Hz, 4H, 1-H, 2-H), 3.26 (d, J = 6.7 Hz, 2H, 1''-H), 1.87 – 1.76 (m, 2H, 2''-H), 1.44 (s, 9H, $C(CH_3)_3$) ppm.

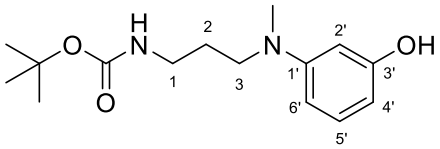
^{13}C NMR (101 MHz, $CDCl_3$): δ = 161.5 (NCOO), 157.6 (C-3'), 156.5 (C-1'), 130.5 (C-5'), 80.0 ($O\underline{C}(CH_3)_3$), 60.4 (C-3''), 51.3 (C-1 or C-2), 48.8 (C-1 or C-2), 38.0 (C-1''), 29.9 (C-2''), 28.5 ($C(\underline{C}H_3)_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3336, 2974, 2934, 1681, 1503, 1454, 1366, 1249, 1160, 1049, 755 cm^{-1}.

HRMS (EI): m/z = calculated for $C_{16}H_{26}N_2O_4$ $[M]^{\cdot+}$: 310.1888; found: 310.1889.

Purity (HPLC): >90% (λ = 210 nm), >96% (λ = 254 nm), Method 1c.

tert-Butyl (3-((3-hydroxyphenyl)(methyl)amino)propyl)carbamate (226)



$C_{15}H_{24}N_2O_3$

M_w = 280.37 g/mol

N-Substituted aromatic amine **226** was prepared following General procedure P, using secondary amine **208** (400 mg, 1.50 mmol, 1.0 eq), methyl iodide (319 mg, 2.25 mmol, 1.5 eq), and $NaHCO_3$ (198 mg, 2.25 mmol, 1.5 eq). The reaction was stirred at 100 °C for 4 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:3 EtOAc/isohexanes), yielding tertiary amine **226** (157 mg, 0.560 mmol, 37%) as a colourless solid.

R_f: 0.49 (1:1 EtOAc/isohexanes).

Mp: 105 – 108 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.09 – 7.03 (m, 1H, 5'-H), 6.29 – 6.25 (m, 1H, 2'-H), 6.20 – 6.15 (m, 2H, 4'-H, 6'-H), 3.37 – 3.29 (m, 2H, 3-H), 3.16 (d, J = 7.1 Hz, 2H, 1-H), 2.89 (s, 3H, CH_3), 1.76 (p, J = 7.0 Hz, 2H, 2-H), 1.45 (s, 9H, $C(CH_3)_3$) ppm.

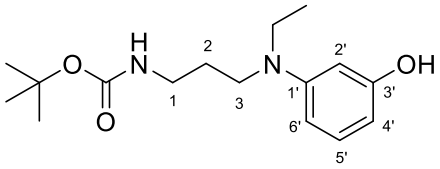
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.0 (C-3'), 156.2 (NCOO) 150.9 (C-1'), 130.3 (C-5'), 105.2 (C-6'), 103.5 (C-4'), 99.5 (C-2'), 79.6 ($O\underline{C}(CH_3)_3$), 50.3 (C-3), 38.6 (CH_3), 38.8 (C-1), 28.6 ($C(\underline{C}H_3)_3$), 27.6 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3341, 2981, 2962, 1679, 1509, 1446, 1388, 1364, 1287, 1139, 753 cm^{-1}.

HRMS (ESI): *m/z* = calculated for: $C_{15}H_{23}N_2O_3$ $[M-H]^-$: 279.1714; found: 279.17141.

Purity (HPLC): >97% (210 nm), 100% (254 nm), Method 1c.

tert-Butyl (3-(ethyl(3-hydroxyphenyl)amino)propyl)carbamate (227)



$C_{16}H_{26}N_2O_3$

M_w = 294.39 g/mol

N-Substituted aromatic amine **227** was prepared following General procedure P, using secondary amine **208** (346 mg, 1.30 mmol, 1.0 eq), ethyl iodide (406 mg, 2.60 mmol, 2.0 eq), and $NaHCO_3$ (218 mg, 2.60 mmol, 1.5 eq). The reaction was stirred at 120 °C for 16 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:1 EtOAc/isohexanes), yielding tertiary amine **227** (237 mg, 0.805 mmol, 62%) as a colourless solid.

R_f: 0.68 (1:1 EtOAc/isohexanes).

Mp: 108 – 109 °C.

^{1}H NMR (500 MHz, CDCl3): δ = 7.05 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.25 (dd, *J* = 8.4, 2.4 Hz, 1H, 6'-H), 6.18 – 6.13 (m, 2H, 2'-H, 4'-H), 3.32 (q, *J* = 7.0 Hz, 2H, 1-H), 3.27 (t, *J* = 7.4 Hz, 2H, 3-H), 3.22 – 3.14 (m, 2H, $C\underline{H}_2CH_3$), 1.77 (p, *J* = 7.1 Hz, 2H, 2-H), 1.45 (s, 9H, $C(CH_3)_3$), 1.13 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

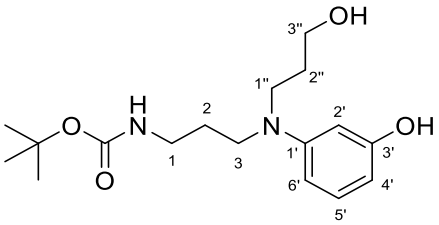
^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.1 (C-3'), 156.2 (NCOO), 149.5 (C-1'), 130.3 (C-5'), 105.0 (C-6'), 103.0 (C-4'), 99.3 (C-2'), 79.6 ($O\underline{C}(CH_3)_3$), 47.9 (C-3), 45.3 (C-1), 38.8 ($\underline{C}H_2CH_3$), 28.6 (C-2), 28.3 ($C(\underline{C}H_3)_3$), 12.4 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3343, 3231, 2970, 1682, 1447, 1282, 1136, 1011, 750 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{16}H_{26}N_2O_3$ $[M]^{\cdot+}$: 294.1938; found: 294.1933.

Purity (HPLC): 100% (λ = 210 nm), >99% (λ = 254 nm), Method 1c.

tert-Butyl (3-((3-hydroxyphenyl)(3-hydroxypropyl)amino)propyl)carbamate (228)



$C_{17}H_{28}N_2O_4$

M_w = 324.42 g/mol

N-Substituted aromatic amine **228** was prepared following General procedure P, using secondary amine **208** (450 mg,1.69 mmol, 1.0 eq), 3-bromo-1-propanol (470 mg, 3.38 mmol, 2.0 eq), and $NaHCO_3$ (342 mg, 3.38 mmol, 2.0 eq). The reaction was stirred at 120 °C for 16 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding tertiary amine **228** (169 mg, 0.552 mmol, 31%) as a brown oil.

R_f: 0.37 (1:2 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.08 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.34 (dd, *J* = 21.0, 12.8 Hz, 3H, 2'-H, 4'-H, 6'-H), 3.70 (t, *J* = 5.8 Hz, 2H, 3''H), 3.39 (q, *J* = 7.7 Hz, 2H, 1''-H), 3.29 (q, *J* = 6.7 Hz, 2H, 3-H), 3.14 (d, *J* = 7.8 Hz, 2H, 1-H), 1.81 (q, 2H, 2''-H), 1,76 (q, 2H, 2-H) ppm.

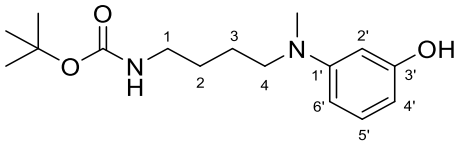
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.6 (NCOO), 153.2 (C-1'), 132.2 (C-3'), 130.5 (C-5'), 106.9 (C-4'), 106.3 (C-6'), 100.2 (C-5') 79.7 (O<u>C</u>$(CH_3)_3$), 60.5 (C-3''), 47.8 (C-3), 47.3 (C-1''), 38.6 (C-1), 29.9 (C-2''), 28.6 (C(<u>C</u>$H_3)_3$), 27.7 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 2936, 2870, 1681, 1577, 1462, 1450, 1393, 1237, 1165, 813, 753 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{17}H_{27}N_2O_4$ $[M-H]^-$: 323.1976; found: 323.19752.

Purity (HPLC): >96% (λ = 210 nm), >95% (λ = 254 nm), Method 1c.

tert-Butyl (4-((3-hydroxyphenyl)(methyl)amino)butyl)carbamate (229)



$C_{16}H_{26}N_2O_3$

M_w = 294.39 g/mol

N-Substituted aromatic amine **229** was prepared following General procedure P, using secondary amine **209** (449 mg, 1.60 mmol, 1.0 eq), methyl iodide (341 mg, 2.40 mmol, 1.5 eq),

and $NaHCO_3$ (202 mg, 2.40 mmol, 1.5 eq). The reaction was stirred at 100 °C for 4 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:2 EtOAc/isohexanes), yielding tertiary amine **229** (267 mg, 0.908 mmol, 57%) as a light yellow oil.

R_f: 0.70 (1:1 EtOAc/isohexanes).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.06 (t, *J* = 8.2 Hz, 1H, 5'-H), 6.24 (d, *J* = 6.9 Hz, 2H, 4'-H, 6'-H), 6.20 – 6.16 (m, 1H, 2'-H), 3.30 – 3.25 (m, 2H, 4-H), 3.19 (q, *J* = 6.7 Hz, 2H, 1-H), 2.89 (s, 3H, NCH_3), 1.62 – 1.54 (m, 2H, 3-H), 1.50 (p, *J* = 7.0 Hz, 2H, 2-H), 1.44 (s, 9H, $C(CH_3)_3$) ppm.

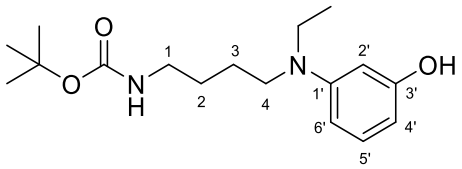
^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.2 (C-3'), 156.4 (NCOO), 150.1 (C-1'), 130.2 (C-5'), 104.7 (C-4' or C-6'), 103.4 (C-4' or C-6'), 99.4 (C-2'), 79.7 ($O\underline{C}(CH_3)_3$), 52.3 (C-4), 40.3 (C-1), 38.4 (NCH_3), 28.6 ($C(\underline{C}H_3)_3$), 27.7 (C-2 and C-3) ppm.

IR (ATR): $\tilde{\nu}$ = 3341, 2976, 2933, 1678, 1504, 1451, 1391, 1239, 1163, 1006. 754 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{16}H_{27}N_2O_3$ $[M+H]^+$: 295.2017; found: 295.20158.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

tert-Butyl (4-(ethyl(3-hydroxyphenyl)amino)butyl)carbamate (230)



$C_{17}H_{28}N_2O_3$

M_w = 308.42

N-Substituted aromatic amine **229** was prepared following General procedure P, using secondary amine **209** (480 mg, 1.71 mmol, 1.0 eq), ethyl iodide (533 mg, 3.42 mmol, 2.0 eq), and $NaHCO_3$ (287 mg, 3.42 mmol, 1.5 eq). The reaction was stirred at 120 °C for 19 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (1:2 EtOAc/isohexanes), yielding tertiary amine **230** (328 mg, 1.06 mmol, 62%) as a light yellow oil.

R_f: 0.67 (1:1 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.04 (dd, *J* = 8.7, 7.9 Hz, 1H, 5'-H), 6.22 (dq, *J* = 5.4, 1.7 Hz, 2H, 4'-H, 6'-H), 6.16 – 6.11 (m, 1H, 2'-H), 3.32 (q, *J* = 7.0 Hz, 2H, 4-H), 3.23 (q, *J* = 6.3 Hz, 4H, 1-H, $C\underline{H}_2CH_3$), 1.65 – 1.47 (m, 6H, 2-H, 3-H, 2-H), 1.44 (s, 9H, $C(CH_3)_3$), 1.13 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

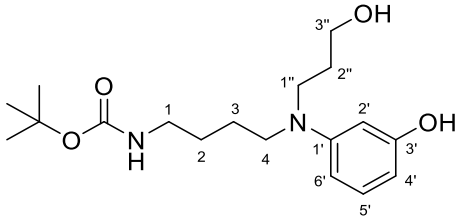
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.3 (C-3'), 156.4 (NCOO), 149.5 (C-1'), 130.3 (C-5'), 104.4 (C-4' or C-6'), 102.7 (C-4' or C-6'), 99.0 (C-2'), 79.7 (O<u>C</u>$(CH_3)_3$), 50.0 (C-4), 45.1 (C-1), 40.2 (<u>C</u>H_2CH_3), 28.6 (C(<u>C</u>$H_3)_3$), 27.7 (C-2 or C-3), 24.4 (C-2 or C-3), 12.5 (CH_2<u>C</u>H_3) ppm.

IR (ATR): $\tilde{\nu}$ = 3345, 2973, 2933, 1679, 1503, 1453, 1392, 1365, 1163, 752 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{17}H_{28}N_2O_3$ $[M]^{\cdot+}$: 308.2095; found: 308.2092.

Purity (HPLC): >96% (λ = 210 nm), >96% (λ = 254 nm), Method 1c.

tert-Butyl (4-((3-hydroxyphenyl)(3-hydroxypropyl)amino)butyl)carbamate (231)



$C_{18}H_{30}N_2O_4$

M_w = 338.45 g/mol

N-Substituted aromatic amine **231** was prepared following General procedure P, using secondary amine **209** (454 mg,1.62 mmol, 1.0 eq), 3-bromo-1-propanol (450 mg, 3.24 mmol, 2.0 eq), and $NaHCO_3$ (272 g, 3.24 mmol, 2.0 eq). The reaction was stirred at 120 °C for 16 h. The mixture was extracted with EtOAc, and the crude purified by flash column chromatography (100:0.5 EtOAc/acetone), yielding tertiary amine **231** (149 mg, 0.440 mmol, 27%) as a brown oil.

R_f: 0.68 (99.5:0.5 EtOAc/acetone).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.07 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.37 (s, 1H, 2'-H), 6.33 – 6.20 (m, 2H, 4'-H, 6'-H), 3.71 (t, *J* = 5.9 Hz, 2H, 3''-H), 3.40 (t, *J* = 7.1 Hz, 2H, 4-H), 3.31 – 3.23 (m, 2H, 1''-H), 3.17 (d, *J* = 7.1 Hz, 2H, 1-H), 1.82 (p, *J* = 6.3 Hz, 3H, 2''-H), 1.61 (p, *J* = 7.3 Hz, 2H, 3-H), 1.50 (q, *J* = 6.9 Hz, 2H, 2-H), 1.44 (s, 9H, $C(CH_3)_3$) ppm.

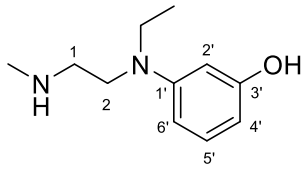
^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.2 (NCOO), 157.4 (C-3'), 157.0 (C-1'), 130.4 (C-5'), 77.4 (O<u>C</u>$(CH_3)_3$), 60.6 (C-3''), 51.6 (C-1''), 48.6 (C-4), 40.1 (C-1), 30.0 (C-2''), 28.6 (C(<u>C</u>$H_3)_3$), 27.6 (C-2), 23.8 (C-3) ppm.

IR (ATR): $\tilde{\nu}$ = 3332, 2933, 2872, 1679, 1504, 1453, 1365, 1249, 1161, 754 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{18}H_{30}N_2O_4$ $[M]^{\cdot+}$: 338.2201; found: 338.2201.

Purity (HPLC): ND.

3-(Ethyl(2-(methylamino)ethyl)amino)phenol (232)



$C_{11}H_{18}N_2O$

M_w = 194.28 g/mmol

Secondary amine **232** was synthesised following General procedure R from carbamate **212** (263 mg, 0.938 mmol, 1.0 eq). The filtrate was extracted with EtOAc (3 x 20 mL). The crude product was purified by flash column chromatography (100:5:1 → 100:10:1 DCM/MeOH/25% NH_3 aq. solution), yielding methyl amine **232** (111 mg, 0.572 mmol, 61%) as a beige solid.

R_f: 0.23 (100:20:1 DCM/MeOH/25% NH_3 aq. solution).

Mp: 87 – 93 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.04 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.31 – 6.25 (m, 1H, 1' -H), 6.22 (t, *J* = 2.4 Hz, 1H, 2' -H), 6.15 (ddd, *J* = 8.0, 2.2, 0.8 Hz, 1H, 4' -H), 3.42 (t, *J* = 6.7 Hz, 2H, 2-H), 3.35 (q, *J* = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 2.81 (t, *J* = 6.7 Hz, 2H, 1-H), 2.48 (s, 3H, NCH_3), 1.12 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

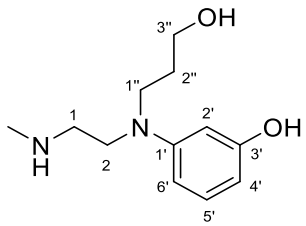
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.7 (C-3'), 149.7 (C-1'), 130.3 (C-5'), 104.8 (C-6'), 103.7 (C-4'), 99.8 (C-2'), 49.9 (C-2), 49.6 (C-1), 45.5 ($\underline{C}H_2CH_3$), 36.4 (NCH_3), 12.2 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3268, 2926, 2871, 1591, 1472, 1448, 1374, 1223, 1151, 744 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{11}H_{19}N_2O$ $[M+H]^+$: 195.1492; found: 195.14910.

Purity (HPLC): >98% (λ = 210 nm), >99% (λ = 254 nm), Method 1c.

3-((3-Hydroxypropyl)(2-(methylamino)ethyl)amino)phenol (233)



$C_{12}H_{20}N_2O_2$

M_w = 224.3 g/mmol

Secondary amine **233** was synthesised following General procedure R from carbamate **225** (69 mg, 0.22 mmol, 1.0 eq). The filtrate was evaporated under reduced pressure and this

procedure was repeated as many times as necessary. The crude product was purified by flash column chromatography (100:20:2 DCM/MeOH/25% NH_3 aq. solution), yielding product **233** (44 mg, 0.20 mmol, 89%) as a grey oil.

R_f: 0.36 (100:20:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (500 MHz, CD_3OD): δ = 7.04 (t, *J* = 8.2 Hz, 1H, 5'-H), 6.37 (dd, *J* = 8.2, 2.5 Hz, 1H, 4'-H), 6.32 (t, *J* = 2.4 Hz, 1H, 2'-H), 6.23 (dd, *J* = 8.0, 2.2 Hz, 1H, 6'-H), 3.66 (t, *J* = 6.4 Hz, 2H, 1''-H), 3.61 (t, *J* = 6.4 Hz, 2H, 3''-H), 3.57 (dd, *J* = 7.6, 5.0 Hz, 2H, 2-H), 3.19 (t, *J* = 6.5 Hz, 2H, 1-H), 2.72 (s, 3H, NCH_3), 1.76 (ddt, *J* = 15.0, 12.8, 6.3 Hz, 4H, 2''-H, NH, OH) ppm.

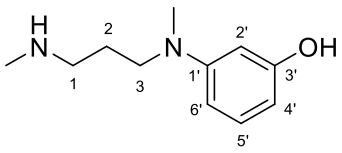
^{13}C NMR (126 MHz, CD_3OD): δ = 158. 3 (C-3'), 148.9 (C-1'), 129.8 (C-5'), 105.8 (C-4' or C-6'), 105.1 (C-4' or C-6'), 101.3 (C-2'), 61.4 (C-1'') 59.1 (C-3''), 58.62 (C-2),46.7 (C-1), 32.6 (NCH_3), 29.0 (C-2') ppm.

IR (ATR): $\tilde{\nu}$ = 1631, 1461, 1402, 1218, 1171, 1051 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{12}H_{20}N_2O_2$ $[M]^{\cdot+}$: 224.1520; found: 224.1522.

Purity (HPLC): ND.

3-(Methyl(3-(methylamino)propyl)amino)phenol (234)



$C_{11}H_{18}N_2O$

M_w = 194.28 g/mol

Secondary amine **234** was synthesised following General procedure R from carbamate **226** (127 mg, 0.452 mmol, 1.0 eq). The filtrate was extracted with EtOAc (3 x 20 mL). The crude product was purified by flash column chromatography (100:5:2 → 100:20:2 DCM/MeOH/25% NH_3 aq. solution), yielding product **234** (34 mg, 0.18 mmol, 39%) as a light brown oil.

R_f: 0.22 (100:10:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (500 MHz, CD_3OD): δ = 7.04 (t, *J* = 8.0 Hz, 1H, 5'-H), 6.26 – 6.21 (m, 1H, 6'-H), 6.18 (t, *J* = 2.3 Hz, 1H, 2'-H), 6.16 (ddd, *J* = 7.9, 2.3, 0.8 Hz, 1H, 4'-H), 3.33 (t, *J* = 7.2 Hz, 2H, 1-H), 2.86 (s, 3H, NCH_3), 2.67 (t, *J* = 7.1 Hz, 2H, 3-H), 2.45 (s, 3H, $NHC\underline{H}_3$), 1.81 (p, *J* = 7.2 Hz, 2H, 2-H) ppm.

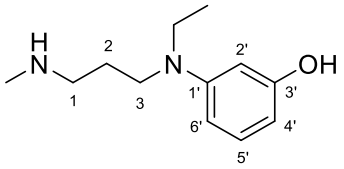
^{13}C NMR (126 MHz, CD_3OD): δ = 157.5 (C-3'), 150.7 (C-1'), 130.0 (C-5'), 104.3 (C-6'), 103.7 (C-4'), 99.5 (C-2'), 50.6 (C-1), 49.4 (C-3), 38.2 ($NHCH_3$), 35.9 (NCH_3), 26.5 (C-2) ppm.

IR (ATR): $\tilde{\nu}$ = 3286, 2938, 2866, 1572, 1471, 1451, 1356, 1237, 1157, 818, 751 cm^{-1}.

HRMS (EI): *m/z* = calculated for: $C_{11}H_{18}N_2O$ $[M]^{\cdot+}$: 194.1419; found: 194.1412.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1c.

3-(Ethyl(3-(methylamino)propyl)amino)phenol (235)



$C_{12}H_{20}N_2O$

M_w = 208.3 g/mol

Secondary amine **235** was synthesised following General procedure R from carbamate **227** (217 mg, 0.737 mmol, 1.0 eq). The filtrate was extracted with EtOAc (3 x 20 mL). The crude product was purified by flash column chromatography (100:5:2 → 100:20:2 DCM/MeOH/25% NH_3 aq. solution), yielding product **235** (103 mg, 0.494 mmol, 67%) as a light brown oil.

R_f: 0.21 (100:20:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.03 (td, *J* = 8.0, 0.6 Hz, 1H, 5'-H), 6.25 – 6.18 (m, 2H, 4'-H, 6'-H), 6.14 (ddd, *J* = 7.9, 2.2, 0.9 Hz, 1H, 2'-H), 3.29 (q, *J* = 7.4 Hz, 4H, 3-H, $C\underline{H}_2CH_3$), 2.71 (t, *J* = 7.1 Hz, 2H, 1-H), 2.48 (s, 3H, NCH_3), 1.84 (p, *J* = 7.2 Hz, 2H, 2-H), 1.11 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

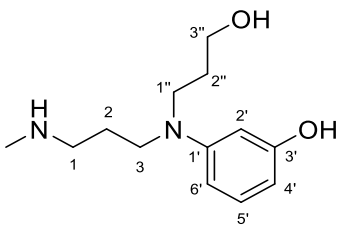
^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.8 (C-3'), 149.4 (C-1'), 130.3 (C-5'), 104.5 (C-6'), 103.4 (C-4'), 99.6 (C-2'), 49.4 (C-1), 48.4 (C-3 or $\underline{C}H_2CH_3$), 45.3 (C-3 or $\underline{C}H_2CH_3$), 35.8 (NCH_3), 27.0 (C-2), 12.4 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3286, 2966, 2869, 1571, 1467, 1453, 1370, 1210, 750 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{21}N_2O$ $[M+H]^+$: 209.1649; found: 209.1647.

Purity (HPLC): >99% (λ = 210 nm), >99% (λ = 254 nm), Method 1c.

3-((3-Hydroxypropyl)(3-(methylamino)propyl)amino)phenol (236)



$C_{13}H_{22}N_2O_2$

M_w = 238.33 g/mol

Secondary amine **236** was synthesised following General procedure R, using carbamate **228** (406 mg, 1.25 mmol, 1.0 eq). The filtrate was extracted with $CHCl_3$/isopropanol (3:1), yielding product **236** (195 mg, 0.818 mmol, 66%) as a dark brown oil.

R_f: 0.53 (100:15:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, CD_3OD): δ = 6.99 (t, *J* = 8.1 Hz, 1H, 5'-H), 6.29 (ddd, *J* = 8.3, 2.5, 0.8 Hz, 1H, 4'-H), 6.23 (t, *J* = 2.3 Hz, 1H, 2'-H), 6.15 (ddd, *J* = 7.9, 2.2, 0.8 Hz, 1H, 6'-H), 3.64 – 3.59 (m, 3H, 3''-H), 3.39 (td, *J* = 7.2, 5.0 Hz, 4H, 3-H, 1''-H), 3.06 – 3.01 (m, 2H, 1-H), 2.69 (s, 3H, $C(CH_3)_3$), 1.95 (dt, *J* = 15.9, 7.3 Hz, 2H, 2''-H), 1.82– 1.74 (m, 3H, 2-H) ppm.

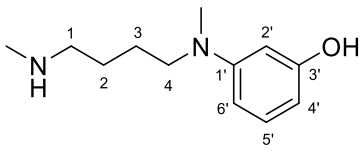
^{13}C NMR (101 MHz, CD_3OD): δ = 159.5 (C-3'), 150.6 (C-1'), 131.0 (C-5'), 106.3 (C-4'), 105.2 (C-6'), 101.5 (C-2'), 60.5 (C-3''), 49.3 (C-3), 48.5 (C-1), 47.4 (C-1''), 33.7 (NCH_3), 31.0 (C-2), 25.3 (C-2'') ppm.

IR (ATR): $\tilde{\nu}$ = 3282, 2937, 2869, 1571, 1501, 1462, 1355, 1166, 753 cm^{-1}**.**

HRMS (EI): *m/z* = calculated for $C_{13}H_{22}N_2O_2$ $[M]^{\cdot+}$: 238.1676; found: 238.1678.

Purity (HPLC): 100% (λ = 210 nm), >97% (λ = 254 nm), Method 1c.

3-(Methyl(4-(methylamino)butyl)amino)phenol (237)



$C_{12}H_{20}N_2O$

M_w = 208.30 g/mol

Secondary amine **237** was synthesised following General procedure R, using carbamate **229** (233 mg, 0.790 mmol, 1.0 eq). The filtrate was extracted with $CHCl_3$/isopropanol (3:1). The crude product was purified by flash column chromatography (100:15:2 → 100:20:2 DCM/MeOH/25% NH_3 aq. solution), yielding product **237** (85 mg, 0.41 mmol, 52%) as a light brown oil.

R_f: 0.21 (100:10:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.06 – 7.01 (m, 1H, 5'-H), 6.22 (ddd, *J* = 8.4, 2.4, 0.9 Hz, 1H, 6´-H), 6.16 – 6.12 (m, 2H, 2'-H, 4'-H), 3.27 (t, *J* = 6.8 Hz, 2H, 4-H), 2.87 (s, 3H, NCH_3), 2.64 (t, *J* = 6.7 Hz, 2H, 1-H), 2.45 (s, 3H, $NHC\underline{H}_3$), 1.63 – 1.50 (m, 4H, 2-H, 3-H) ppm.

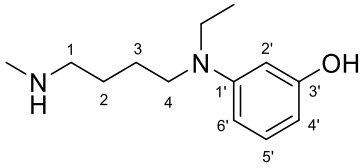
^{13}C NMR (101 MHz, CD_3OD): δ = 157.7 (C-3'), 150.8 (C-1'), 130.0 (C-5'), 104.4 (C-4' or C-6'), 103.6 (C-4' or C-6'), 99.7 (C-2'), 52.6 (C-4), 51.5 (C-1), 38.4 (NCH_3), 35.9 ($NHCH_3$), 26.7 (C-2 or C-3), 24.5 (C-2 or C-3) ppm.

IR (ATR): $\tilde{\nu}$ = 2985, 2937, 2829, 1579. 1463, 1449, 1399, 1382, 1237, 1166, 1109 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{12}H_{21}N_2O$ $[M+H]^+$: 209.1649; found: 209.16469.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

3-(Ethyl(4-(methylamino)butyl)amino)phenol (238)



$C_{13}H_{22}N_2O$

M_w = 222.33 g/mol

Secondary amine **238** was synthesised following General procedure R from carbamate **230** (304 mg, 0.986 mmol, 1.0 eq). The filtrate was extracted $CHCl_3$/isopropanol (3:1, 3 x 40 mL) The crude product was purified by flash column chromatography (100:15:2 → 100:20:2 DCM/MeOH/25% NH_3 aq. solution), yielding product **238** (107 mg, 0.481 mmol, 49%) as a dark brown oil.

R_f: 0.21 (100:20:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.02 (t, *J* = 8.0 Hz, 1H, 5'-H), 6.22 – 6.16 (m, 2H, 4'-H, 6'-H), 6.12 (ddd, *J* = 7.9, 2.2, 0.8 Hz, 1H, 2'-H,), 3.29 (q, *J* = 7.1 Hz, 2H, 4-H), 3.20 (t, *J* = 6.8 Hz, 2H, $C\underline{H}_2CH_3$), 2.68 (t, *J* = 6.5 Hz, 2H, 1-H), 2.46 (d, *J* = 4.4 Hz, 3H, $NHC\underline{H}_3$), 1.60 (d, *J* = 7.1 Hz, 4H, 2-H, 3-H), 1.11 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

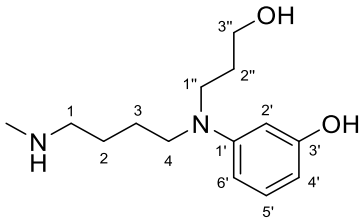
^{13}C NMR (101 MHz, CD_3OD): δ = 157.8 (C-3'), 149.5 (C-1'), 130.2 (C-5'), 104.3 (C-4' or C-6'), 103.1 (C-4' or C-6'), 99.4 (C-2'), 51.3 (C-4), 50.4 ($\underline{C}H_2CH_3$), 45.2 (C-1), 35.7 ($NHCH_3$), 26.7 (C-2 or C-3), 25.4 (C-2 or C-3), 12.5 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2932, 2866, 1572, 1463, 1369, 1211, 1020, 751 cm^{-1}.

HRMS (ESI): *m/z* = calculated for $C_{13}H_{23}N_2O$ $[M+H]^+$: 223.1805; found: 223.18041.

Purity (HPLC): >83% (λ = 210 nm), >82% (λ = 254 nm), Method 1c.

3-((3-Hydroxypropyl)(4-(methylamino)butyl)amino)phenol (239)



$C_{14}H_{24}N_2O_2$

M_w = 252.36 g/mol

Secondary amine **239** was synthesised following General procedure R from carbamate **231** (246 mg, 0.727 mmol, 1.0 eq). The filtrate was evaporated under reduced pressure, dissolved in MeOH/DCM (1:1) and filtered again. This procedure was repeated four times. The crude product was purified by flash column chromatography (100:20:2 DCM/MeOH/25% NH_3 aq. solution), yielding product **239** (170 mg, 0.674 mmol, 93%) as a grey oil.

R_f: 0.47 (100:20:1 DCM/MeOH/25% NH_3 aq. solution).

^{1}H NMR (400 MHz, CD_3OD): δ = 6.98 (p, *J* = 4.1 Hz, 1H, 5'-H), 6.28 (d, *J* = 8.3 Hz, 1H, 4'-H), 6.23 (s, 1H, 2'-H), 6.13 (d, *J* = 8.0 Hz, 1H, 6'-H), 3.62 (t, *J* = 6.2 Hz, 2H, 3''-H), 3.42 – 3.36 (m, 4H, 4-H, 1''-H), 3.01 (dd, *J* = 8.2, 6.6 Hz, 2H, 1-H), 2.68 (s, 3H, NCH_3), 1.84 – 1.63 (m, 6H, 2-H, 3-H, 2''-H) ppm.

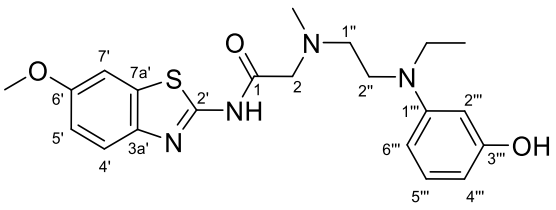
^{13}C NMR (101 MHz, CD_3OD): δ = 159.2 (C-3'), 157.2 (C-1'), 131.0 (C-5'), 105.0 (C-4'), 103.5 (C-6'), 101.1 (C-2'), 60.6 (C-3''), 50.3 (C-1, C-1''), 33.6 (NCH_3), 31.0 (C-2 or C-3), 30.1 (C-2 or C-3), 25.4 (C-2'') ppm.

IR (ATR): $\tilde{\nu}$ = 1637, 1509, 1463, 1407, 1375, 1167, 1019 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{14}H_{24}N_2O_2$ $[M]^{\cdot+}$: 252.1833; found: 252.1832.

Purity (HPLC): >94% (λ = 210 nm), >97% (λ = 254 nm), Method 1c.

2-((2-(Ethyl(3-hydroxyphenyl)amino)ethyl)(methyl)amino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (240)



$C_{21}H_{26}N_4O_3S$

M_w = 414.53 g/mol

Tertiary amine **240** was prepared following General procedure F, using secondary amine **232** (94 mg, 0.48 mmol, 1.0 eq), chloroacetamide **67** (136 mg, 0.531 mmol, 1.1 eq), and triethylamine (0.074 mL, 0.53 mmol, 1.1 eq). The mixture was stirred for 16 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding product **240** (58 mg, 0.14 mmol, 29%) as a beige solid.

R_f: 0.56 (1:3 EtOAc/isohexanes).

Mp: 133 – 137 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 8.9 Hz, 1H, 4'-H), 7.28 (d, *J* = 2.6 Hz, 1H, 7'-H), 7.09 – 7.04 (m, 2H, 5'-H, 5'''-H), 6.36 (t, *J* = 2.3 Hz, 1H, 2'''-H), 6.31 (dd, *J* = 8.3, 2.4 Hz, 1H, 4'''-H,), 6.23 (ddd, *J* = 8.0, 2.3, 0.8 Hz, 1H, 6'''-H), 3.88 (s, 3H, OCH_3), 3.48 (t, *J* = 6.8 Hz, 2H, 2''-H), 3.34 (q, *J* = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 3.31 (s, 2H, 2-H), 2.72 (t, *J* = 6.8 Hz, 2H, 1''-H), 2.39 (s, 3H, NCH_3), 1.16 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

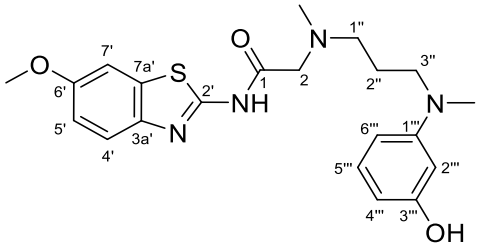
^{13}C NMR (126 MHz, $CDCl_3$): δ = 169.7 (C-1), 157.7 (C-6'), 157.2 (C-3'''), 155.9 (C-2'), 149.6 (C-1'''), 142.2 (C-3a'), 133.2 (C-7a'), 130.7 (C-5'''), 121.7 (C-4'), 115.6 (C-5'), 105.7 (C-4'''), 104.8 (C-6'''), 104.5 (C-7'), 100.2 (C-2'''), 62.2 (C-2), 56.0 (OCH_3), 55.8 (C-1''), 49.6 (C-2''), 45.2 ($\underline{C}H_2CH_3$), 43.6 (NCH_3), 12.6 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3280, 2960, 1704, 1535, 1451, 1350, 1274, 1187, 1026, 828 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{21}H_{27}N_4O_3S$ $[M+H]^+$: 415.1799; found: 415.17925.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

2-((3-((3-Hydroxyphenyl)(methyl)amino)propyl)(methyl)amino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (242)



$C_{21}H_{26}N_4O_3S$

M_w = 414.53 g/mol

Tertiary amine **242** was prepared following General procedure F, using secondary amine **234** (32 mg, 0.16 mmol, 1.0 eq), chloroacetamide **67** (46 mg, 0.18 mmol, 1.1 eq), and triethylamine (0.025 mL, 0.18 mmol, 1.1 eq). The mixture was stirred for 16 h, and the extraction was

conducted with EtOAc. The residue was purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding product **242** (38 mg, 0.091 mmol, 56%) as a beige solid.

R_f: 0.55 (1:3 EtOAc/isohexanes).

Mp: 159 – 161 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 8.8 Hz, 1H, 4'-H), 7.27 (d, *J* = 2.6 Hz, 1H, 7'-H), 7.12 (t, *J* = 8.1 Hz, 1H, 5'''-H), 7.05 (dd, *J* = 8.9, 2.6 Hz, 1H, 5'-H), 6.58 (t, 1H, 2'''-H), 6.31 (dd, *J* = 14.3, 8.1 Hz, 2H, 4'''-H, 6'''-H), 3.87 (s, 3H, OCH_3), 3.44 (t, *J* = 6.8 Hz, 2H, 3''-H), 3.31 (s, 2H, 2-H), 2.88 (s, 3H, (C-1''')NCH_3), 2.56 (t, 2H, 1''-H), 2.22 (s, 2H, (C-2)NCH_3), 1.79 (t, 2H, 2''-H) ppm.

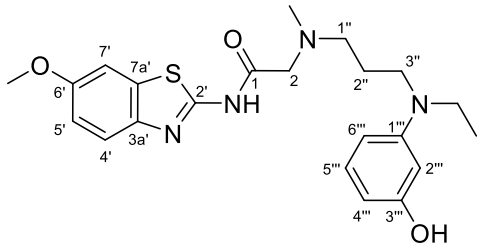
^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.0 (C-1), 158.1 (C-6'), 157.3 (C-3'''), 156.0 (C-2'), 149.2 (C-1'''), 141.1 (C-3a'), 133.0 (C-7a'), 130.8 (C-5'''), 121.7 (C-4'), 115.7 (C-6'), 109.0 (C-4'''), 104.5 (C-7', C-6'''), 100.6 (C-2'''), 58.5 (C-2), 56.0 (OCH_3), 54.2 (C-1''), 49.9 (C-3''), 43.9 ((C-2)N<u>C</u>H_3), 37.5 ((C-1''')N<u>C</u>H_3) ppm.

IR (ATR): $\tilde{\nu}$ = 1686, 1621, 1540, 1471, 1377, 1262, 1219, 1024, 999, 822, 752 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{21}H_{26}N_4O_3S$ $[M]^{\cdot+}$: 414.1721; found: 414.1729.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

2-((3-(Ethyl(3-hydroxyphenyl)amino)propyl)(methyl)amino)-N-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (243)



$C_{22}H_{28}N_4O_3S$

M_w = 428.56 g/mol

Tertiary amine **243** was prepared following General procedure F, using secondary amine **235** (85 mg, 0.41 mmol, 1.0 eq), chloroacetamide **67** (116 mg, 0.451 mmol, 1.1 eq), and triethylamine (0.063 mL, 0.45 mmol, 1.1 eq). The mixture was stirred for 16 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding product **243** (87 mg, 0.20 mmol, 49%) as a beige solid.

R_f: 0.44 (1:3 EtOAc/isohexanes).

Mp: 117 – 119 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.78 (d, *J* = 8.9 Hz, 1H, 4'-H), 7.29 (d, *J* = 2.5 Hz, 1H, 7'-H), 7.10 (t, *J* = 8.1 Hz, 1H, 5'''-H), 7.06 (dd, *J* = 8.9, 2.6 Hz, 1H, 5'-H), 6.42 (t, *J* = 2.4 Hz, 1H, 2'''-H), 6.28 (td, *J* = 7.6, 2.3 Hz, 2H, 6'''-H), 3.88 (s, 3H, OCH_3), 3.38 (t, 2H, 3''-H), 3.31 (q, *J* = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 3.26 (s, 2H, 2-H), 2.53 (t, *J* = 6.3 Hz, 2H, 1''-H), 2.25 (s, 3H, NCH_3), 1.77 (p, *J* = 6.5 Hz, 2H, 2''-H), 1.16 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H}_3$) ppm.

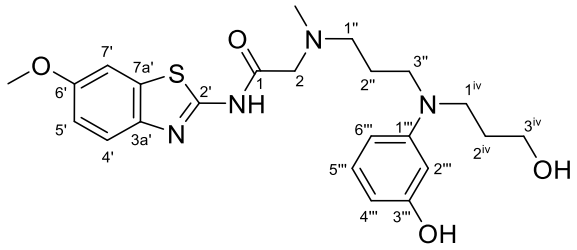
^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.7 (C-1), 158.0 (C-6'), 157.2 (C-3'''), 155.8 (C-2'), 150.0 (C-1'''), 142.1 (C-3a'), 133.1 (C-7a'), 130.7 (C-5'''), 121.8 (C-4'), 115.6 (C-5'), 105.0 (C-4'''), 104.5 (C-6'''), 104.4 (C-7'), 100.2 (C-2'''), 60.5 (C-2), 56.0 (OCH_3), 55.0 (C-1''), 47.7 (C-3''), 44.1 ($\underline{C}H_2CH_3$), 43.9 (NCH_3), 26.0 (C-2''), 12.6 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 3340, 2963, 2877, 1680, 1538, 1471, 1451, 1352, 1260, 1219, 751 cm^{-1}.

HRMS (ESI): *m/z* = calculated for: $C_{22}H_{29}N_4O_3S$ $[M+H]^+$: 429.1955; found: 429.1948.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

2-((3-((3-Hydroxyphenyl)(3-hydroxypropyl)amino)propyl)(methyl)amino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (244)



$C_{23}H_{30}N_4O_4S$

Mw = 458.58 g/mol

Tertiary amine **244** was prepared following General procedure F, using secondary amine **236** (154 mg, 0.645 mmol, 1.0 eq), chloroacetamide **67** (182 mg, 0.710 mmol, 1.1 eq), and triethylamine (0.099 mL, 0.71 mmol, 1.1 eq). The mixture was stirred for 16 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding product **244** (95 mg, 0.21 mmol, 32%) as a brown waxy solid.

R_f: 0.33 (1:3 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.71 (d, *J* = 8.9 Hz, 1H, 4'-H), 7.28 (d, *J* = 2.5 Hz, 1H,7'-H), 7.10 – 7.05 (m, 2H, 5'''-H), 7.04 (d, *J* = 2.6 Hz, 2H, 5'-H), 6.36 (d, *J* = 2.6 Hz, 1H, 2'''-H), 6.30 (dd, *J* = 8.3, 2.3 Hz, 1H, 6'''-H), 6.23 (dd, *J* = 8.0, 2.2 Hz, 1H, 4'''-H), 3.87 (s, 3H, OCH_3), 3.76

(t, *J* = 5.6 Hz, 2H, 3^{iv}-H), 3.50 (td, *J* = 6.8, 4.5 Hz, 4H, 3-H, 1^{iv}-H), 3.27 (s, 2H, 2-H), 2.55 (t, *J* = 6.1 Hz, 2H, 1''-H), 2.30 (s, 3H, NCH_3), 1.81 (m, 8H, 3''-H, 2''-H, 2^{iv}-H) ppm.

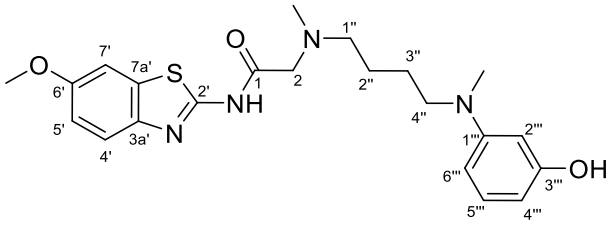
^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 169.8 (C-1),157.7 (C-6'), 157.2 (C-3'''), 156.2 (C-2'), 149.7 (C-1'''), 141.9 (C-3a'), 133.0 (C-7a'), 130.57 (C-5'''), 121.6 (C-4'), 115.6 (C-5'), 105.1 (C-6'''), 104.4 (C-4'''), 100.0 (C-2'''), 60.8 (C-2), 59.8 (C-1^{iv}), 56.0 (OCH_3), 54.9 (C-1''), 48.4 (C-3''), 46.8 (C-3^{iv}), 43.6 (NCH_3), 30.0 (C-2'' or C-1^{iv}), 25.4 (C-2'' or C-1^{iv}) ppm.

IR (ATR): $\tilde{\nu}$ = 3251, 2935, 2872, 1658, 1537, 1468, 1385, 1258, 1220, 1026, 821, 752 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{23}H_{30}N_4O_4S$ $[M]^{\cdot+}$: 458.1976; found: 458.1983.

Purity (HPLC): >92% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

2-((4-((3-Hydroxyphenyl)(methyl)amino)butyl)(methyl)amino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (245)



$C_{22}H_{28}N_4O_3S$

M_w = 428.56 g/mol

Tertiary amine **245** was prepared following General procedure F, using secondary amine **237** (77 mg, 0.37 mmol, 1.0 eq), chloroacetamide **67** (104 mg, 0.405 mmol, 1.1 eq), and triethylamine (0.041 mL, 0.29 mmol, 1.1 eq). The mixture was stirred for 16 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding product **245** (80 mg, 0.19 mmol, 51%) as a brown oil.

R_f: 0.67 (1:3 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 7.73 (d, *J* = 8.9 Hz, 1H, 4'-H), 7.28 (d, *J* = 2.6 Hz, 1H, 7'-H), 7.08 – 7.05 (m, 1H, 5'''-H), 7.05 – 7.02 (m, 1H, 5'-H), 6.31 (t, 1H, 2'''-H), 6.28 – 6.24 (m, 1H, 6'''-H), 6.24 – 6.19 (m, 1H, 4'''-H), 3.87 (s, 3H, OCH_3), 3.30 (t, *J* = 7.2 Hz, 4H, 1''-H, 4''-H), 2.89 (s, 3H, (C-1''')NCH_3), 2.56 (t, 2H, 2-H), 2.39 (s, 2H, (C-2)NCH_3), 1.64 (p, *J* = 7.1 Hz, 2H, 2''-H or 3''-H), 1.57 – 1.47 (m, 2H, 2''-H or 3''-H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 169.6 (C-1), 157.1 (C-6'), 157.0 (C-3'''), 155.5 (C-2'), 150.7 (C-1'''), 142.2 (C-3a'), 133.2 (C-7a'), 130.2 (C-5'''), 121.6 (C-4'), 115.4 (C-5'), 105.0 (C-4'''),

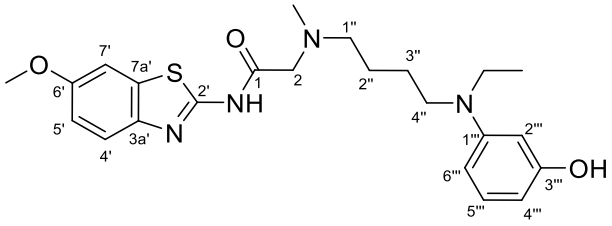
104.3 (C-6’’’), 104.1 (C-7’), 99.9 (C-2’’’), 60.5 (C-1’’), 60.4 (C-4’’), 57.8 (C-2), 55.8 (OCH_3), 43.7 ((C-2)NCH_3), 38.7 ((C-1’’’)NCH_3), 24.9 (C-2’’), 24.2 (C-3’’) ppm.

IR (ATR): $\tilde{\nu}$ = 2925, 2857, 1711, 1612, 1539, 1472, 1449, 1368, 1150, 1061, 1022, 813 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{22}H_{28}N_4O_3S$ $[M]^{\cdot+}$: 428.1877; found: 428.1882.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

2-((4-(Ethyl(3-hydroxyphenyl)amino)butyl)(methyl)amino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (246)



$C_{23}H_{30}N_4O_3S$

M_w = 442.58 g/mol

Tertiary amine **246** was prepared following General procedure F, using secondary amine **238** (87 mg, 0.39 mmol, 1.0 eq), chloroacetamide **67** (110 mg, 0.428 mmol, 1.1 eq), and triethylamine (0.060 mL, 0.43 mmol, 1.1 eq). The mixture was stirred for 16 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (2:1 EtOAc/isohexanes), yielding product **246** (51 mg, 0.12 mmol, 30%) as a brown oil.

R_f: 0.67 (1:3 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 73 (d, *J* = 8.9 Hz, 1H, 4’-H), 7.28 (d, *J* = 2.6 Hz, 1H, 7’-H), 7.08 – 7.01 (m, 2H, 5’-H, 5’’’-H), 6.32 – 6.24 (m, 2H, 2’’’-H), 6.21 (d, *J* = 7.9 Hz, 1H, 4’’’-H), 3.87 (s, 3H, OCH_3), 3.31 (q, *J* = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 3.28 – 3.21 (m, 4H, 2-H, 4’’-H), 2.54 (t, *J* = 7.1 Hz, 2H, 1’’-H), 2.36 (s, 4H, NCH_3), 1.65 (m, 2H, 2’’-H), 1.53 (p, *J* = 7.2 Hz, 2H, 3’’-H), 1.12 (t, *J* = 7.0 Hz, 2H, $CH_2C\underline{H}_3$) ppm.

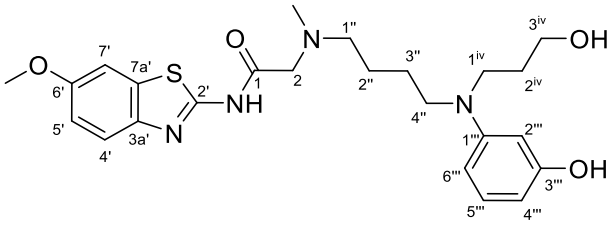
^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.9 (C-1), 157.5 (C-6’), 157.1 (C-3’’’), 155.7 (C-2’), 142.4 (C-3a’), 133.3 (C-7a’), 130.4 (C-5’’’), 121.7 (C-4’), 115.5 (C-5’), 105.1 (C-4’’’), 104.4 (C-6’’’), 103.9 (C-7’), 100.0 (C-2’’’) 60.7 (C-2), 57.9 (C-1’’), 56.0 (OCH_3), 50.4 (C-4’’), 46.0 ($\underline{C}H_2CH_3$), 43.8 (NCH_3), 25.2 (C-3’’), 25.1 (C-2’’), 12.5 ($CH_2\underline{C}H_3$) ppm.

IR (ATR): $\tilde{\nu}$ = 2933, 2866, 1697, 1604, 1536, 1468, 1371, 1259, 1059, 1026, 818, 750 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{23}H_{30}N_4O_3S$ $[M]^{\cdot+}$: 442.2034; found: 442.2037.

Purity (HPLC): 100% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

2-((4-((3-Hydroxyphenyl)(3-hydroxypropyl)amino)butyl)(methyl)amino)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (247)



$C_{24}H_{32}N_4O_4S$

M_w = 472.61 g/mol

Tertiary amine **247** was prepared following General procedure F, using secondary amine **239** (180 mg, 0.715 mmol, 1.0 eq), chloroacetamide **67** (202 mg, 0.786 mmol, 1.1 eq), and triethylamine (0.110 mL, 0.786 mmol, 1.1 eq). The mixture was stirred for 16 h, and the extraction was conducted with EtOAc. The residue was purified by flash column chromatography (1:5 → 1:2 EtOAc/isohexanes), yielding product **247** (30 mg, 0.064 mmol, 9%) as a light yellow oil.

R_f: 0.45 (1:2 EtOAc/isohexanes).

^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 7.73 (d, *J* = 9.2 Hz, 1H, 4'-H), 7.28 (d, *J* = 2.5 Hz, 1H, 7'-H), 7.08 – 7.03 (m, 2H, 5'-H, 5'''-H), 6.31 (s, 2H, 2'''-H), 6.29 (s, 1H, 6'''-H), 6.21 (d, *J* = 7.9 Hz, 1H, 4'''-H), 3.87 (s, 3H, OCH_3), 3.72 (t, *J* = 5.8 Hz, 2H, 3^{iv}-H), 3.42 (t, *J* = 7.0 Hz, 2H, 1^{iv}-H), 3.33 – 3.26 (m, 2H, 4''-H), 3.25 (s, 2H, 2-H), 2.54 (t, *J* = 6.8 Hz, 2H, 1''-H), 2.35 (s, 3H, NCH_3), 1.83 (p, *J* = 6.3 Hz, 2H, 2^{iv}-H), 1.71 (q, *J* = 8.0 Hz, 2H, 3''-H), 1.54 (p, *J* = 7.1 Hz, 3H, 2''-H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 69.9 (C-1), 157.3 (C-6'), 157.2 (C-3'''), 155.9 (C2'), 149.0 (C-1'''), 142.2 (C-3a'), 133.3 (C-7a'), 130.3 (C-5'''), 121.7 (C-4'), 115.6 (C-5'), 104.4 (C-4', C-7'''), 100.3 (C-2''', C-6'''), 61.0 (C-2), 60.5 (3^{iv}-H), 57.8 (C-1''), 56.0 (OCH_3), 43.8 (NCH_3), 30.3 (2^{iv}-H), 25.0 (C-4''), 24.8 (C-2'') ppm.

IR (ATR): $\tilde{\nu}$ = 2931, 2862, 1665, 1467, 1369, 1467, 1369, 1259, 1165, 1058, 853, 749 cm^{-1}.

HRMS (EI): *m/z* = calculated for $C_{24}H_{32}N_4O_4S$ $[M]^{\cdot+}$: 472.2139; found: 472.2137.

Purity (HPLC): >97% (λ = 210 nm), 100% (λ = 254 nm), Method 1c.

6 Appendices

6.1 Abbreviation

2-DOS	2-deoxystreptamine
AC	all-culture
ACE2	angiotensin-converting enzyme 2
ACN	acetonitrile
aq.	aqueous
ASAP	atmospheric solids analysis probe
ASO	antisense oligonucleotide
br	broad (NMR)
conc.	concentrated
COSY	homonuclear correlation spectroscopy
COVID-19	coronavirus disease 2019
CPMG	Carr-Purcell-Meiboom-Gill
CQ	chloroquine
CSP	chemical shift perturbation
d	doublet (NMR)
DAP	3,5-diaminopiperidine
DBAD	*tert*-butyl azodicarboxylate
DCM	dichloromethane
dd	double doublet (NMR)
ddd	double double doublet (NMR)
DEAD	diethyl azodicarboxylate
DEPT	distortionless enhancement by polarisation transfer
DIBAL-H	diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DSMZ	Mikroorganismen und Zellkulturen GmbH
DSS	3-(trimethylsilyl)propane-1-sulfonate
dt	double triplet (NMR)
DTT	dithiothreitol
EI	electron ionisation
eq	equivalents

ESI	electron spray ionisation
FBS	fetal bovine serum
FCC	flash column chromatography
Et	ethyl
GL	good leaving group
h	hours
HBS	HEPES-buffered solution
HCQ	hydroxychloroquine
HEK293	human embryonic kidney cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
HTS	high-throughput screening
Hz	hertz
IR	infrared
IVL	intraluminal vesicles
KPi	potassium phosphate
LNA	locked nucleic acid
LSD	lysosomal storage disorders
m	multiplet (NMR)
M	molar
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
mmol	millimole
mp	melting points
MsCl	methanesulfonyl chloride
ms	millisecond
mTOR	mechanistic target of rapamycin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MVB	multi-vesicular bodies
MWCO	molecular weight cut-off
NAADP	nicotinic acid adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
ND	not determined
NMR	nuclear magnetic resonance
NsCl	nosyl chloride
Nsp1	non-structural protein 1

ORF	open reading frames
P-PMOs	peptide-conjugated morpholino oligomers
$PI(3,5)P_2$	phosphatidylinositol 3,5 bisphosphate
PIKfyve	FYVE finger-containing phosphoinositide kinase
PG	protecting group
Ph	phenyl
ppm	parts per million
q	quartet (NMR)
QC	quality control
quant.	quantitative
RdRp	RNA-dependent RNA polymerase
R_f	retardation factor
RFP	red fluorescent protein
RNA	ribonucleic acid
RP	reverse-phase
rt	room temperature
s	singlet (NMR)
SAR	structure-activity relationship
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SARS CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SEM	standard error of the mean
S_NAr	nucleophilic aromatic substitution
SOGGY	solvent-optimised double gradient spectroscopy
t	triplet (NMR)
T_2	relaxation time
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TDO	thiourea dioxide
TFA	trifluoroacetic acid
TFEB	transcription factor EB
THF	tetrahydrofuran
TM	transmembrane
TMEM	transmembrane protein
TOCSY	total correlation spectroscopy
TPC	two pore channel
TRPML	mucolipin receptor family
TsCl	4-toluenesulfonyl chloride
TsIm	*N*-(*p*-toluenesulfonyl) imidazole

UTR	untranslated region
VEGF	vascular endothelial growth factors
V-ATPase	vacuolar-type ATPase
WHO	World Health Organization
wLOGSY	water-ligand observed *via* gradient spectroscopy
wt%	percentage by weight

6.2 References

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