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Benzoxazepine-type inhibitors for the CBP/p300 bromodomains

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aus

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Erklärung

Diese Dissertation wurde im Sinne von § 7 der Promotionsordnung vom 28. November 2011 von Herrn Prof. Dr. Franz Bracher betreut.

Eidesstattliche Versicherung

Diese Dissertation wurde eigenständig und ohne unerlaubte Hilfe erarbeitet.

München, den 11.03.2016

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Chapter I – Introduction

1. Epigenetics and bromodomains

In the middle of the 20th century, in times of war and the rise of the superpowers, an exciting race for the discovery of the chemical composition of our inheritable information thrilled the scientific communities. As a key event in 1944 Avery *et al*. published evidence that DNA contains the genetic information of bacteria.[1] The Hershey–Chase experiments[2] in 1952 supported the DNA-hypothesis and shortly later the DNA‘s double helix structure was resolved by Crick and Watson. Prior to that, due to their greater structural complexity, proteins were favored by most researchers as the carriers of genetic information. In a special edition issued in 1979 for the 35th birthday of Avery’s publication, the president of the Rockefeller University states in his foreword:

> „Furthermore, the chemical studies of Phoebus A. T. Levene [on nucleic acids with the proposal of circular tetranucleotides; added by Popp] pointed to a monotonous homogeneity of structure, manifestly inconsistent with the specificity (today we would say informational capacity) of nucleic acids. No wonder that most biologists of the era spoke vaguely of ‚nucleoproteins‘ as the most likely composition of genes.“[3]

Also from today’s point of view, that uncertainty does not seem so unjustified (again?). Undoubtly our genetic information is stored on the DNA. However, the clear and simple concept with exclusively the DNA carrying our inheritable information has been shattered. For example so called ‚nucleoproteins‘ like histones do not only „store“ the DNA, but also direct gene transcription etc. and are finely tuned. Over the recent decades the research field of epigenetics revealed new mechanisms and further hereditary processes that do not involve alteration of the DNA sequence. Arthur Riggs and colleagues defined epigenetics as “the study of mitotically and/or meiotically inheritable changes in gene function that cannot be explained by changes in DNA

sequence".[4] There are three major types of epigenetic modifications: Methylation of the bases that make up the DNA double strand; nucleosome positioning; and modifications of the histones around which the DNA is wrapped. An overview on these mechanisms will be given in the following pages. Other regulatory mechanisms like microRNA expression etc. seem to be downstream results of these three principles.[5]

1.1 DNA methylation

The term DNA methylation almost exclusively describes the methylation of cytosine to 5-methylcytosine in CpG dinucleotides[5] (Figure 1.1). This process occurs seldom but with large impact in regions of the DNA that are called CpG islands.[6] Those CpG islands are regions with > 200 bases, a content of cytosine and guanine > 50 %, a CpG frequency > 0.6, and often contain gene promotors.[5-6] If CpG island methylation occurs, that is generally associated with long-term gene silencing[5]. The process of DNA methylation is essential for X-chromosome inactivation and genomic imprinting: Hypermethylation of one of the two parent alleles enables monoallelic expression.[7]

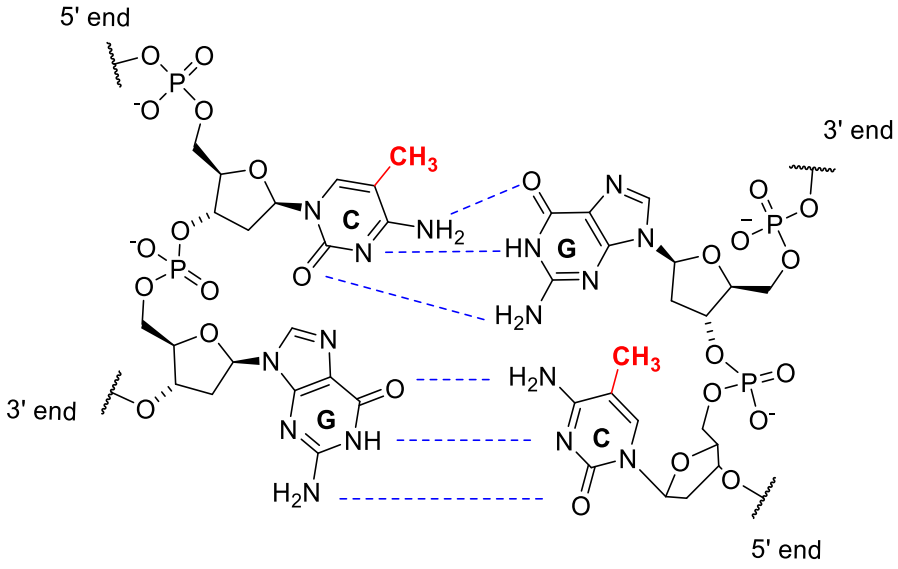


Figure 1.1. The CpG dinucleotide of one DNA strand forming hydrogen bonds (blue) to the corresponding base pairs of the other DNA strand. Cytosine has been methylated at position 5 (red).

1.2 Nucleosome positioning

The nucleosome is the repetitive unit of the chromatin and contains most of the DNA (Figure 1.2). It consists of approximately 166 base pairs of the DNA backbone, which is wrapped twice around one histone octamer and an additional H1 histone protein. The octamer is formed of two of each of the histone proteins H2A, H2B, H3, and H4. The histone proteins are positively charged at cellular pH through protonation of basic amino acid side chains. This is essential for the attraction of the negatively charged phosphate-sugar backbone of the DNA. The nucleosomes are connected via linker DNA, which is approximately 20 base pairs long.[8] Obviously packaging the genetic information tightly within nucleosomes itself affects gene transcription. The DNA and transcription start sites are shielded from transcription factors and activators, and the elongation of transcripts by polymerases is inhibited.[5] Nucleosome positioning has also been linked to DNA methylation.[9]

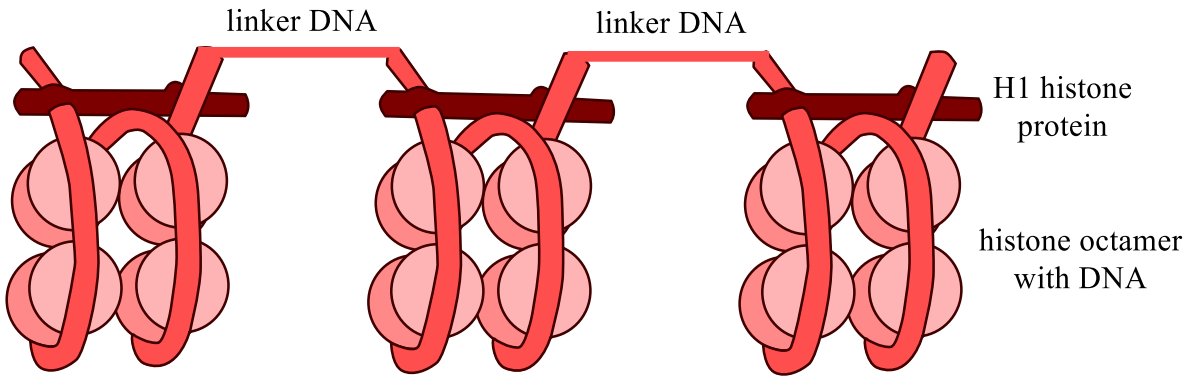
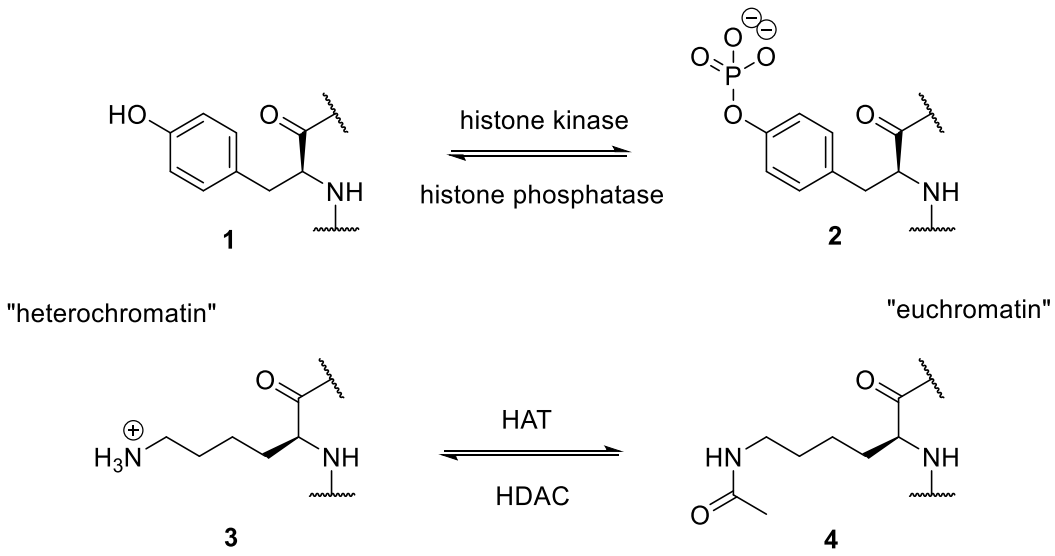


Figure 1.2. Three nucloeosomes with connecting linker DNA.

1.3 Histone post-translational modifications

The posttranslational modification of histones is a process known since the 1960s[10], but more and more means of modification are found and we are just beginning to understand these processes.[11] This chapter is only intended to give a concise and very simplified overview on some of these complex and tightly cross-linked processes:

One of the modifications involved is the multiple or single methylation of lysine and arginine amino acids of the side chains of the histones. In 2004 two groups simultanoeusly showed that the methylation of arginine is antagonized via the conversion into citrulline.[12] Since then demethylases are known that selectively demethylate tri-, di-, and monomethylated lysine moieties.[13] Processes like ubiquitinylation, histone tail clipping, and histone-phosphorylation have also been described.[11] Histone-phosphorylation is mediated by histone kinases, which are able to phosphorylate the hydroxyl group of the side chain of serines, threonines and tyrosines using ATP[14] (**1** & **2**, Scheme 1.1). As a result the net charge of the histone is reduced, faciliating the detachment of the negatively charged DNA double strand. The heterochromatin decoils to euchromatin, is accessible by DNA-, RNA-polymerases, and transcription factors and gene transcription is activated[15]. This process is reversed by phosphatases.[11]



Scheme 1.1. Simplified depiction of reactions influencing chromatin structure and gene transcription.

Another major and very dynamic process influencing the net charge of histones is histone-acetylation (**3** & **4**, Scheme 1.1). The degree of acetylation is increased by histone acetyltransferases (HATs) and decreased by histone deacetylases (HDACs). HATs catalyse the transfer of an acetyl group from cofactor acetyl CoA to the ε-amino group of basic lysine side chains.[11] As one possible result, the acetylated lysine is no longer positively charged, resulting in reduced interaction with the negatively charged

DNA backbone and fostering the formation of euchromatin. Obviously aberrant degrees of histone acetylation and thus gene activation are associated with various diseases such as cancer. Accordingly, HDACs have been recognized as possible drug targets[16], and EMA and the FDA have approved several successful HDAC inhibitors for cancer therapy[17]. However, due to their pleitotropic anticancer effects, these drugs didn't provide a much deeper understanding of the underlying biological mechanisms, possibly limiting their optimal use.[15b, 18]

1.4 The histone acetyltransferases p300/CBP and the bromodomains of CBP and p300

Depending on their occurance, HATs are grouped into A-type HATs (nucleus) and B-type HATs (cytoplasm). Type-A HATs are further subdivided into at least five families, one of them being p300/CBP.[19] Actually CBP (CREB (cAMP responsive element binding protein) binding protein (CREBBP)) and p300 (adenovirus E1A-associated 300-kD protein) are two different acetyltransferase enzymes occuring in man and most eukaryotes[20], but because of the very high sequence homology, the two enzymes are often embraced as p300/CBP.[20-21] These are described as „key enzymes in higher eukaryotes"[20] and acetylate lysines (K → K_{ac}) of all histone core proteins using their HAT-domain.[22] p300/CBP also catalyses the acetylation of non-histone proteins such as transcription factors, in total 100 proteins.[20, 23] Apart from their HAT domain, the p300/CBP proteins contain further domains for the interaction with more than 400 proteins[20, 24], enabling them to act as transcriptional co-activators for RNA polymerase II and others.[25] Likewise CBP/p300 is involved in many signaling pathways such as the cAMP pathway, Notch- and NFκB-signalling.[20] Another domain of p300/CBP is able to recognize ε–N-acetylated lysine moieties, for example on tumor supressor protein p53.[26] Recognition of these ε–N-acetylated lysine motifs of histones is a key step in the reading process of epigenetic marks and is exclusively accomplished by domains named bromodomains.[27]

61 different bromodomains are known on 46 nuclear proteins like methyl transferases, transcriptional coactivators and regulators, ATP-dependent chromatin-remodeling

complexes, helicases, and the HATs.[27b] For most of the bromodomains and the corresponding specific K_{ac} the binding affinities were determined and found to be in the order of µM.[22b] Bromodomains are clustered into eight families, but all share one conserved fold, which consists of a left-handed bundle of four α helices (α_Z, α_A, α_B, α_C) (Figure 1.3). These helices are linked by ZA and BC loops of varying length and amino acid sequence, lining the K_{ac} binding site and influencing the binding specificity.[27b]

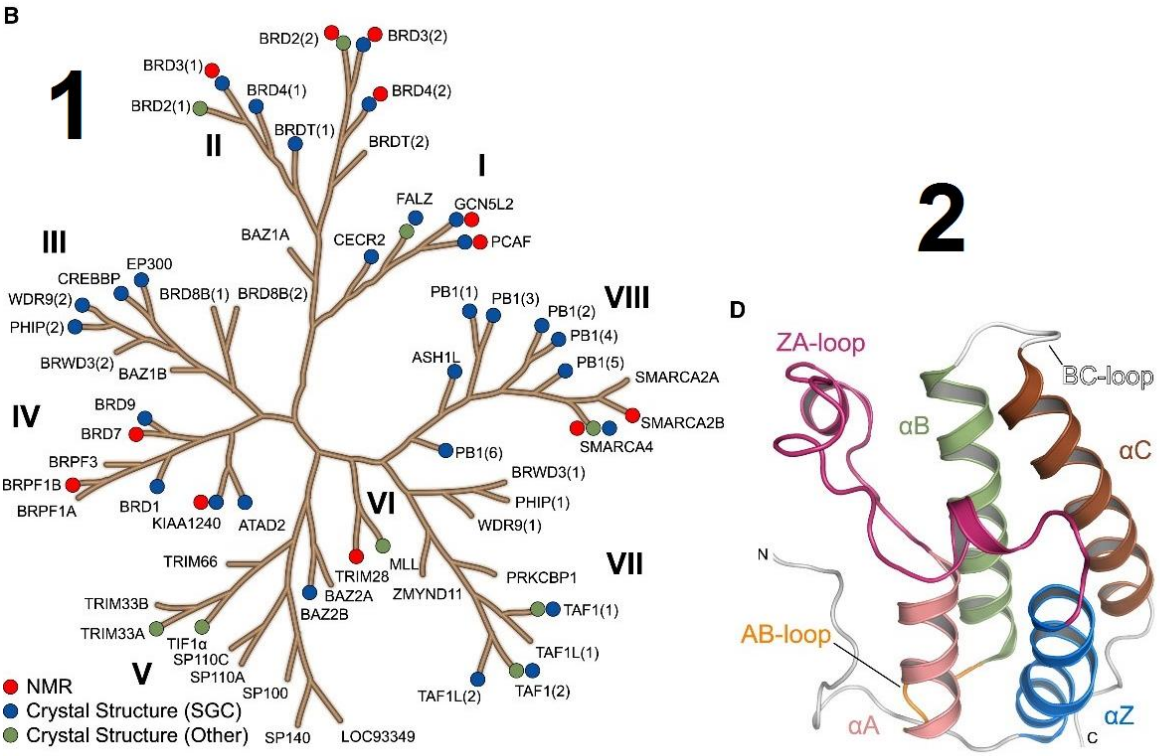


Figure 1.3.* (1) The bromodomain families and availability of structural information. CBP (CREBBP) and p300 (EP300) belong to familiy III. (2) General structure of bromodomains for the example of BRD4(1): The four conserved α helices and the variable ZA and BC loops.

* Cutout from figure 1 of: Histone recognition and large-scale structural analysis of the human bromodomain family[27b]. Further modified by addition of numbers 1 & 2. Under public license; Creative Commons Attribution License (CC BY); Elsevier.

Owen *et al.* demonstrated in 2000 with a co-crystallization that the Gcn5p bromodomain recognizes K_{ac} via hydrogen bonds from Asn407.[28] Meanwhile corresponding interaction has been confirmed for further bromodomains including the CBP bromodomain and its Asn1168 (located on the BC loop).[29] At the same time a few, but essential water molecules remain at a shallow depression at the end of the bromodomain's binding pocket (Figure 1.4) and mediate further hydrogen bonds.[28-29]

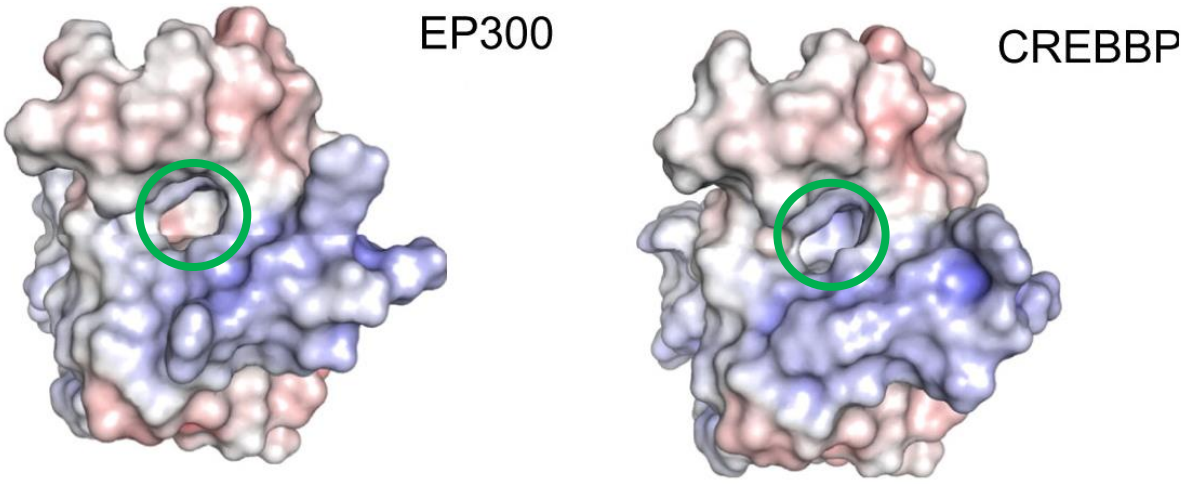


Figure 1.4.* Electrostatic surface potential of the p300 (EP300) and CBP (CREBBP) bromodomains based on crystal structures. Positive charge is blue, negative charge is red. The binding pockets for K_{ac} are surrounded by green circles.

* Cutout from figure 2 of: Histone recognition and large-scale structural analysis of the human bromodomain family[27b]. Further modified by circles around the binding sites. Under public license; Creative Commons Attribution License (CC BY); Elsevier.

Data indicates that recognition of ε–N-acetylated lysine by p300/CBPs‘ bromodomains leads to positive feedback and further acetylation via the HAT domain.[30] CBP is essential for regulation of hematopoietic stem cell (HSC) self-renewal[31], and chromosomal translocations of CBP or p300 with MOZ or MLL have been observed in acute myeloid leukemia.[32] Besides leucemias[33] CBP and p300 have also been linked to carcinomas[34] and the Rubinstein-Taybi syndrome, with patients suffering from broad thumbs, cranio-facial and cardiac abnormalities, as well as mental retardation and cancer predisposition.[35] Consequently academia and the pharmaceutical industry extended their research to HATs and bromodomains for the elucidation of biological mechanisms and the development of novel drugs.

Chapter II – Project and strategy

2.1 Aim of this thesis

> „Selective small molecule inhibitors (chemical probes) have a major impact on our understanding of human biology and for the validation of novel disease associated targets for the development of new treatment therapies. However, the development and characterization of chemical probes is a cost intensive multidisciplinary process requiring significant efforts in medicinal chemistry, structural biochemistry, screening and cell biology that can rarely be accomplished by an isolated laboratory‘[36]‘.
>
> … to combine expertise and resources in different areas of chemical biology we formed a large multinational group involving academic research laboratories and also currently 8 large pharmaceutical companies. This consortium was established based on the Structural Genomics Consortium (SGC) open access model, which distributes and publishes reagents promptly and without constrains imposed by intellectual property.“[37]

Following this approach to a more efficient research model, the SGC has managed to develop a number of impressive probes for protein kinases[38] and more recently a comprehensive set of probes for the bromodomains[15b]. The effort to develop bromodomain inhibitors first focused on bromodomains of the BET family,[15b] which were predicted[39] and proven to be easily druggable[40]. The probe coverage for other bromodomain families has also been expanding rapidly, and more and more publications and patent applications have been filed concerning the various bromodomains.[41]

Friendship and cooperation between Prof. Franz Bracher’s medicinal chemistry group and Prof. Stefan Knapp’s groups at the SGC at the University of Oxford has developed into a fruitful tradition. This tradition yielded the potent and selective kinase inhibitor KH-CB19[38b] and gave further insight into the inhibition of bromodomains of the BET

family through benzodiazepines and benzotriazepines[42]. With the general research focus shifting towards bromodomains outside the BET family, our research cooperation followed: Based on the screening of a commercial substance library and some custom made compounds, the SGC developed the potent and to some extent selective benzoxazepine-type inhibitor **I-CBP112** for the CBP and p300 bromodomains (Figure 2.1).[43]

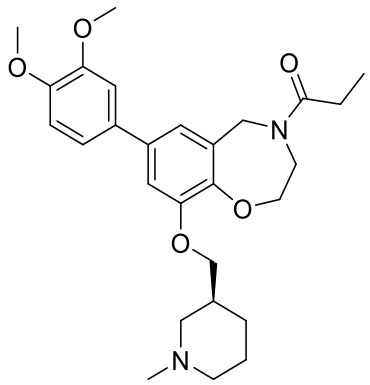


Figure 2.1. CBP/p300 bromodomain inhibitor **I-CBP112**.

Some of this compound's moieties were assumed not to be fully optimized and aim of this thesis was the further refinement of this inhibitor: Especially the residual activity towards the BET family remained an issue of this probe. Selectivity is essential to clearly understand and prove the biological mechanisms of epigenetics, which may be difficult enough with CBP and p300, due to the promiscuity of these proteins described above. This residual activity towards the BET family could not be completely eradicated by other inhibitors, which were published during the course of this thesis. These were based on different scaffolds such as dihydroquinoxalinone[44], acetylbenzene[45], or benzimidazole[46]. However, an 34-fold selectivity of benzimidazole compound CBP30 over BRD4(1) was published by Hay *et al.* from the SGC in 2014.[46] Inhibition of CBP/p300 by this compound leads to suppression of the human Th17 responses, making it an interesting compound for use against ankylosing spondylitis or psoriatic arthritis.[47] Meanwhile the alternative inhibitor **I-CBP112** has been provided as a research tool and has become commercially available.[43] **I-CBP112** was proven to be effective against mouse and human leukemic cell lines *in vitro* and *in vivo*. Synergistic

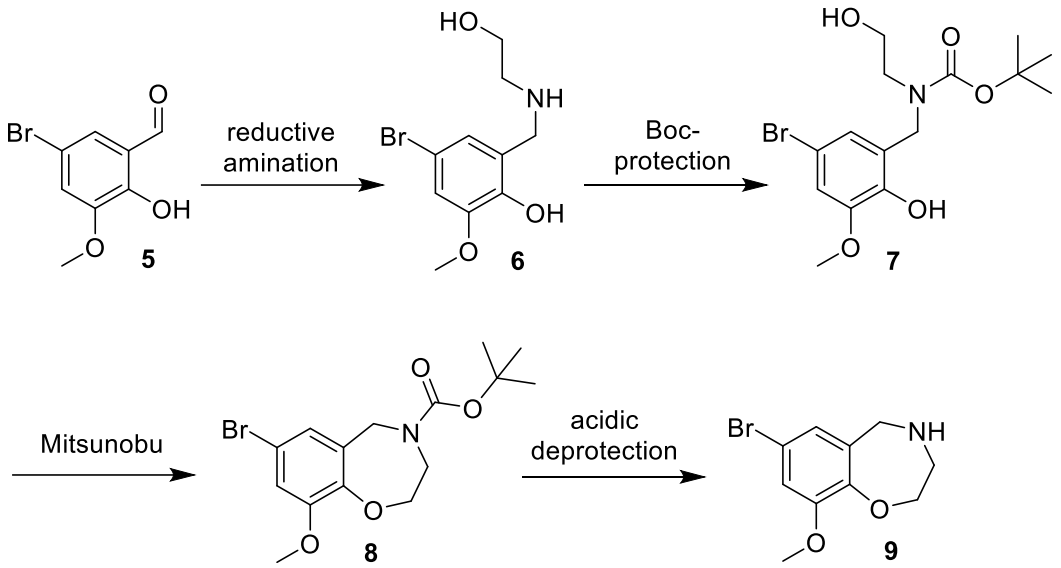
effects were shown with standard therapeutics (doxorubicin) or with BET-inhibitors, and **I-CBP112** may be an interesting candidate for clinical evaluation.[48]

Obviously bromodomains and their inhibition is currently a hot topic, which may yield an understanding of and cure for horrible diseases. Accordingly competition is great and the pace is quick. The preparation of a large number of inhibitors will be necessary to find those that are most suitable for therapy. It is not yet clear, whether the selective or the unselective ones will be more interesting for clinical approaches. The aim of this thesis was to further investigate **I-CBP112**-type inhibitors, to learn more about the SAR, and to be able to functionalize this molecule for different purposes.

2.2 Strategy of synthesis: Published syntheses and innovative approaches

2.2.1 Preparation of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with methyl ether at C-9 and first compounds

The 1,4-benzoxazepine element is reported as a scaffold of various substances with anti-inflammatory[49], anti-thrombotic[50], anti-tumor[51], and anti-amyloid-beta plaque activity[52]. Accordingly, various synthetic strategies have been published. The favoured scaffold for this thesis seemed readily available following an established approach to a 2,3,4,5-tetrahydro-1,4-benzoxazepine backbone[51b, 51c], but starting with 5-bromo-2-hydroxy-3-methoxybenzaldehyde **5** instead of 5-bromo-2-hydroxybenzaldehyde (Scheme 2.1). Preliminary data from the SGC suggested that the voluminous C-9 ether moiety of lead structure **I-CBP112** was pointing towards the solvent and away from the protein, so this moiety did not seem vital for the compound's activity. Thus the intention was to test first variations of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold on the simplified backbone **9** (Scheme 2.1) bearing a methoxy moiety at C-9. This intermediate was planned as a versatile building block that would allow flexible introduction of different moieties at C-7 via cross coupling and various N-functionalizations at N-4 in any order. This flexibility would be important to then optimize the moieties at C-7 and N-4.



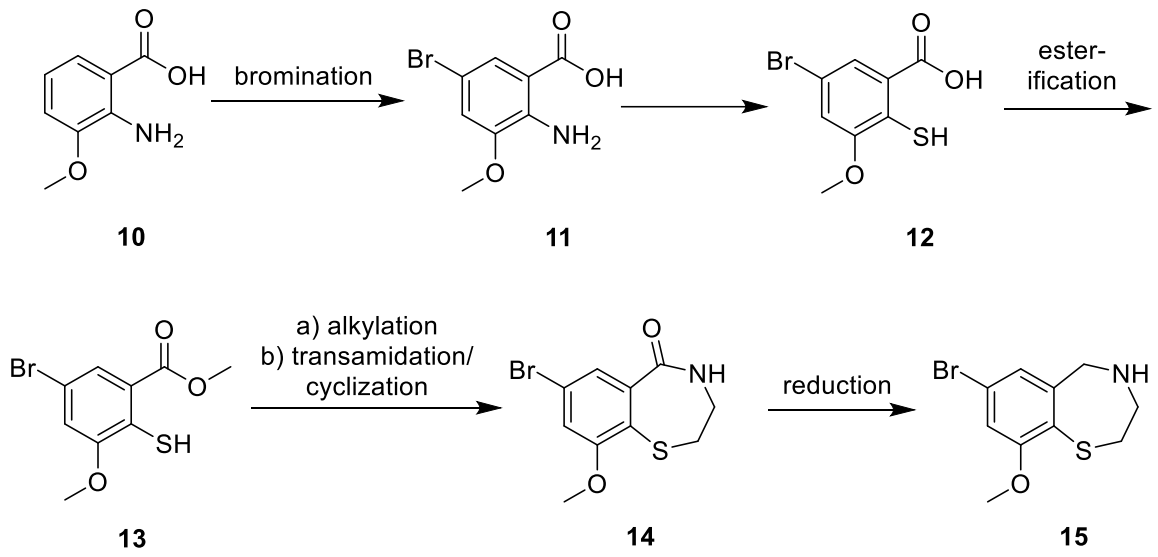
Scheme 2.1. Planned synthesis of the simplified 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold.

Since the initial investigations conducted by the SGC on purchased 2,3,4,5-tetrahydro-1,4-benzoxazepines had already revealed that only very small residues on N-4 were tolerated, further modifications of the hitherto most promising acyl group should be performed without increasing the size of the group too much. We mainly aimed at the introduction of more polar groups of similar size, to enable further hydrogen bonds mediated through the conserved water molecules at the end of the binding pocket. Attempts to replace these essential water molecules were fruitless so far as they form a network of hydrogen bonds mediating K_{ac} binding. For moieties at C-7 it was planned to test all kinds of groups, since the purchased molecules mainly comprised chloro- and methoxyphenyl substituents.

2.2.2 Preparation of 2,3,4,5-tetrahydro-1,4-benzothiazepine analogues

In order to investigate whether the 2,3,4,5-tetrahydro-1,4-benzothiazepine ring could act as a a more selective scaffold, benzothiazepine analogues bearing promising moieties at both C-7 and N-4 and a methoxy group at C-9 were planned. Starting with bromination of 2-amino-3-methoxybenzoic acid (**10**, Scheme 2.2),[53] a number of standard reactions should be adapted to lead to a versatile 1,4-benzothiazepine

building block **15**. Those reactions include a standard multistep reaction for the preparation of thiols from anilines: Diazotation, reaction with potassium ethyl xanthate, and alkaline hydrolysis[54] to obtain novel mercaptobenzoic acid **12**. The acid-catalyzed conversion of other mercaptobenzoic acids into the corresponding methyl esters is described for the synthesis of benzothiophenes.[54b] Subsequent thioetherification of differently substituted methyl mercaptobenzoates with 2-chloroethylamine and base-mediated lactamization is also described.[55] It was planned to obtain benzothiazepinone **14** via **13** in the same manner. Reduction of the lactam function[55] would provide the versatile 1,4-benzothiazepine intermediate **15**.

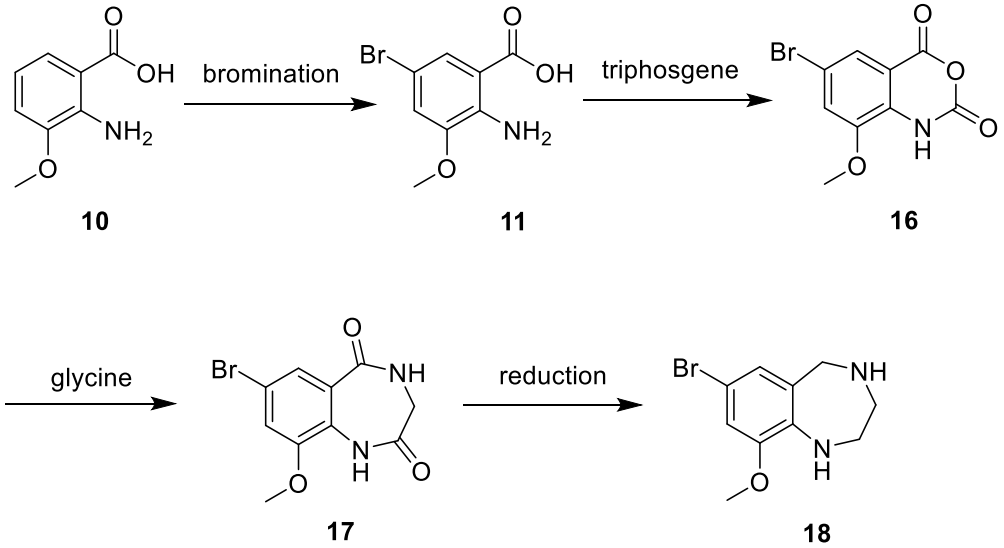


Scheme 2.2. Planned synthesis of the 2,3,4,5-tetrahydro-1,4-benzothiazepine scaffold.

2.2.3 Preparation of 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine analogues

The oxygen at position 1 of the 1,4-benzoxazepine scaffold is capable to act as a hydrogen bond acceptor. The secondary amine at N-1 of the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold is capable to act as hydrogen bond acceptor and donor, making this structure particularly interesting. The 1,4-benzodiazepine scaffold has been intensively studied over many decades now, and has been designated a “privileged scaffold” in drug development.[56] Depending on the degree of unsaturation

of the diazepine ring and the substitution pattern of the two rings, compounds with a broad spectrum of pharmacological activities have been designed.[56a] Consequently, a large number of approaches towards the 1,4-benzodiazepine ring system have been published[56b, 57]. Most of the sophisticated syntheses of the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine ring system, including aziridine ring opening reactions[58], aminoalkylstannane-based routes[59], Pd-catalyzed cyclizations[60], metal-catalyzed hydrogen-transfer reactions[61], chlorosilane-promoted cyclizations of *N*,*O*-acetals[57] were unattractive for our purposes, since these protocols necessarily included the introduction of undesired alkyl or aryl residues at either N-1, N-4, C-2 or C-3. Thus, for the preparation of analogues for our purposes, we initially pursued a classical, short, and very drastic approach[62] via activation of the anthranilic acid 2-amino-5-bromo-3-methoxybenzoic acid (**11**, Scheme 2.3) with triphosgene to the isatoic acid anhydride **16**. Conversion with glycine and reduction of the obtained dilactam **17** was expected to yield the 1,4-benzodiazepine **18** as useful intermediate. Thanks to the higher nucleophilicity of N-4 (secondary aliphatic amine) compared to N-1 (secondary aromatic amine), selective functionalization of N-4 in presence of the unsubstitited N-1 was expected to be feasible.[62e]

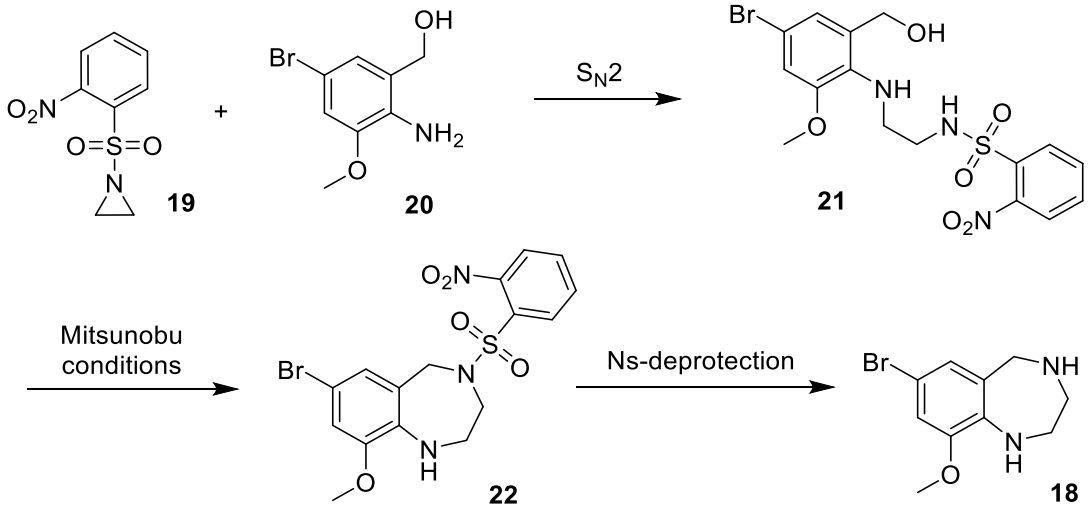


Scheme 2.3. Planned synthesis of the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold.

However, the rection conditions for the reduction of the dilactam are extremely harsh (lengthy refluxing with BH_3 or $LiAlH_4$ in THF) and not compatible with all functional

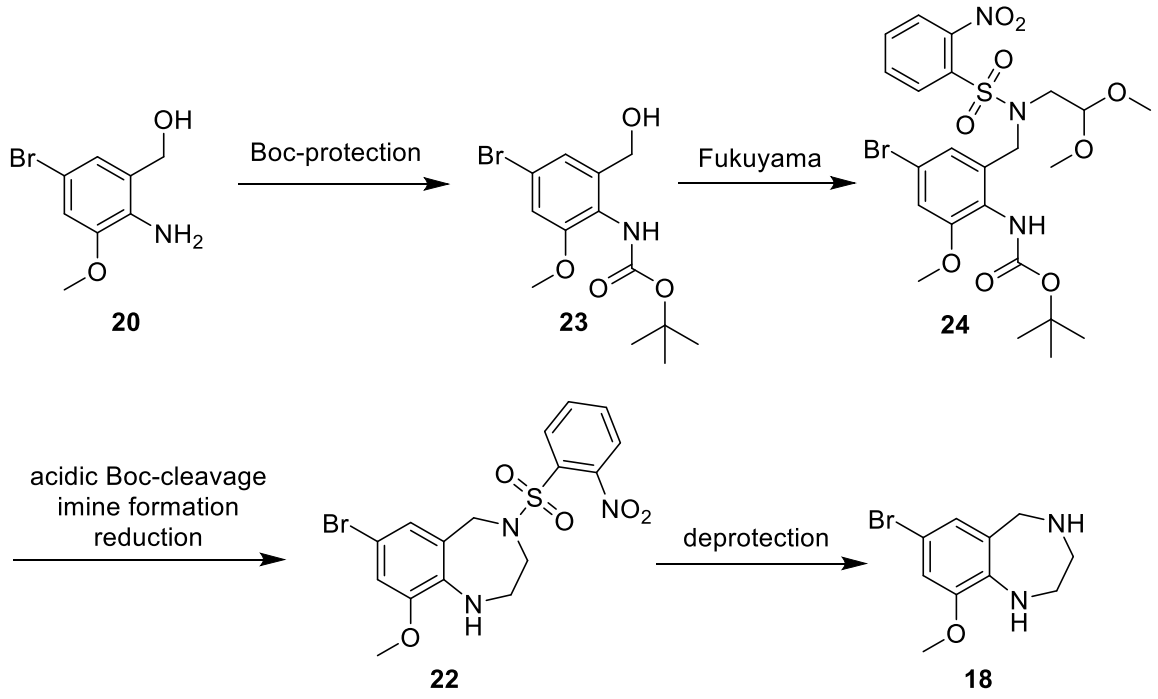
groups. Even debromination at C-7 is observed and reported in literature[51c]. It was decided to test this classical approach for our purposes, but we kept two alternative and innovative ideas in mind and soon strived to develop a novel and mild approach to 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines.

One of these ideas was a two step approach: The first step being a S_N2-reaction of reactive *N*-nosylaziridine[63] and 2-aminobenzyl alcohol (**19** & **20**, Scheme 2.4), with the aniline's nitrogen acting as nucleophile, resulting in intermediate **21**. This idea was inspired by a publication on the use of *N*-tosylaziridines for the one-pot synthesis of tosylated 1,4-benzodiazepin-5-ones.[64] Following this published synthesis was not an option, because the tosyl protected 1,2,3,4-tetrahydro-1,4-benzodiazepin-5*H*-5-one that is obtained was expected to be difficuilt to reduce. Furthermore, cleavage of the tosyl group is performed under reductive conditions and can also be very problematic. The aziridine idea was adopted, but the *N*-nosylaziridine was chosen, because the nosyl protecting group is cleaved very smoothly using thiophenol or thioglycolic acid[65]. We also decided to implement a second reaction step: Fukuyama amine synthesis[65] with the acidic sulfonamide function acting as nucleophile under Mitsunobu conditions[66] should lead to fused compound **22**. Recently the use of *N*-tosylates and Mitsunobu conditions has been published for the synthesis of 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines.[50] However this synthesis is lengthy and also results in di-tosyl-protected compounds.



Scheme 2.4. Proposed alternative route I to the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold.

The other idea for a novel, mild, and short approach to 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines also involved Fukuyama amine synthesis[65]: *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide[67] was expected to react with a *N*-Boc protected 2-aminobenzyl alcohol (**23**, Scheme 2.5) under Mitsunobu conditions to give compound **24**. The intermolecular alkylation of anilines using dimethyl acetals of aldehydes in a triethylsilane – trifluoroacetic acid mixture had been described as giving good yields.[68] To obtain compound **22**, an attempt should be made to use this acidic mixture for Boc-cleavage, intramolecular imine formation, and reduction in a one-pot reaction. Again the nosyl group could finally be cleaved smoothly with thiophenol[65] to obtain intermediate **18**.

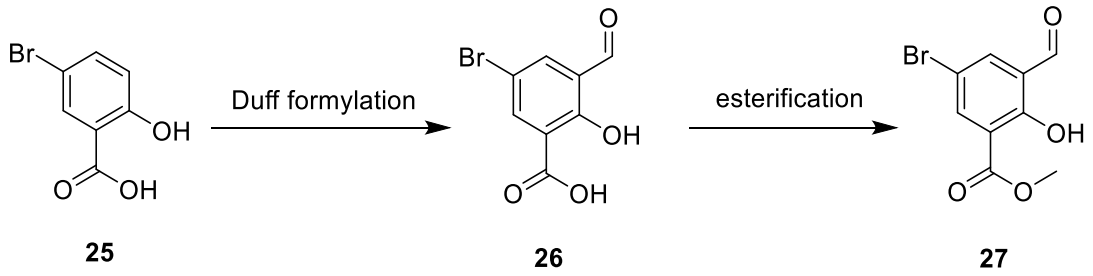


Scheme 2.5. Proposed alternative route II to the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold.

Both novel approaches would offer one cunning feature: The resulting 2,3,4,5-1*H*-tetrahydro-1,4-benzodiazepines would have a protecting group on the more nucleophilic nitrogen (N-4), thus leaving the possibility of functionalizing the less nucleophilic aniline (N-1) prior to deprotection of the more reactive amine.

2.2.4 Preparation of a versatile 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with ester function at C-9

The hitherto used „simplified“ 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold should also be refined at C-9 to introduce further moieties. We decided to embed a carbonyl function and replace **I-CBP112**'s ether function with an amide bond. Of course, this new carbonyl function would also allow introduction of a large number of other functional groups following reactions such as Curtius-rearrangment, Schmidt reaction, amide reduction after transamidiation, etc. Synthesis of this backbone was planned exactly as for the simplified scaffold (Scheme 2.1), but required synthesis of the intermediate methyl 5-bromo-3-formyl-2-hydroxybenzoate (bearing an ester group) beforehand (**27**, Scheme 2.6). Inexpensive 5-bromosalicylic acid (**25**) had been described to undergo Duff formylation and subsequent esterification in large scale and good yields[69] and was selected as starting point.



25 **26** **27**

Scheme 2.6. Known preparation of the educt for the synthesis of 2,3,4,5-tetrahydro-1,4-benzoxazepines with ester function at C-9.

Chapter III - Highlighted methods for the characterization of the compounds

Almost all compounds synthesized for the inhibition of the CBP/p300 bromodomains were screened by differential scanning fluorimetry (DSF, chapter 3.1) for an approximate determination of their potency. The potency of one inhibitor was determined by Alphascreen assay, which gives an IC_{50} value (chapter 3.2). Following the hints obtained through these screenings, the compounds were further optimized. Final compounds which proved more optimized and interesting were further characterized. ITC (isothermal titration calorimetry, chapter 3.3) was conducted for determination of the K_d of one inhibitor. A Co-crystallization was analyzed for this advanced compound for the exact determination of its binding mode (chapter 3.4), and the effectiveness of a final compound in living cells was proven via a FRAP assay (chapter 3.5). All these experiments were conducted at the SGC at the University of Oxford, under supervision of Stefan Knapp, Oleg Fedorov, Catherine Rogers, Cynthia Tallant Blanco and co-workers. Thanks to these and Franz Bracher, I was able to visit the SGC and assist in some of the assays. Moreover all synthesized and novel compounds were routinely tested in our group for cytotoxicity by the MTT assay (chapter 3.6), and for antibacterial and antifungal activity by agar diffusion test (chapter 3.7). These assays were mainly conducted by Martina Stadler.

3.1 Differential scanning fluorimetry (DSF)

Differential scanning fluorimetry is a fast and inexpensive method for the determination of relative binding affinities of small compounds to purified proteins. The melting temperature (T_m) of a protein in a solution with a potential inhibitor / ligand is observed and compared to the T_m in a solution of pure protein. Generally proteins are most stable at moderate temperatures, and susceptible to low temperatures and freezing. Likewise at high temperatures proteins denature, they unfold and lose their function. The state

of equilibrium, with equal concentrations of folded and unfolded protein is defined as the melting temperature (T_m) of proteins (although the process of unfolding is not reversible for many proteins). A potent ligand stabilizes the protein and conserves its folded state. In a solution with protein and ligand this generally results in an elevated melting temperature (T_m), compared to a solution with protein only.[70]

The DSF assay can be run as a high throughput method using devices, which were originally designed for PCR.[71] The increase of a protein's melting temperature is detected by gradual heating of mixtures of protein, ligand candidates, and a suitable fluoresecent dye from room temperature to high temperatures. For example SYPRO® orange is a suitable dye as it is highly fluorescent in a hydrophobic environment, while its fluorescence is quenched in aqueous media. Furthermore, its high excitation wavelength of 492 nm is favorable as it reduces the chance of undesired quenching through other small molecules. For DSF analysis the fluorescence signal is plotted against the rising temperature. Starting at ambient temperature very little fluorescence is noticed. As the temperature rises and protein denatures and unfolds, the protein's internal hydrophobic sites are exposed. As a result the fluorescent dye can bind to those sites and the fluorescence signal rapidly increases to a maximum. Fluorescence later decreases again, as hydrophobic sites are removed through protein aggregation or precipitation.[70]

The T_m of the protein in the solution with the potential ligand can be derived from this plot. Subtraction of the T_m of the pure protein in the reference solution gives ΔT_m ("T_m shift"). Generally the larger ΔT_m, the higher the ligand affinity. However, the magnitude of ΔT_m also depends on the specific protein, the ligand concentration, and various potential modes of binding.[70] Accordingly the magnitude of ΔT_m allows only a comparison of potencies of compounds with similar physicochemical properties and only within the same specific protein (and at best the same batch of protein).

3.2 Alphascreen

Another sophisticated approach to the characterisation of inhibitors is the Alphascreen assay. This assay is useful for the investigation of protein protein interactions and

inhibition thereof. Interference of these interactions allows the determination of the IC_{50} values of inhibitors. Advantages of this assay are a high sensitivity, high specificity, and simple protocols without washing steps. Consequently Alphascreens on microtiter plates have become a tool in high throughput screening.[72]

ALPHA is an abbreviation for „amplified luminescent proximity homogeneous assay“. Its core elements are „donor“ and „acceptor“ polystyrene beads. Each of these beads can bind to the analyte protein in a specific, non-covalent way. Through this interaction with the beads, the protein recruits donor and acceptor beads to its surface and mediates their proximity. The donor bead contains a photosensitizing agent (phthalocyanine), that - under excitation at 680 nm - generates excited singlet oxygen from ambient oxygen at a very high rate, thus amplifying the excitation signal. The acceptor bead typically contains three polycyclic dyes: Thioxene, anthracene and rubrene. If the acceptor bead is within proximity (maximum of 200 nm) of the excitated donor bead, the created singlet oxygen excites the thioxene dye on the acceptor bead. This energy is there converted into light energy, and transferred via anthracene to rubrene. Rubrene finally emits light with a wavelenght of 520 – 620 nm, which can be quantified. Because of the short half life of the singlet oxygen, a dark background, and low bead concentrations, the signal to noise ratio is excellent in this assay.[72]

For the determination of the IC_{50} value of a CBP/p300 bromodomain inhibitor, the donor bead is coated with streptavadin, the acceptor bead with Ni^{2+}-chelators. The following non-covalent bindings result in the signal cascade described above: Streptavadin firmly binds to added biotinylated peptides, and these contain acetylated lysines. The studied target bromodomain (CBP, p300, etc.) then binds to acetylated lysine and remains in proximity to the donor bead. The bromodomain itsself contains a polyhistidine-tag, whose histidine moieties bind to the acceptor bead via Ni^{2+}-complexes. Donor and acceptor beads are thus immobilized and luminescence will be observed, when the donor is excited. This signal cascade and thus the detection of luminescence can be interrupted by the inhibition of binding of the bromodomain to acetylated lysine. Depending on the bromodomain inhibitor concentration and potency, the donor bead will not be recruited into the proximity of the acceptor bead and no or very little luminescence will be observed. The IC_{50} value can be derived from a corresponding plot.[73]

3.3 Isothermal titration calorimetry (ITC)

The dissociation constant (K_d) of a ligand from a protein is an important characteristic of an inhibitor. The smaller the value, the stronger the binding. Unlike ΔT_m obtained from DSF, K_d values may be used to compare the potency of inhibitors across substance classes and different proteins. K_d values of protein inhibitors can be determined accurately using a technique called ITC. The enthalpy change upon binding of the ligand to the protein is measured, thus dissecting ΔG (free energy of binding) into contributions from ΔH (free enthalpy change) and ΔS (entropy change) and allowing calculation of ΔG and ΔS. This is the major advantage over DSF and has contributed to the elucidation of relationships between thermodynamics, structure and function.[74] For this titration two adiabatic cells are simultaneously heated very slowly (< 0.1 °C/h). The reference cell contains buffer solution, the sample cell protein solution. Ligand is titrated into the sample cell. This results in temperature effects that are recognised and lead to adaption of the heating rate for the sample cell and an electronic signal. Initially, due to the surplus of protein, most of the ligand binds upon release, giving a value for ΔH. As titration goes on, more and more binding sites are occupied and ligands remain unbound, although binding sites are still available. This point gives a good estimate of K_d. Obviously this titration also allows conclusions on the stochiometry of the binding[74] (Figure 3.1).

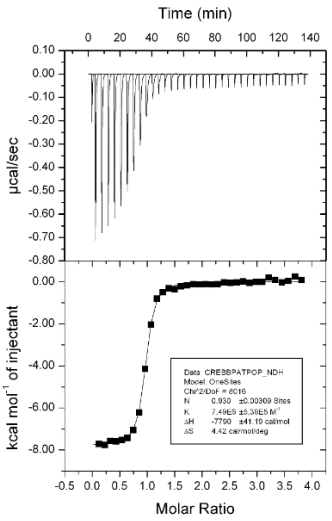


Figure 3.1. Typical graph of an ITC experiment. The top panel depicts the relative power applied to the sample cell compared to the reference cell in order to maintain the same temperature in both cells. The bottom panel shows the integrals from the peaks of the top panel with a line of best fit, necessary to derive ΔH, K_d and the binding stoichiometry.

3.4 Co-crystallization

Differential scanning fluorimetry, isothermal titration calorimetry and Alphascreen data give a comprehensive picture of a compound's potency and selectivity. However, this data contains no information on the binding modes and binding sites. To explain the potency and to undoubtly describe the binding modes, it is necessary to obtain co-crystallizations of inhibitors with the corresponding bromodomain. Furthermore, co-crystallizations may reveal new potential interaction sites and thus give hints for the introduction of new moieties or for the derivatization of present functional groups.

3.5 FRAP

The experiments described so far allow a good characterization of an inhibitor's potency and selectivity. However, these experiments are mainly conducted in vitro on the purified protein. To go a step further and to prove efficacy in living cells, the FRAP (fluorescence recovery after photobleaching) assay has been established. FRAP has been developed since the 1970s, and allows studies of protein protein interactions and protein mobility in living cells.[75] Novel techniques for protein labeling and fluorescence microscopy turned this assay into a powerful tool in the field of epigenetics.[75-76] Figure 3.2 depicts the course of a typical FRAP experiment with a confocal microscope:

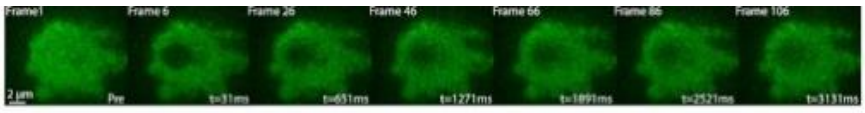


Figure 3.2*. Typical course of a FRAP experiment: A cell with good fluroescence signal arising from excitation of the fluorescence tagged target protein is focused (1st photograph from left). The fluorescence dye of the tagged target protein is then photobleached within a small area (2nd photograph). Afterwards the rate of recovery of the fluorescence signal through protein migration is measured (3rd photograph and so on).

* Cutout from figure 5 of: Munc18-1 protein molecules move between membrane molecular depots distinct from vesicle docking sites.[77] Under public license; Creative Commons Attribution License (CC BY).

FRAP can be employed to study the interaction and binding of a bromodomain to chromatin. The bromodomain must be tagged with a fluorescent dye such as green fluorescent protein (GFP). First all GFP tagged bromodomains are photobleached within a small area of the nucleus with a high-intensity laser pulse. Then the rate of recovery of the fluorescence signal in that area is measured. The duration of recovery is obviously dependant on the rate of migration of GFP tagged bromodomains into the bleached area and thus the bromodomain mobility. Bromodomain mobility itsself is influenced by the binding to K_{ac} of the chromatin. This binding again is also dependant on the degree of lysine acetylation. To obtain a firm binding of the bromodomains to the chromatin and consequently clearer results, the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) is added in experiments, resulting in increased global acetylation. The presence of potent inhibitors of the bromodomain's K_{ac} binding site will release the GFP tagged bromodomains from the chromatin and result in rapid migration and recovery of the fluorescence signal.[75-76, 78]

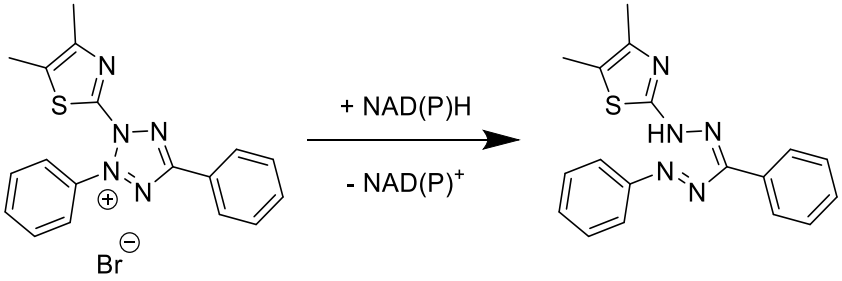
For our purposes, the FRAP assay was performed with human osteosarcoma cells (U2OS). This assay may still be an in vitro assay on isolated cells, but proof of cell permeability and intracellular compound efficacy is essential for potential drug candidates.[76]

3.6 MTT assay

All synthesised and novel compounds were also tested for cell toxicity using the standard MTT assay, following the protocol of Mosmann.[79] This well established assay is suitable to differentiate between living and dead cells. It allows the determination of a substance's cytotoxicity via measurement of cellular metabolic activity. Advantages of this assay are a high precision, a protocol without washing steps and a high throughput using multiwell scanning spectrophotometers.

The assay is based on the conversion of the water soluble, yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, **28**, Scheme 3.1) to an insolube dark blue formazan dye (**29**). This reduction is catalysed in the cytosol of living, metabolically active cells and requires nicotinamide adenine dinucleotide

(NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as reductive agents. After incubation of lymphoma cells with MTT, the insoluble formazan dye is redissolved and photometrically quantified. The amount of formazan dye correlates with the number of living cells. For our purposes human leucemia cells (HL-60) are used, and the nonionic detergent Triton X-100 is applied for positive control. The assay is performed with several suitable dilutions of a stock solution of the test compound. When plotting the dilutions against the percentage of living cells, a sigmoidal curve is obtained, from which the IC_{50} of the substance can be derived. The IC_{50} of cisplatin is around 5 µM. For this thesis IC_{50} values were only exactly determined when smaller than 50 µM.



28 **29**

Scheme 3.1. Water soluble MTT is reduced into the insoluble formazan dye.

3.7 Agar diffusion test

Not linked to the main topic of this thesis, but as part of a routine screening of our group, novel substances were tested for antibacterial and antifungal activity against eight model microorganisms, namely *Escherichia coli* and *Pseudomonas marginalis* for Gram-negative bacteria, and *Staphylococcus equorum* and *Streptococcus entericus* for Gram-positive bacteria. Furthermore *Yarrowia lipolytica* and *Candida glabrata* for yeasts, as well as the fungi *Aspergillus niger* and *Hyphopichia burtonii*. For the agar diffusion test, these organisms are seeded on appropriate agar plates. Tiny platelets containing either the synthesized compounds or **tetracycline** as reference for

antibacterial potency and **clotrimazole** as reference for antifungal potency are added to the plate (Figure 3.3).

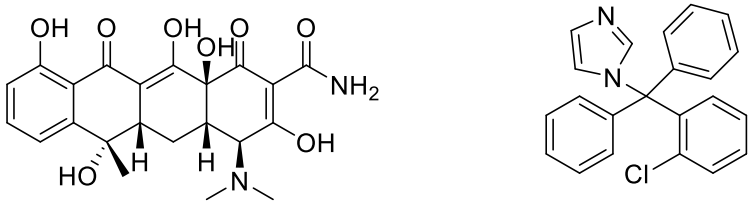
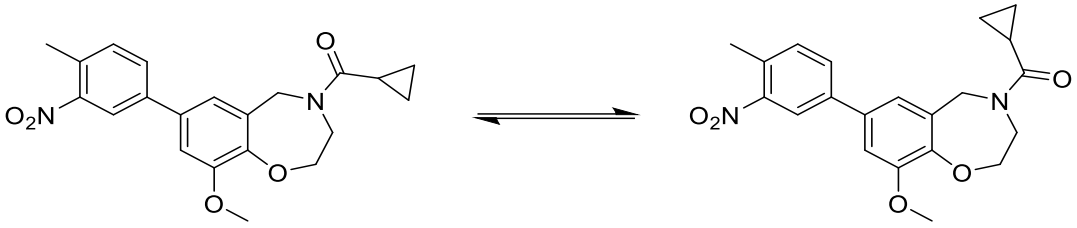


Figure 3.3. Reference substances tetracyline (left-hand) and clotrimazole (right-hand).

After incubation circular growth inhibition is measured around the reference plateletes and the diameter of growth inhibition is compared to the diameter of colony free areas arround the plateletes with the synthesized compounds. This test allows just a rough estimate of the antibacterial and antifungal potency of novel compounds, but is well enough to detect possible new lead structures.

3.8 High-temperature NMR

Recently scale-up experiments for the preparation of a kinase inhibitor with 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold were published. This mTOR inhibitor bears an amide function at N-4, and two rotational isomers were observed in ^{1}H and ^{13}C NMR spectra. Two separate signals for the isomeric protons (and carbon atoms) and thus a double set of signals were found. This mTOR inhibitor was characterized at room temperature and the ratio of the two isomers could be deducted from comparison of their integrals[51c], although this ratio may be different in other solvents or the cellular medium. This well-known phenomenon[80] is caused by a hindered rotation about the amide bond. It was observed in all synthesized CBP inhibitors of this work, which bear an amide, thioamide, carbamate, or thiourea function at N-4, for example in amide **30** (Scheme 3.2).



Scheme 3.2. The two rotational isomers of synthesized compound **30**.

For some of these compounds NMR spectra were recorded at a high temperature of 100 °C (in DMSO-d_6) or 110 °C (in $C_2D_2Cl_4$). At these temperatures sufficient energy is available to smoothly overcome the energy barrier between the two isomers and a coalescence of the NMR signals was observed. The ^{1}H NMR spectra of **30** in $C_2D_2Cl_4$ at room temperature and at 110 °C can be compared in Figure 3.4.

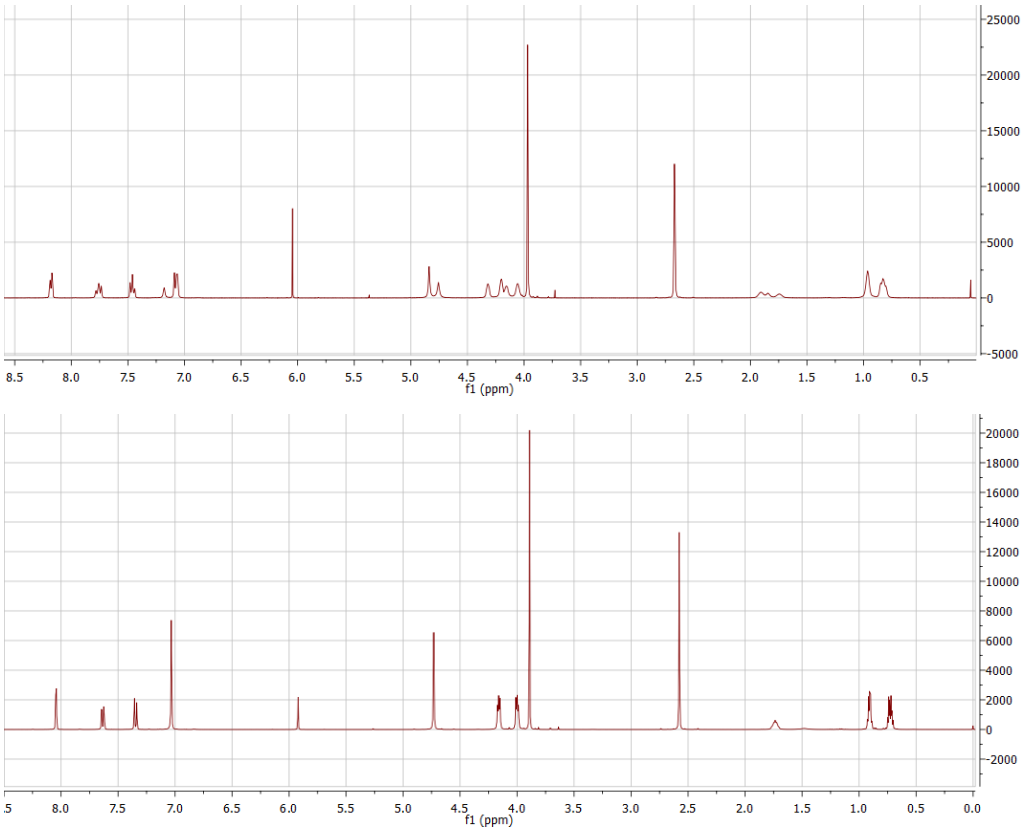


Figure 3.4. ^{1}H NMR spectra of amide **30** at room temperature (top panel) and 110 °C (bottom panel).

For thioamide **31** the energy barrier was particularly high, with the signals from the methylene groups between 4.0 and 5.5 ppm not even coalescing at 110 °C (Figure

3.5). The carbon-nitrogen bond in thioamides shows an increased double bond character compared to amides.[81] Although energy barriers differ from solvent to solvent, generally higher energy barriers for signal coalescence for thioamides (81 – 103 kJ/mol) than for amides (66 – 88 kJ/mol) are described in literature.[82]

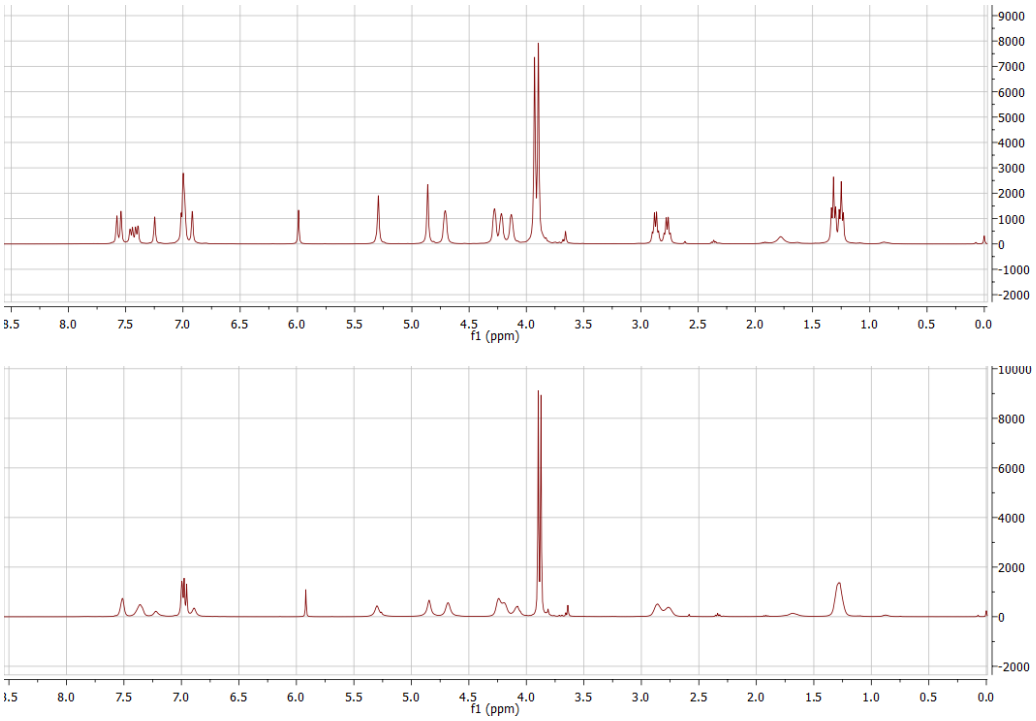


Figure 3.5. ^{1}H NMR spectra of thioamide **31** at room temperature (top panel) and 110 °C (bottom panel).

In 2014 Opatz *et al.* described two very stable rotational isomers of a tetrahydroisoquinoline derivative with formamide function: They could even be chromatographically separated and equilibrium was restored very slowly at 20 °C. Even at 150 °C no signs of the onset of coalescence of the formyl proton resonances were found. The energy barrier for the transformation of these isomers was found to be approximately 90 kJ/mol.[83] Rotational isomers are also important in medicinal chemistry. Only one of the two conformers may bind to the target and ΔG will be smaller for the inhibitor-protein complex, since conversion of the inactive into the active isomer requires energy and entropy is lost by forcing the molecule into one conformation. In the course of the development of HIV-1 integrase inhibitor L-870810, approximately 21 kJ/mol are reported for overcoming of the rotational barrier of a corresponding amide bond. The conformation of the inactive form was favoured and the net difference

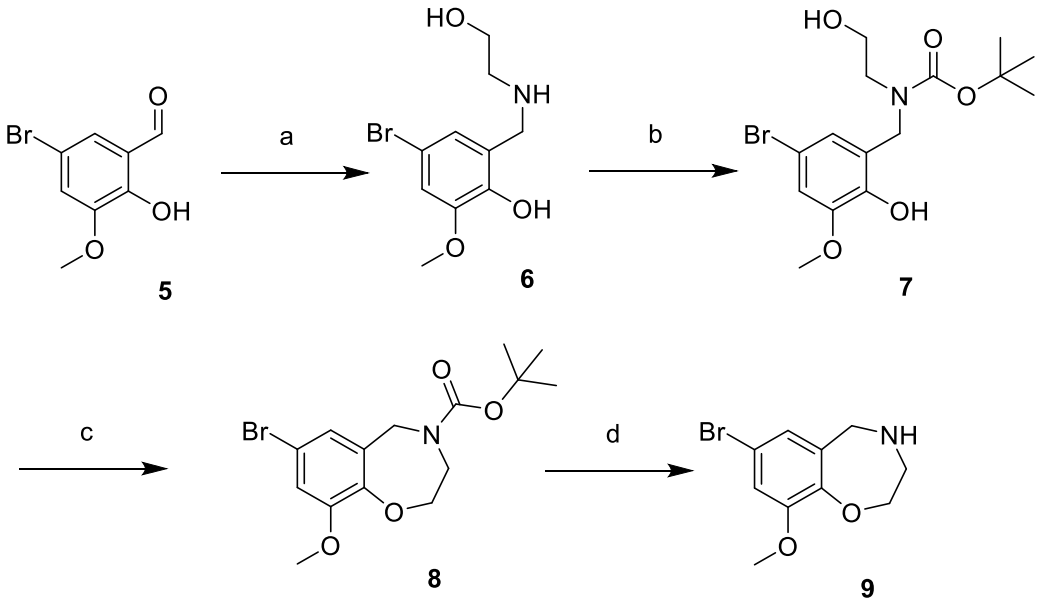
between inactive and active form was + 7.1 kJ/mol only, however a 29-fold increase in the potency (by IC_{50}) was accomplished through rigidification (lactamization) of model compounds.[84] CBP inhibitors with rigid or swiftly rotatable binding elements like CBP30[85] do not pay a comparable energy penalty and the occurance of rotameric isomers may be a limitation of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold. However **I-CBP112** is already a potent lead structure with high affinity and its scaffold is certainly worth further refinement without giving up its 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold and without adapting rigid scaffolds known from many other bromodomain inhibitors.

Chapter IV – Synthesis, results & discussion

4.1 The 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with methyl ether at C-9

4.1.1 Synthesis of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with methyl ether at C-9

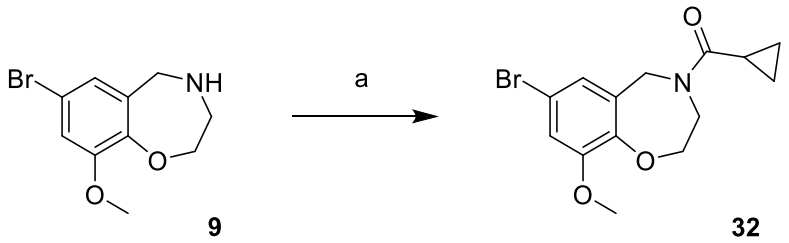
Preparation of scaffold **9** was accomplished as planned, following the established approach to the 2,3,4,5-tetrahydro-1,4-benzoxazepine backbone[51b, 51c], but starting with 5-bromo-2-hydroxy-3-methoxybenzaldehyde (**5**, Scheme 4.1) instead of 5-bromo-2-hydroxybenzaldehyde. Reductive amination of 5-bromo-2-hydroxy-3-methoxybenzaldehyde (**5**) with 2-aminoethanol and $NaBH_4$ in THF/MeOH gave secondary amine **6** in 93 % yield. After Boc-protection of the amino group, ring closure of phenol **7** to the benzoxazepine **8** was performed in 91 % yield using the Mitsunobu reagents DIAD and triphenylphosphine. Acidic *N*-deprotection gave an almost quantitative yield of the 1,4-benzoxazepine **9**. The central building block **9** could thus be synthesized within few steps in large scale. This was important, because plenty variations were planned.



Scheme 4.1. Synthesis of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with methyl ether at C-9. Reagents and conditions: (a) 2-aminoethanol, $NaBH_4$, THF, MeOH, rt, 93 %; (b) di-*tert*-butyl dicarbonate, $NaHCO_3$ solution, EtOAc, rt, 69 %; (c) PPh_3, DIAD, DCM, rt, 91 %; (d) HCl, 1,4-dioxane, MeOH, reflux, 94 %.

4.1.2 Preparation of compounds for the optimization of the substituent at C-7

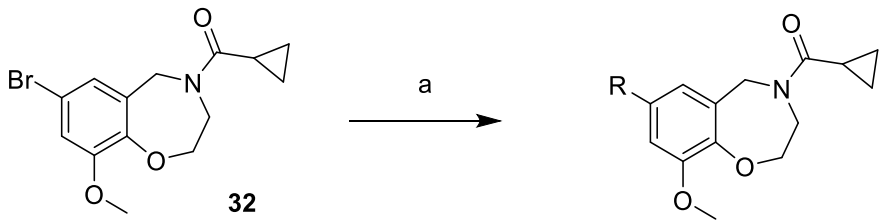
First, for the assessment of the best moiety at C-7 precursor **32** was prepared by *N*-acylation (Scheme 4.2). The cyclopropanecarboxamide moiety was selected as it was among the most promising residues in the set of purchased, prescreened compounds.



9 **32**

Scheme 4.2. Preparation of intermediate **32**. Reagents and conditions: (a) cyclopropanecarbonyl chloride, DIPEA, DCM, 0 °C - rt, 71 %.

Suzuki cross-coupling[51b] of aryl bromide **32** with various boronic acids and boronic acid pinacol esters under Pd(dppf)Cl_2 catalysis gave the biaryls **30**, and **33** - **46** (Scheme 4.3).



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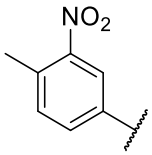
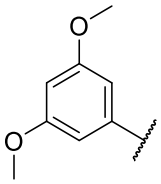
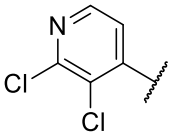
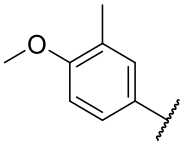
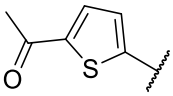
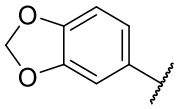
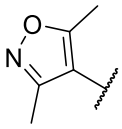
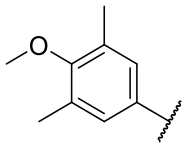
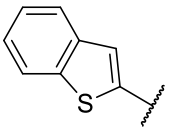
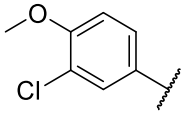
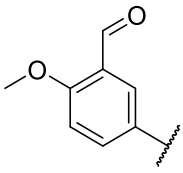
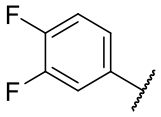
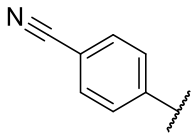
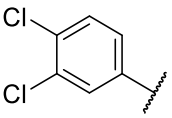
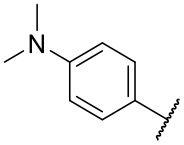
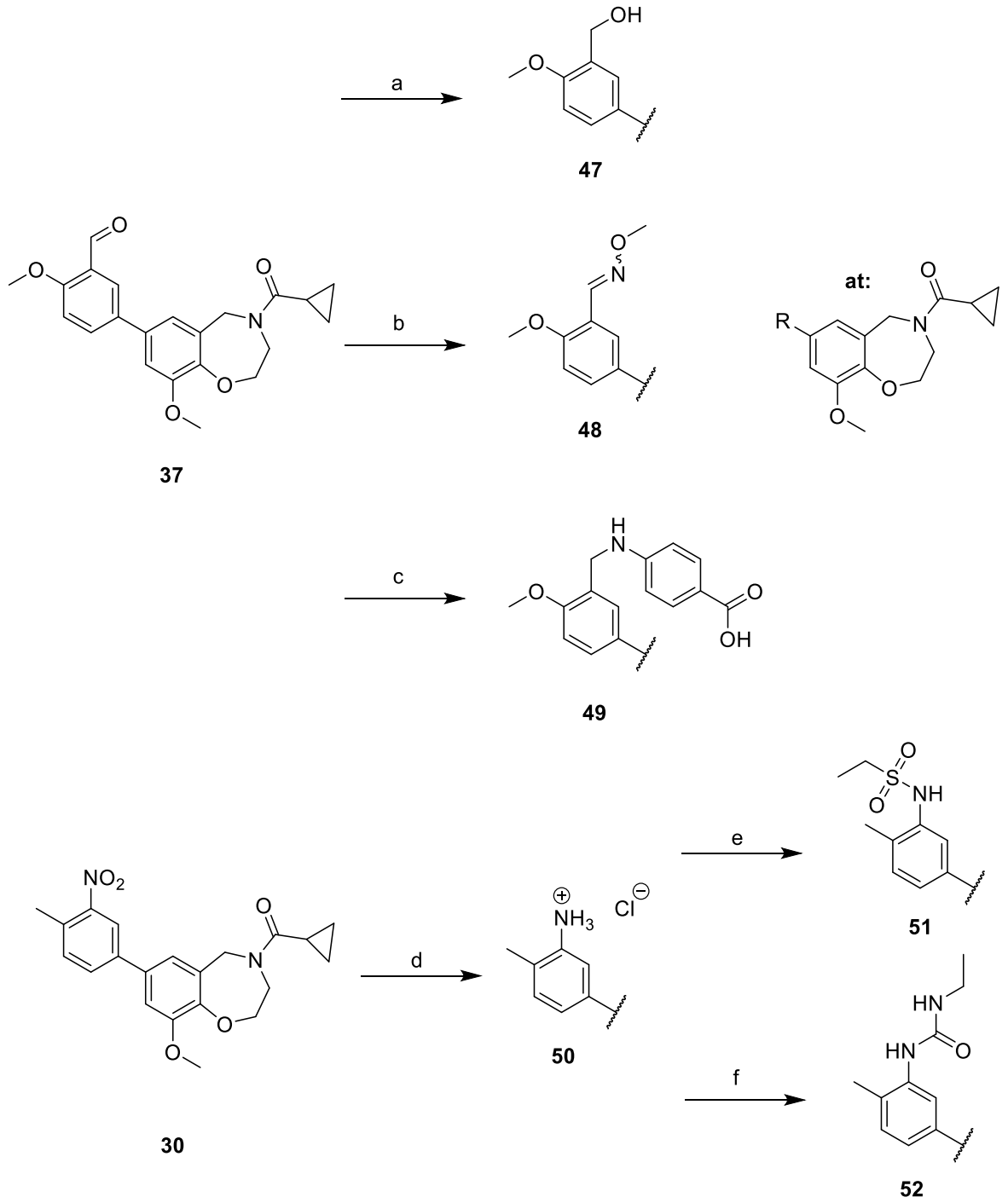
	**30[a]**		**40[a]**
	**33[a]**		**41[a]**
	**34[a]**		**42[a]**
	**35[b]**		**43[a]**
	**36[a]**		**44[a]**
	**37[a]**		**45[a]**
	**38[a]**		**46[a]**
	**39[a]**		

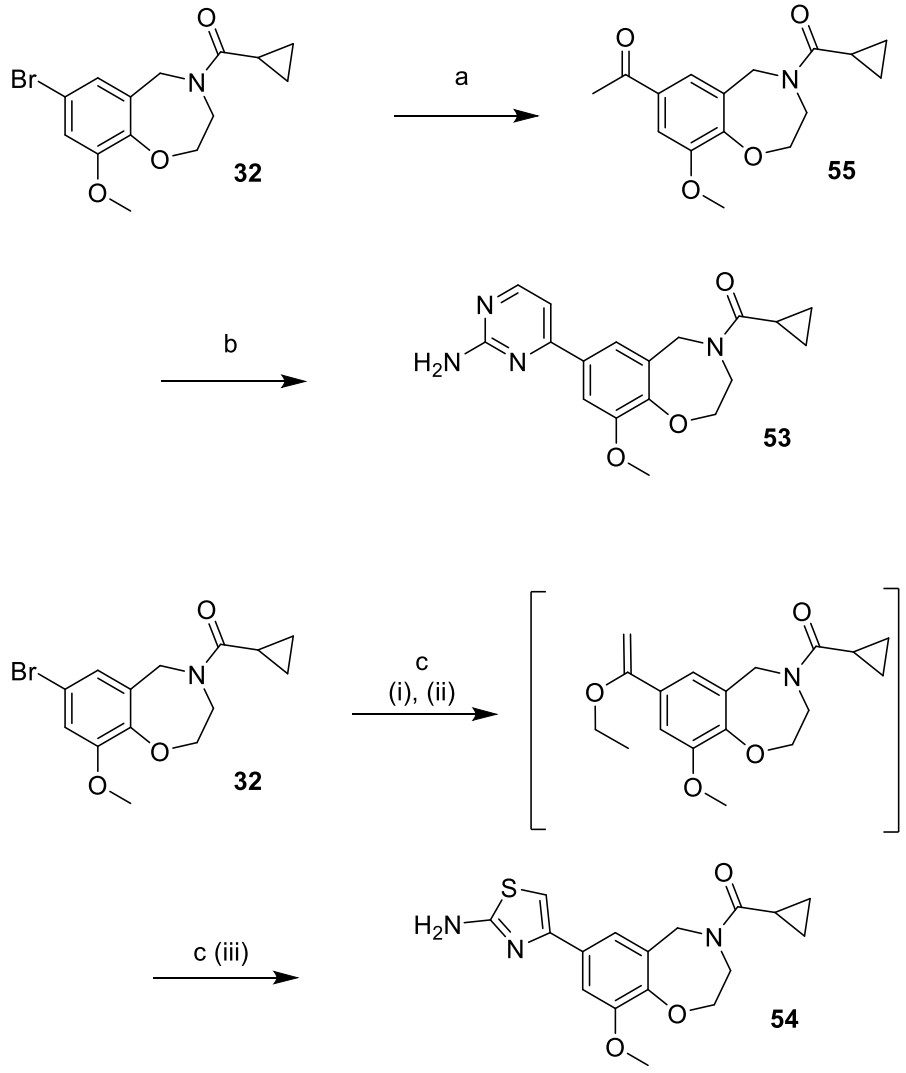
Scheme 4.3. Preparation of compounds from intermediate **32**. Reagents and conditions: (a) various boronic acids[a] or boronic pinacol ester[b], Pd(dppf)Cl_2 x DCM, DIPEA, H_2O/1,4-dioxane, 95 °C, 31 – 94 %.

Additionally, the aromatic aldehyde **37** and the nitrophenyl compound **30** were also used as intermediates for further modifications (Scheme 4.4). The aromatic aldehyde **37** was reduced to the benzyl alcohol **47** using $NaBH_4$ with 97 % yield, and converted quantitatively with *O*-methylhydroxylamine into the *O*-methyloxime **48**. A reductive amination with 4-aminobenzoic acid and $NaCNBH_3$ gave the N-aryl compound **49** in good yield. The nitrophenyl compound **30** was reduced in a transfer hydrogenation to the corresponding aniline **50** using Raney-nickel and hydrazine in 38 % yield. This aniline was further functionalized with mediocre yields into the sulfonamide **51** using ethanesulfonyl chloride and the urea derivative **52** using ethyl isocyanate.



Scheme 4.4. Preparation of 2,3,4,5-tetrahydro-1,4-benzoxazepine inhibitors with different moieties at C-7. Reagents and conditions: (a) $NaBH_4$, DCM, MeOH, rt, 97 %; (b) *O*-methylhydroxylamine, K_2CO_3, EtOH, rt, 99 %; (c) 4-aminobenzoic acid, $NaCNBH_3$, DCM, MeOH, rt, 80 %; (d) Raney-nickel, N_2H_4, EtOH, reflux, 38 %; (e) ethanesulfonyl chloride, DMAP, pyridine, 0 °C - rt, 60 %; (f) ethyl isocyanate, DIPEA, DCM, rt, 47 %.

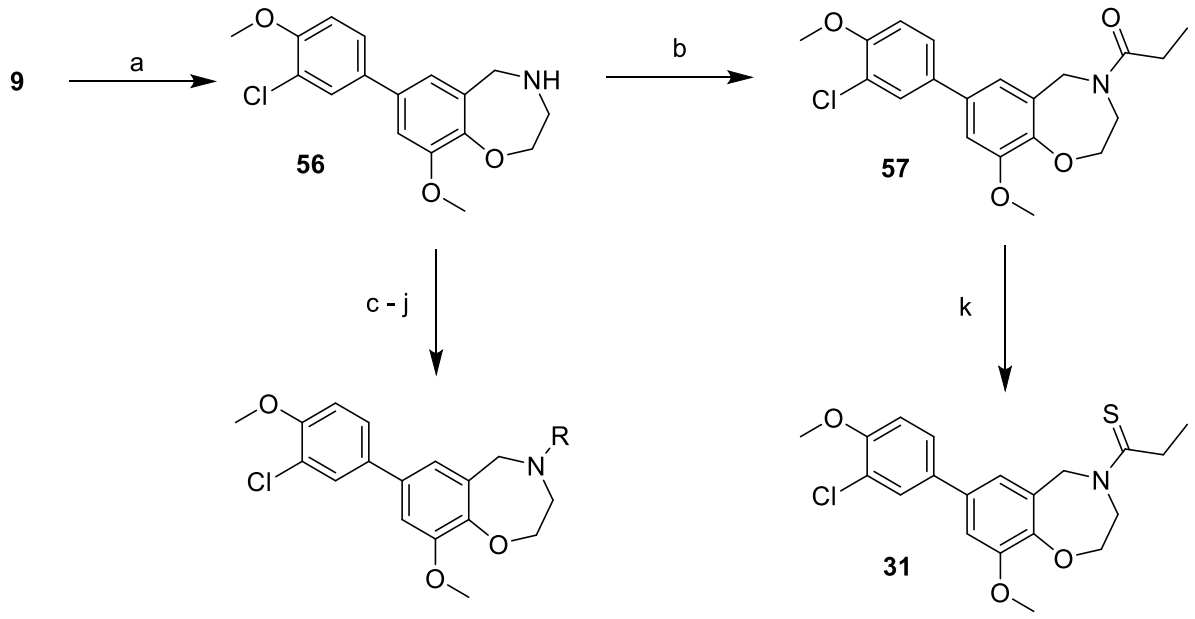
We also strived for two compounds that were not accessible with boronic acids: Aminopyrimidine **53** and aminothiazole **54**. Both compounds could be synthesized in several steps starting with Stille cross-coupling and different aqueous workups. Stille cross-coupling of **32** with tributyl(1-ethoxyvinyl)tin under $Pd(PPh_3)_2Cl_2$ catalysis (Scheme 4.5) followed by acidic workup[86] gave the methyl ketone **55** in 67 % yield. This was further converted into the aminopyrimidine **53** in 57 %, using Bredereck's reagent and guanidinium carbonate[87]. The same Stille coupling of **32** with tributyl(1-ethoxyvinyl)tin, but with neutral aqueous workup gave an unstable enolether in 71 % yield. After purification by flash column chromatography, this intermediate was treated with NBS giving a bromoacetyl intermediate. Aqueous workup allowed cyclization with thiourea[88] and conversion into the aminothiazole **54** (20 % yield over three steps).

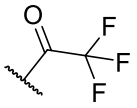
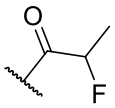
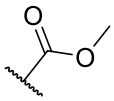
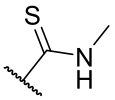
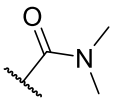
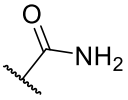
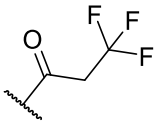
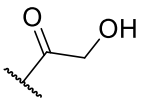


Scheme 4.5. Preparation of 2,3,4,5-tetrahydro-1,4-benzoxazepine inhibitors with different moieties at C-7. Reagents and conditions: (a) (i) $Pd(PPh_3)_2Cl_2$, tributyl(1-ethoxyvinyl)tin, 1,4-dioxane, 140 °C, (ii) 2.7 M HCl_{aq}, rt, 67 %; (b) Bredereck's reagent, DMF, 160 °C, then guanidinium carbonate, K_2CO_3, DMF, 160 °C, 57 %; (c) (i) $Pd(PPh_3)_2Cl_2$, tributyl(1-ethoxyvinyl)tin, 1,4-dioxane, 140 °C, (ii) NBS, THF, H_2O, 0 °C - rt; (iii) thiourea, DMF, rt, 20 % overall yield.

4.1.3 Preparation of compounds for the optimization of the residue at N-4

Next, we turned to derivatisations of N-4. This evaluation of the best moiety at the nitrogen at position 4 was performed with a 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold bearing a 3-chloro-4-methoxyphenyl substituent at C-7, since at that time this substitution pattern looked most promising. Intermediate **9** (Scheme 4.2) was subjected to a Suzuki cross-coupling reaction[51b] with 3-chloro-4-methoxyphenylboronic acid to give biaryl **56** in 48 % yield (Scheme 4.6). Treatment of the secondary amine **56** with acyl chlorides or carboxylic acid anhydrides gave the amides **57**, **58**, carbamate **59**, and urea **60**. EDC as coupling reagent and appropriate carboxylic acids were used to obtain amides **61** and **62**. (Thio)ureas **63** and **64** were accessible through iso(thio)cyanates, and glycolic acid amide **65** was obtained in acceptable yield through transamidation with neat ethyl glycolate. Finally, Lawesson's reagent was used to convert carboxamide **57** into the corresponding thioamide **31**. We thus obtained a neat set of compounds with very small residues at N-4, and several functional groups that had not been tried for CBP inhibition before. The DSF values obtained from the screening of purchased compounds suggested that no groups much larger than a propionyl or a cyclopropanecarboxamide moiety do fit into the end of the bromodomain binding pocket. Other larger residues may simply be too bulky to fit into the pocket, or the substitution of the conserved water molecules at the pocket's end (see Figure 4.8 in Chapter 4.4.5.4), which form essential hydrogen bonds for the recognition of the N-acyl function, is energetically not favoured. The residues in our systematic investigation ranged from very polar (**59**, **63**, **64**, **65**) to less polar (**31**, **57**, **58**, **61**, **62**). Some of them offered the option for creating additional hydrogen bonds to the conserved water molecules in the pocket, but would not be protonated under physiological conditions, thus avoiding an unfavored hydration shell.



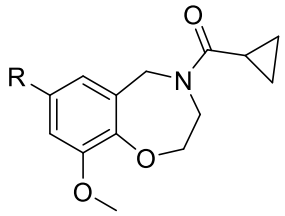
procedure	R	No.	procedure	R	No.
c		**58**	g		**62**
d		**59**	h		**63**
e		**60**	i		**64**
f		**61**	f		**65**

Scheme 4.6. Functionalizations at N-4. Reagents and conditions: (a) 3-chloro-4-methoxyphenylboronic acid, Pd(dppf)Cl_2 x DCM, DIPEA, H_2O/1,4-dioxane, 95 °C, 48 %; (b) propionyl chloride, DIPEA, DCM, 0 °C - rt, 40 %; (c) trifluoroacetic anhydride, DIPEA, DMAP, DCM, 0 °C - rt, 92 %; (d) methyl chloroformate, DIPEA, DCM, 0 °C - rt, 94 %; (e) *N*,*N*-dimethylcarbamoyl chloride, DIPEA, DCM, 0 °C - rt, 74 %; (f) 3,3,3-trifluoropropionic acid, DMAP, EDC x HCl, DCM, 0 °C - rt, 94 %; (g) (±)-2-fluoropropionic acid, DMAP, EDC x HCl, 0 °C - rt, 81 %; (h) NaH, THF, then methyl isothiocyanate, rt, 69 %; (i) (trimethylsilyl)isocyanate, DCM, then HCl in 1,4-dioxane, rt, 87 %; (j) ethyl glycolate, 60 °C, 51 %; (k) Lawesson's reagent, THF, rt, 96 %.

4.1.4 Screening results of compounds from chapters 4.1.2 and 4.1.3

Most of the compounds from these batches were analysed by DSF, the only exception being aminobenzoic acid **49** (Scheme 4.4), which had been prepared by reductive amination from aromatic aldehyde **37**. We were hoping to install a ionic interaction between aminobenzoic acid moiety in **49** and remote arginine 1112 or to form at least a hydrogen bond. **49** was analysed by Alphascreen and the determined value of 5.2 µM was disappointing (compare: 170 nM for **I-CBP112**), so this derivatization was not further considered. The results of the DSF screening of the variations at C-7 are displayed in Table 4.1. All compounds were screened for binding to CBP and exemplarily to BRD4(1), to estimate selectivity over the BET-family. Only a few substances were also tested on p300, since it was not expected to achieve notable selectivity between CBP and p300.

Most of the heteroaromatic variations at C-7 (**33**, **34**, **36**, **53**, **54**) gave only very poorly active compounds. Only electron-rich isoxazole **35** gave a good result. Upon replacement of the phenyl moiety at C-7 by an acetyl moiety (**55**) potency was completely lost. While potency decreased for some compounds bearing electron-deficient phenyl moieties at C-7 (**37**, **38**), the electron-deficient nitrophenyl compound **30** still showed mediocre activity. The electron-rich aminophenyl derivatives **39** and **50** showed similar potency, whereas modifications of the amino group (sulfonamide **51**, urea **52**) led to increased potency. The sulfonamide and the urea moiety were introduced as they are flexible hydrogen bond acceptors and donors, but the outcome of these modifications was not very satisfactory. Regarding the compounds with electron-rich phenyl moiety, **40** with a 3,5-dimethoxyphenyl group was the most potent compound in this series, while related compounds **41**, **42**, **43**, **44**, and **47** still showed acceptable to good potency on CBP. *O*-Methyl benzaldoxime **48** gave the second best result.



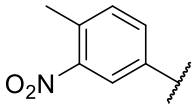
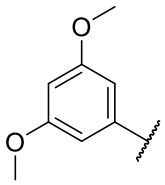
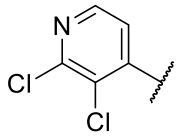
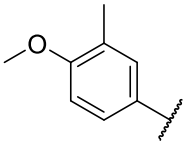
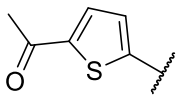
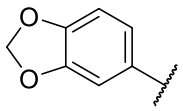
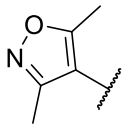
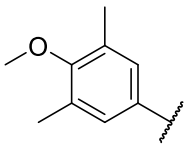
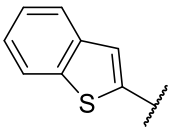
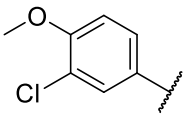
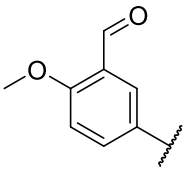
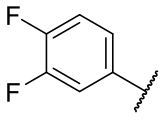
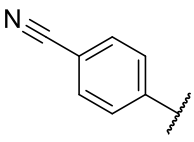
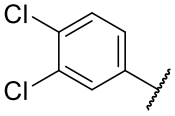
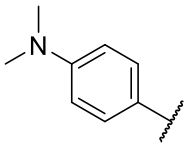
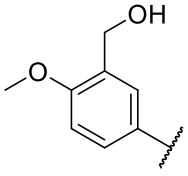
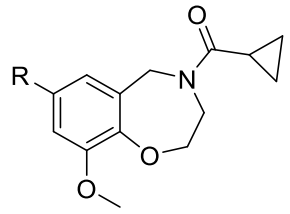
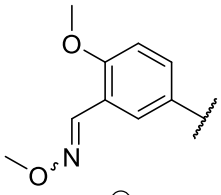
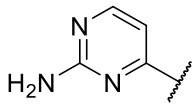
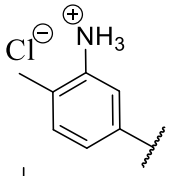
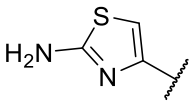
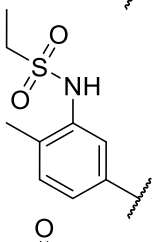
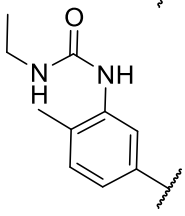
R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)	R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)
	**30**	3.4 (±0.6)	1.5 (±0.3)		**40**	6.7 (±0.9)	4.5 (±0.8)
	**33**	1.7 (±0.3)	1.1 (±0.1)		**41**	4.8 (±0.4)	3.0 (±0.5)
	**34**	2.3 (±0.2)	2.7 (±0.4)		**42**	3.9 (±0.4)	1.6 (±1.2)
	**35**	4.9 (±1.2)	4.1 (±0.7)		**43**	4.5 (±0.6)	2.6 (±0.7)
	**36**	1.5 (±0.9)	1.8 (±0.2)		**44**	4.4 (±0.4)	3.3 (±0.3)
	**37**	1.5 (±2.6)	1.2 (±1.6)		**45**	3.5 (±0.9)	2.6 (±0.5)
	**38**	2.3 (±0.3)	1.9 (±0.3)		**46**	2.7 (±0.9)	2.2 (±1.3)
	**39**	3.3 (±0.2)	2.1 (±0.8)		**47**	4.4 (±0.9)	2.3 (±0.3)

Table 4.1. DSF results for compounds with different moieties at C-7 (n = 3). Continued on the next page.

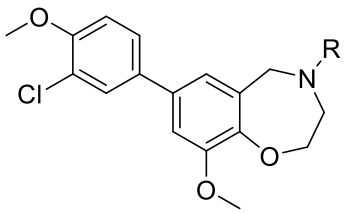


R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)	R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)
	**48**	6.0 (±0.7)	2.5 (±0.6)		**53**	0.3 (±0.2)	0.6 (±0.2)
	**50**	3.1 (±0.6)	2.5 (±0.7)		**54**	2.1 (±0.1)	1.0 (±0.2)
	**51**	4.6 (±0.9)	3.6 (±0.5)		**55**	0.3 (±0.6)	0.1 (±0.3)
	**52**	4.3 (±1.6)	1.5 (±0.9)				

Continutation of Table 4.1. DSF results for compounds with different moieties at C-7 (n = 3).

Regarding variations at N-4, we aimed at using the network of essential, conserved water molecules at the end of the pocket for the formation of new hydrogen bonds between CBP and the new, more or less polar moieties at N-4 of the inhibitor. The outcome is shown in Table 4.2. It is noteworthy that the polar and small ureas **60** and **64** were the only N4-derivatives (except for the sulfonamides, chapter 4.3.3), for which no rotameric isomers were observed in the NMR spectra, hence their poor performance in the DSF screening was particularly disappointing. Due to its capability to act as hydrogen bond acceptor and donator, one of the most promising candidates was the polar, glycolic amide **65**. However this compound was totally inactive. Thiourea **63**, and thioamide **31** showed poor activity, too. The greater van der Waals radius of

sulfur (1.80 Å) compared to oxygen (1.52 Å) and hence steric repulsion could account for inactivity of **31**, but with **63** being somewhat potent, another explanation is more likely: The double bond character of the carbon-nitrogen bond is increased in thioamides[81], and the sulfur in thioamides is a weaker hydrogen bond acceptor than the oxygen of amides.[89] Residual potency of thiourea **63** could result from hydrogen bond donor activity of the NH-group. The introduction of sulfur was not only devastating for the affinity to CBP, but potency to BRD4(1) was not reduced to the same extent (at least for **63**). We had accepted to sacrifice some potency for increased selectivity, but in this case selectivity even decreased. With the thioanalogs and the quite polar moieties having failed, we now aimed at the introduction of less polar, fluorinated residues (compounds **58**, **61**, **62**). The SGC had found that the propionyl moiety at N-4 did fit into the binding pocket, while replacement with an acetyl moiety or a considerably larger moiety than propionyl results in complete inactivity. The trifluoroacetyl moiety of compound **58** is estimated of similar size than the propionyl moiety, but of different geometry and polarization.[90] The trifluoropropionyl moiety of **61** was hoped to fit better into the binding pocket than the propionyl moiety, without being repelled by the conserved, essential water molecules. Monofluorination in α-position of an amide can also affect amide conformation. This effect is particularly strong in amides formed from primary amines, but dipole−dipole interaction between the C−F and C=O bonds should also occur in amides formed from secondary amines.[91] With the conformation of the amide being important for CBP binding, we were hoping for improved binding of compound **62**. The existence and significance of fluorine-hydrogen bonds are still under discussion.[91] In this case all three fluorinated compounds were inactive. Finally, we found that still the propionyl residue (**57**) is most favourable with regard to potency and selectivity, and only the cyclopropanoyl (**44**) and the more polar methyl carbamate (**59**) are nearly as active.



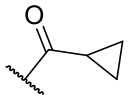
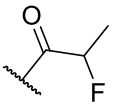
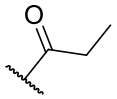
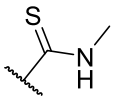
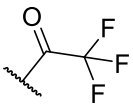
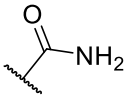
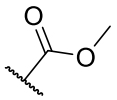
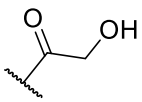
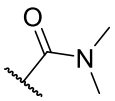
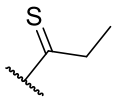
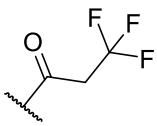
R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)	R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)
	**44**	4.4 (±0.4)	3.3 (±0.3)		**62**	0.5 (±1.4)	0.8 (±0.3)
	**57**	6.0 (±0.1)	2.5 (±0.1)		**63**	3.4 (±0.2)	2.6 (±0.5)
	**58**	0.2 (±0.8)	-0.1 (±0.8)		**64**	0.7 (±0.1)	3.6 (±0.6)
	**59**	4.3 (±1.8)	3.9 (±0.4)		**65**	0.0 (±0.1)	1.4 (±0.3)
	**60**	0.2 (±0.2)	1.5 (±0.1)		**31**	0.7 (±0.6)	0.3 (±0.5)
	**61**	-0.1 (±0.1)	0.5 (±0.1)				

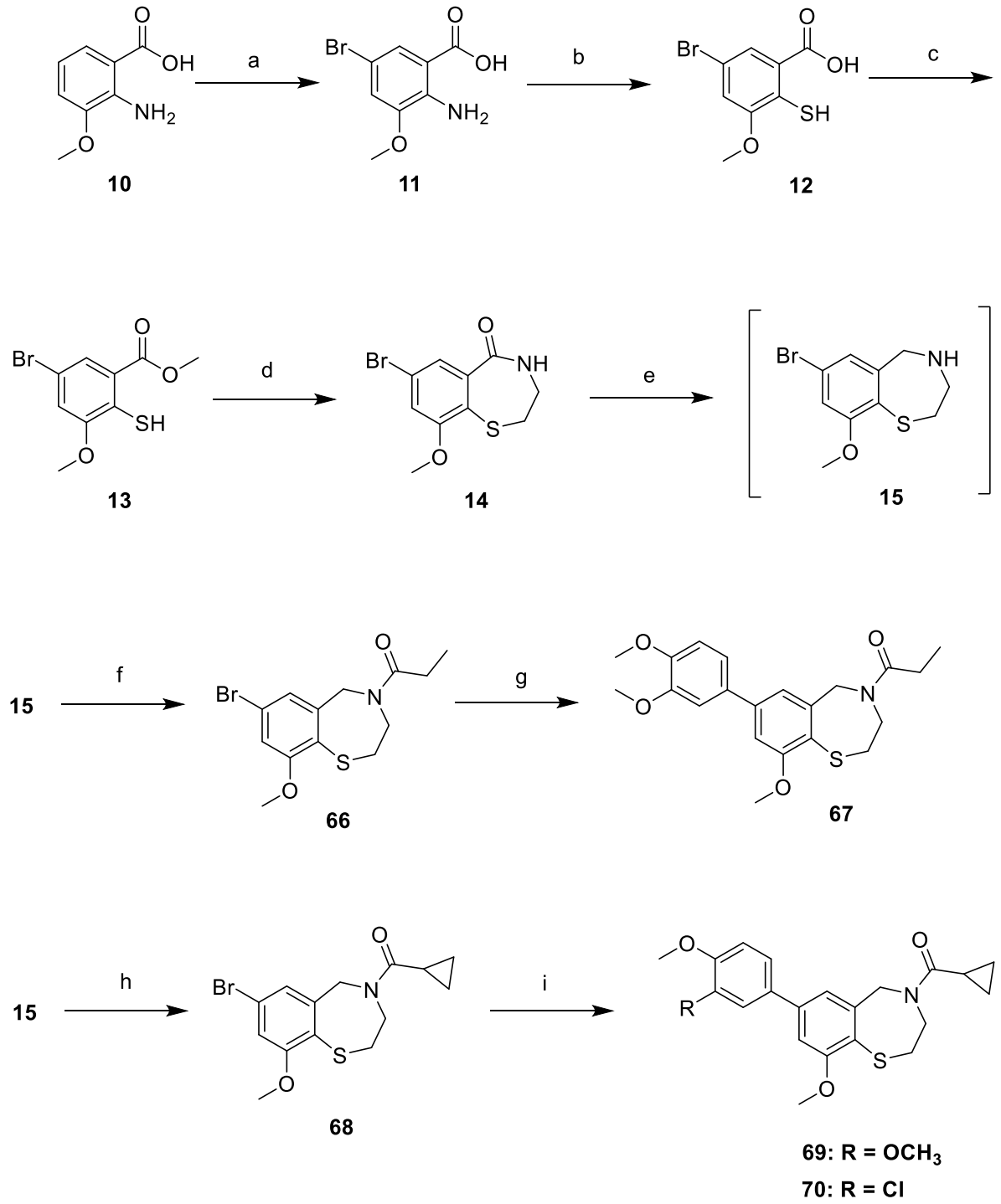
Table 4.2. DSF results for compounds with different *N*-acyl residues (n = 3).

Having further explored these two edges of the molecule, we next turned to the backbone itself through substitution of the oxygen at position 1 by nitrogen or sulfur. We were hoping that the benzothiazepine would act as potent and more selective scaffold. A secondary amine at position 1 of the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold could act as hydrogen bond acceptor and donor. Alternatively additional residues could be introduced by *N*-alkylation at this amino group.

4.2 The 2,3,4,5-tetrahydro-1,4-benzothiazepine scaffold

4.2.1 Synthesis of the 2,3,4,5-tetrahydro-1,4-benzothiazepine scaffold and compounds

The preparation of the 2,3,4,5-tetrahydro-1,4-benzothiazepine scaffold generally went as planned, but consisted of a few labour-intensive reactions. Following quantitative and regioselective bromination[53] of anthranilic acid **10**, conversion[54] of anthranilic acid **11** into mercaptobenzoic acid **12** was accomplished (Scheme 4.7). In this multistep reaction, first a diazonium group was generated from the aniline using sodium nitrite and aqueous hydrogen chloride and a temperature of 0 °C. Then at higher temperatures, nucleophilic substitution took place employing ethyl xanthate, and giving the *S*-arylxanthogenate. Finally alkaline hydrolysis gave thiol **12** with a yield of 62 %. Subsequent acidic esterification[54b] to **13** was accomplished with a satisfactory yield of 72 %. The next alkylation/transamidation reaction did not work as smoothly as described for methyl 2-mercapto-5-methoxy benzoate[55a]: The mere mixing of methyl 5-bromo-2-mercapto-3-methoxybenzoate, 2-chloroethylamine hydrochloride, and sodium methoxide in DMF at 0 °C and the stirring of this mixture overnight at room temperature, did not simply give benzothiazepinone **14**. Only *S*-alkylation was accomplished, but the lactamization had not taken place. To force lactamization, a different procedure[92] was adopted after aqueous workup. *t*BuOK was added to the crude aminoester and the mixture in THF was heated to 45 °C. Benzothiazepinone **14** was now obtained in 63 % yield. Lactam **14** was reduced with BH_3-THF, and the crude 1,4-benzothiazepine was directly acylated at N-4. To get a broader set of compounds, we used both cyclopropanecarbonyl chloride and propionyl chloride for the amidation and thus obtained the two intermediates **66** and **68**. Suzuki cross coupling[51b] was then applied and three exemplary 7-aryl-2,3,4,5-tetrahydro-1,4-benzothiazepines (**67, 69, 70**) were synthesized. The acyl chlorides and boronic acids used would undoubtly give potent inhibitors with the 1,4-benzoxazepine scaffold.



Scheme 4.7. Reagents and conditions: (a) Br_2, $CHCl_3$, 0 °C – rt, 99 %; (b) (i) $NaNO_2$, HCl, H_2O, 0 °C, (ii) KOAc, potassium ethyl xanthate, H_2O, 90 °C, (iii) NaOH, $NaHSO_3$, 85 °C, 62 %; (c) MeOH, H_2SO_4, reflux, 72 %; (d) (i) 2-chloroethylamine, NaOMe, DMF, 0 °C - rt, (ii) *t*BuOK, THF, 0 °C - 45 °C, 63 %; (e) BH_3-THF, -30 °C - reflux; (f) propionyl chloride, DIPEA, DCM, 0 °C - rt, 49 %; (g) 3,4-dimethoxyphenylboronic acid, Pd(dppf)Cl_2 x DCM, DIPEA, H_2O/1,4-dioxane, 95 °C, 59 %; (h) cyclopropanecarbonyl chloride, DIPEA, DCM, 0 °C - rt, 45 %; (i) 3,4-dimethoxyphenylboronic acid or 3-chloro-4-methoxyphenylboronic acid, Pd(dppf)Cl_2 x DCM, DIPEA, H_2O/1,4-dioxane, 95 °C, 85 % and 70 %.

4.2.2. Screening results

The prepared compounds were screened with DSF. The obtained T_m shifts can best be compared with those of benzoxazepine compounds **40** and **44** (Table 4.3). However the obtained T_m shifts are disappointing. Moreover selectivity over BRD4(1) remained unchanged at best. Consequently, this scaffold was discarded.

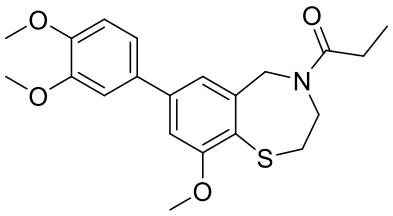
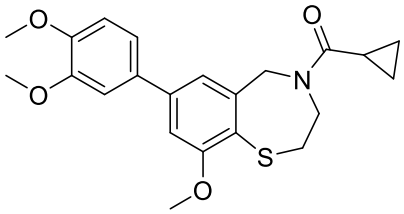
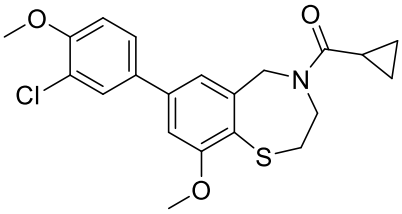
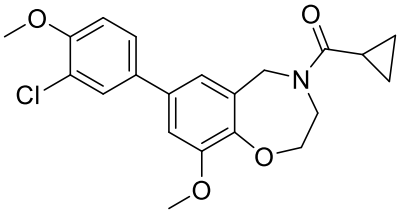
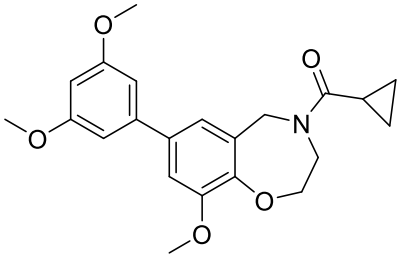
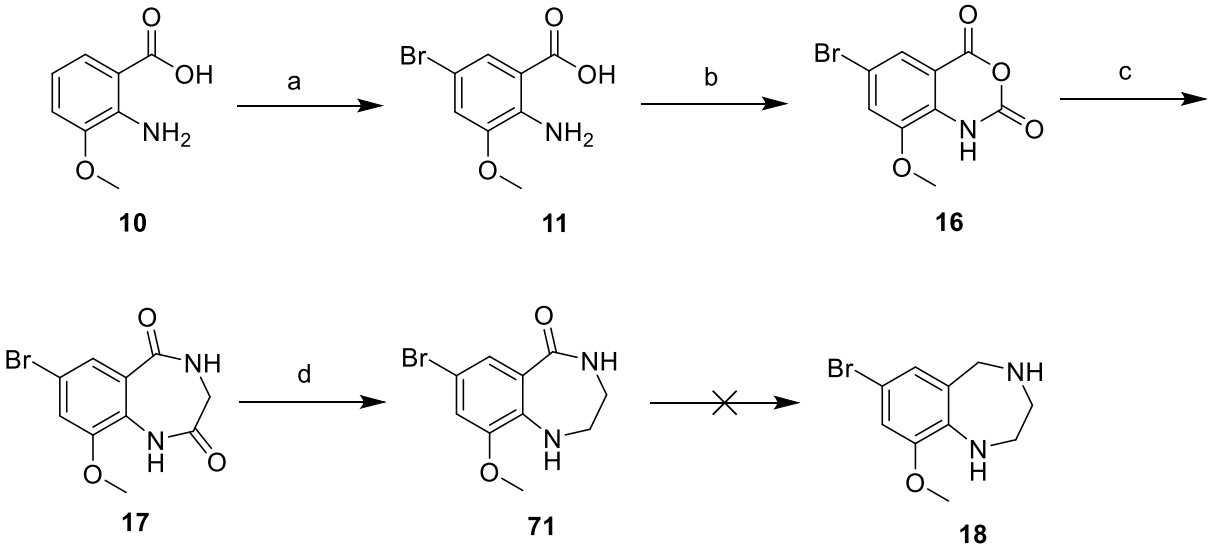
No.	Structure	ΔT_m (°C)
67		CBP: 4.2 (±0.3) BRD4(1):3.1 (±0.9)
69		CBP: 3.0 (±0.1) BRD4(1): 3.3 (±0.8)
70		CBP: 2.0 (±0.4) BRD4(1): 1.4 (±0.2)
44		CBP: 4.4 (±0.4) BRD4(1): 3.3 (±0.3)
40		CBP: 6.7 (±0.8) BRD4(1): 4.5 (±0.8)

Table 4.3. DSF results for 2,3,4,5-tetrahydro-1,4-benzothiazepine compounds (n = 3).

4.3 The 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold

4.3.1 Classic route via isatoic acid anhydride

To get an access to the desired benzodiazepine analogues, compound **18** was regarded as a suitable intermediate. But the initial attempt to obtain the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine **18** following classical approaches via the isatoic acid anhydride **16** was soon discarded (Scheme 4.8). Bromination of commercially available compound **10** was accomplished according to literature[53] in almost quantitative yield and as required earlier for the synthesis of the 1,4-benzothiazepines. The next steps were conducted as described in literature for similar compounds.[62] Activation of **11** with triphosgene to isatoic acid anhydride **16** and subsequent ring transformation with glycine worked well under the harsh conditions described. However, any attempts to reduce compound **17** by refluxing with BH_3-THF resulted in exclusive reduction of the carbonyl group at position 2 and thus conversion into vinylogous urea **71**. The same occured with $LiAlH_4$, and even harsher conditions didn't result in a completer reduction. Instead debromination at C-7 was observed and this reductive dehalogenation is a known problem with this reducing agent.[51c] To circumvent this debromination problem, of course we could have performed the Suzuki reaction with a suitable boronic acid first. But this had led to another problem: Only aryl residues bearing groups that are inert to these reducing agents and the drastic conditions had been applicable then. Thus we now aimed at a novel route to compound **18**.



Scheme 4.8. Discarded synthesis of the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold. Reagents and conditions: (a) Br_2, $CHCl_3$, 0 °C – rt, 99 %; (b) triphosgene, DIPEA, THF, reflux, 79 %; (c) glycine, AcOH, DMF, reflux, 72 %; (d) BH_3-THF, reflux, 73 %.

4.3.2 Novel route via *N*-nosylaziridine

This route was pursued by Edgar Uhl in his master thesis under my supervision. Since details of this approach and compound descriptions are given in his thesis and because this approach did not yield the target scaffold, just a short summary is given here:

Experiments were conducted with two different 2-aminobenzyl alcohols. Commercial 2-aminobenzyl alcohol (**72**) (Figure 4.1) was used for model experiments and known (2-amino-5-bromo-3-methoxyphenyl)methanol (**20**)[53] as educt for the actual scaffold. 1-[(2-Nitrophenyl)sulfonyl]aziridine ("*N*-nosylaziridine") was smoothly synthesized according to literature from 2-aminoethanol and 2-nitrobenzene-1-sulfonyl chloride in three steps.[93] For the Fukuyama type[65] Mitsunobu reaction[66] following upon the *N*-alkylation of the aminobenzyl alcohol, initially diisopropyl azodicarboxylate (DIAD) along with triphenylphosphine were used. Later di-*tert*-butyl azodicarboxylate (DTBAD)[94] and (cyanomethylene)trimethylphosphorane (CMMP)[95] were also tested.

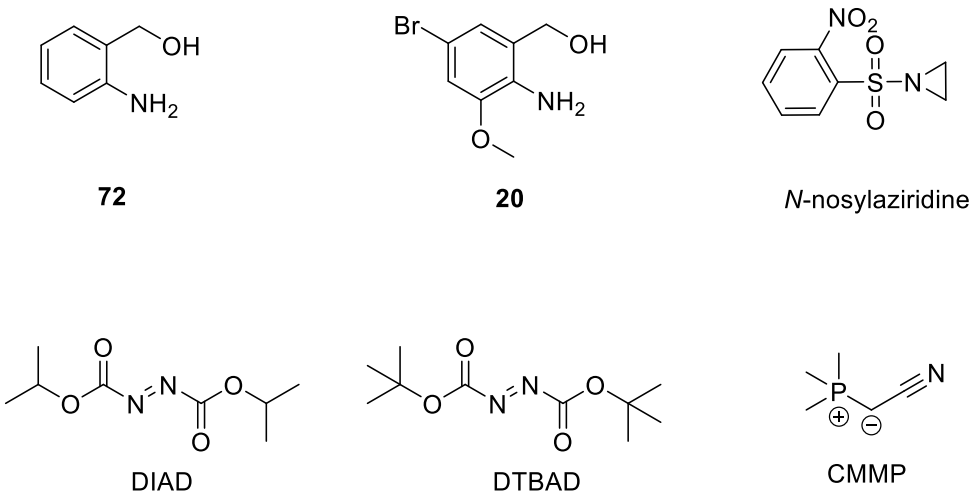
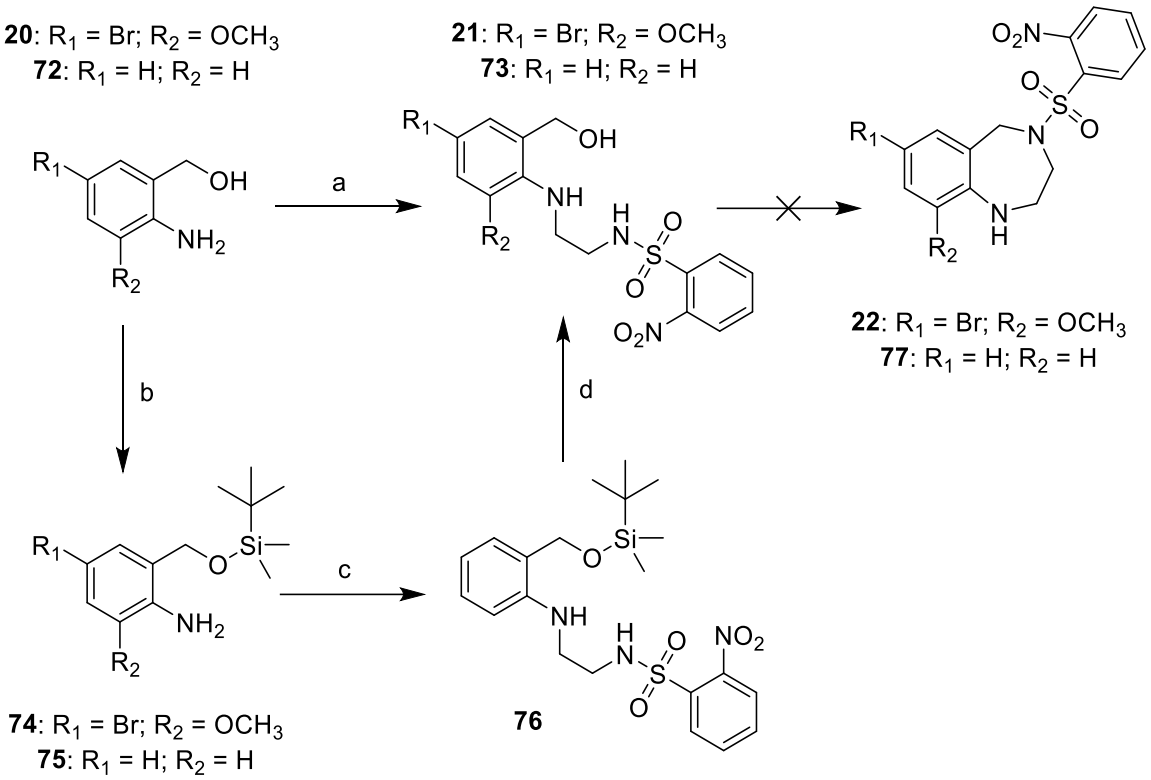


Figure 4.1. Common educts and reagents of the route via *N*-nosylaziridine.

Initial experiments showed that mixing of equimolar amounts of *N*-noslaziridine and aminobenzyl alcohol, aimed at nosylaminoethylation of the aniline, gave several side products and a very poor yield. The conducted experiments suggested that for a proper conversion, either a four-fold excess of the 2-aminobenzyl alcohol should be used (Scheme 4.9, a) or the alcohol function of the 2-aminobenzyl alcohol should be TBDMS-protected (Scheme 4.9, b). This would mean either low efficiency due to wasting large amounts of aminobenzyl alcohol or two additional synthetic steps (protection/deprotection of the alcohol).



Scheme 4.9. Innovative but unaccomplished route with *N*-nosylaziridine. Reagents and conditions: (a) *N*-nosylaziridine, DMF, rt, 46 % (**21**) / 94 % (**73**); (b) *tert*-butyldimethylsilyl chloride, imidazole, DMF, rt, 15 % (**74**) / 95 % (**75**); (c) *N*-nosylaziridine, rt, 93 %; (d) tetrabutylammonium fluoride, THF, rt, 91 %.

These problems were tolerable, but the next reaction posed new problems, that could not be overcome: Ring closure under Mitsunobu conditions was not observed, although many different reaction conditions (different orders of addition, various concentrations, molar ratios, and temperatures) and reagents (DIAD, DTBAD, CMMP) were applied. Only two byproducts could be isolated among many others: These suggested that reaction of the alcohol group with the Mitsunobu reagents may be favoured (Figure 4.2; left side: product from experiment with DIAD; right side: product from experiment with CMMP). DTBAD was then employed to facilitate workup but use of this sterically more hindered reagent resulted in no reaction at all. Consequently this approach was discarded as well.

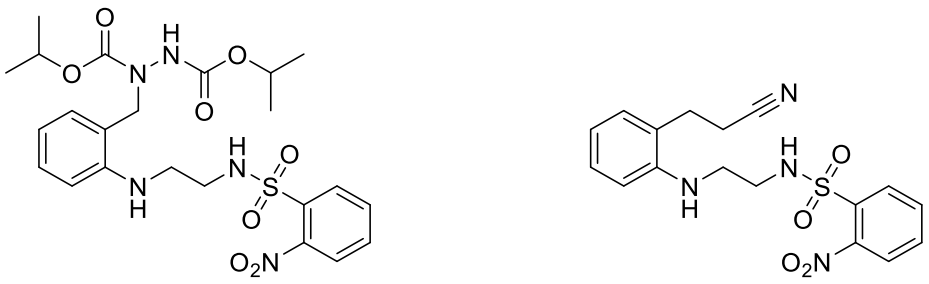


Figure 4.2. Left side: Product obtained by conversion of 2-aminobenzyl alcohol **72** with DIAD under Mitsunobu conditions; right side: product obtained from conversion of 2-aminobenzyl alcohol **72** with CMMP under Mitsunobu conditions.

4.3.3 A new approach to monoprotected 1,4-benzodiazepines *via* a one-pot *N*-deprotection/reductive cyclization procedure

With the previous innovative approach towards 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines having failed, we focussed on an alternative route using *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide[67] (Figure 4.3) as an alternative building block for introduction of C-2, C-3, and N-4 of the attempted 1,4-benzodiazepine scaffold. Initial experiments for this approach were also conducted under my auspices by Edgar Uhl for his master thesis. His experiments with model educt **72** yielded compounds **78**, **79**, **80**, **81**, and **95**. Moreover he applied his findings on (2-amino-5-bromo-3-methoxyphenyl)methanol (**20**) and prepared and characterized intermediates **23**, **24**, and **22**. For a completer picture of the story, his compounds are also shown in this chapter and the spectral data is displayed in the experimental section. They are also found in our joint paper "A new approach to monoprotected 1,4-benzodiazepines via a one-pot *N*-deprotection/reductive cyclization procedure"[96] or Uhl's master thesis.

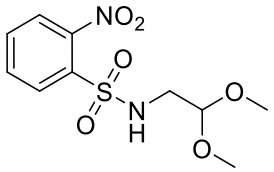
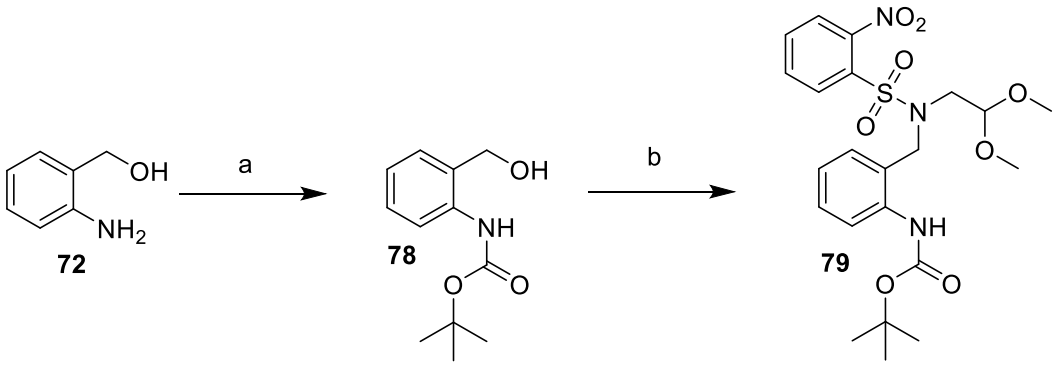


Figure 4.3. *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide

Again first model experiments were conducted with unsubstituted 2-aminobenzyl alcohol. It was attempted to perform a Fukuyama-type[65] Mitsunobu[66] reaction of *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide with unprotected 2-aminobenzyl alcohol (**72**), but an inseparable mixture of products was obtained. So the amino function of **72** was protected with the Boc group in almost quantitative yield[97] (Scheme 4.10). Using this intermediate **78** and after inspiration from an ultrasound-assisted protocol[98] Mitsunobu reaction proceeded well to give the desired product **79** in 50% yield.

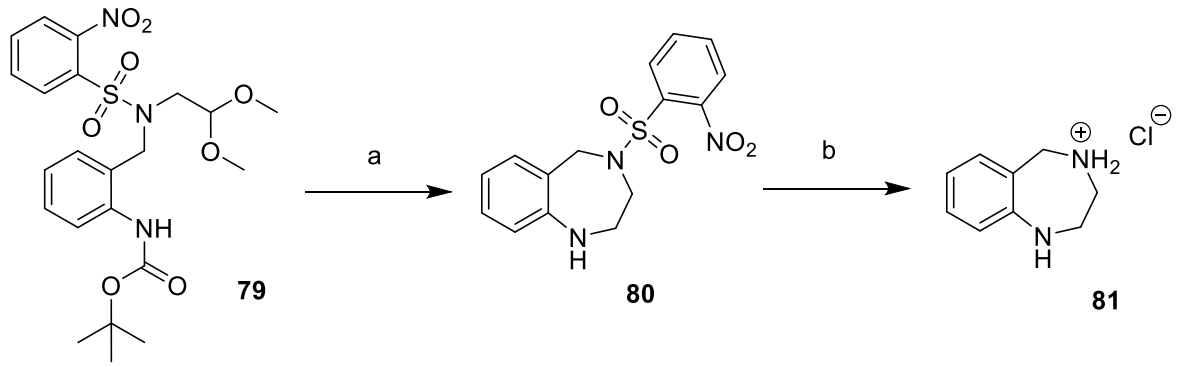


Scheme 4.10. Innovative approach to 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines using *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide, part I. Reagents and conditions: (a) di-tert-butyl dicarbonate, THF, 40 °C, 99 %; (b) PPh_3, DIAD, *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide, THF, rt, 50 %.

Having reversed the reaction order from the previous nosylaziridine approach and thus having accomplished the Mitsunobu reaction as first crucial step, we could now deal with the ring closure. Obviously a cyclization reaction with reductive amination protocol involving acetal and N-1 required cleavage of the *N*-Boc group first. For the construction of an annulated azepine, a lengthy three step deprotection/reductive amination protocol (*N*-deprotection with TFA, acetal hydrolysis with aqueous acid and spontaneous formation of a cyclic imine, reduction of the imine) has been described.[99] We considered to develop a one-pot procedure for *N*- deprotection and ring closure by reductive amination. This idea was boosted by the outcome of a preliminary experiment for acidic cleavage of the Boc protective group of **79**. Using a standard mixture of trifluoroacetic acid and methylene chloride, a poor yield below 50 % was obtained.

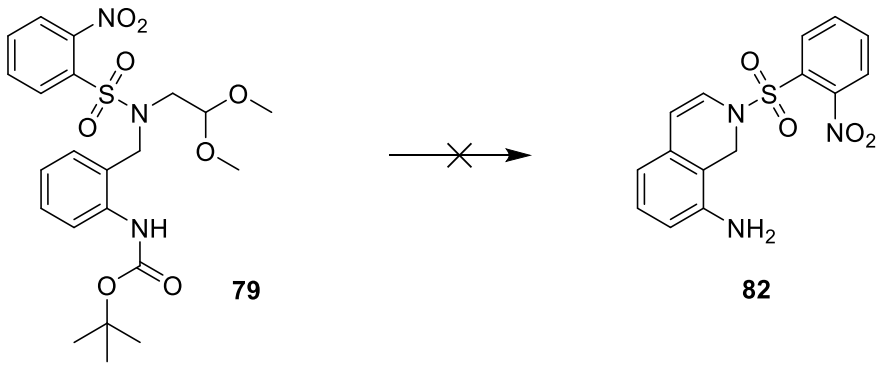
Because Boc cleavage inevitably requires a strong acid, we chose one of the few reducing agents, which is stable under acidic conditions: triethylsilane. In fact the organosilane-trifluoroacetic acid couple has been shown to be suitable for the direct reductive amination of acetals in intermolecular reactions.[68, 100] And truely, treatment of intermediate **79** with 2.5 equivalents of triethylsilane in a trifluoroacetic acid-dichloromethane mixture at ambient temperature gave the desired *N*-nosyl 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine **80** (Scheme 4.11) in 93 % yield (overall yield over 3 steps: 46 %).

To prove feasibility of this approach for the synthesis of unsubstituted 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines, cleavage of the Nosyl protective group from N-4 of **80** remained the last challenge. This was accompished under standard conditions[65] with thiophenol and potassium carbonate and gave **81** (isolated as the hydrochloride).



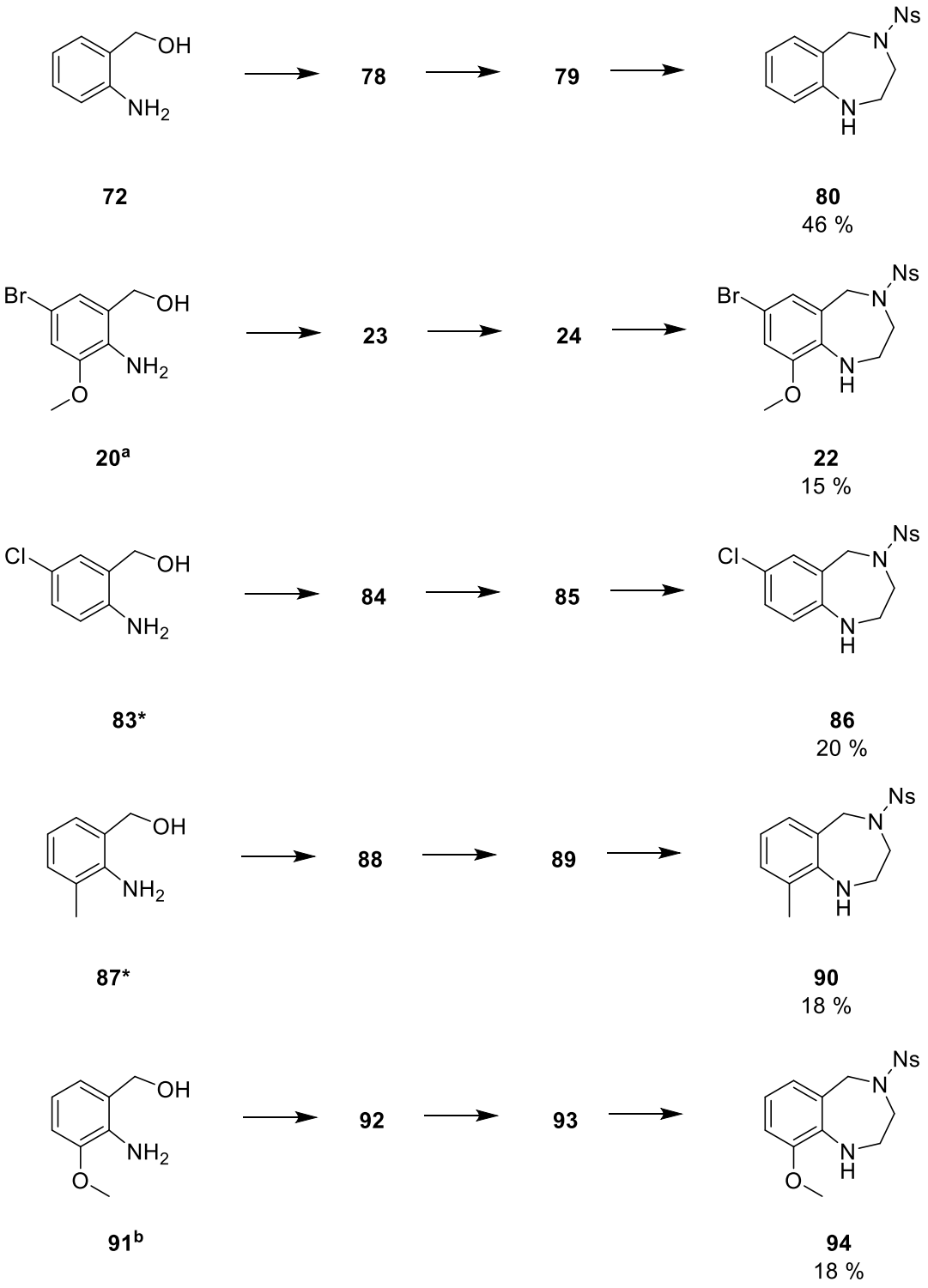
Scheme 4.11. Innovative approach to 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines using *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide, part II. Reagents and conditions: (a) TFA, Et_3SiH, DCM, rt, 93 %; (b) (i) thiophenol, K_2CO_3, MeCN, 50 °C; (ii) 4 M HCl in 1,4-dioxane, MeOH, -18 °C, 61 %.

The smooth benzodiazepine formation from **79** to **80** and the lack of by-products was finally a great relief. Specifically no by-products from a conceivable Pomeranz-Fritsch-type cyclization[101] were found (Scheme 4.12). This was considered as a possible competing reaction, with an acid-triggered electrophilic attack of a cationic species arising from protonation of the acetal moiety at C-3 giving an 8-aminodihydroisoquinoline **82**.



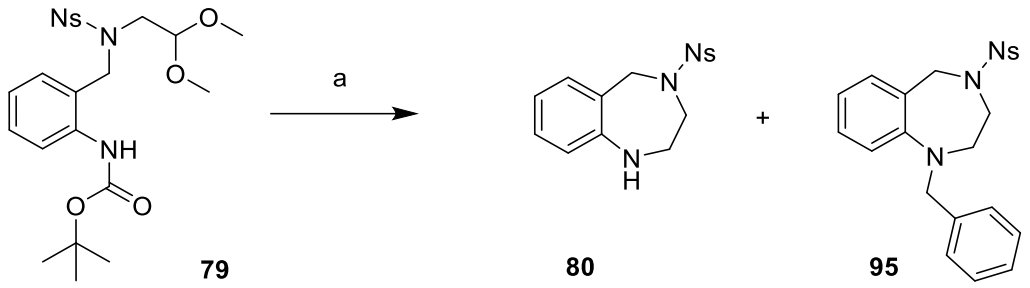
Scheme 4.12. Pomeranz-Fritsch-type cyclization as conceivable competing reaction.

General applicability of this approach was attempted for altogether five different 2-aminobenzyl alcohols. All of them were successfully converted within three steps from the 2-aminobenzyl alcohol into the monoprotected 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine, which is a versatile building block for further reactions. The educts, the obtained monoprotected 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines, the yields over the three steps, and the compound numbers of the corresponding intermediates are shown in Scheme 4.13.



Scheme 4.13. Educts, synthesized monoprotected 2,3,4,5-tetrahdyro-1*H*-1,4-benzodiazepines with total yields, and intermediate numbers; *Commercially available; [a] prepared from 2-amino-3-methoxybenzoic acid by bromination and reduction according to literature[53]; [b] prepared from 2-amino-3-methoxybenzoic acid by reduction according to literature[102].

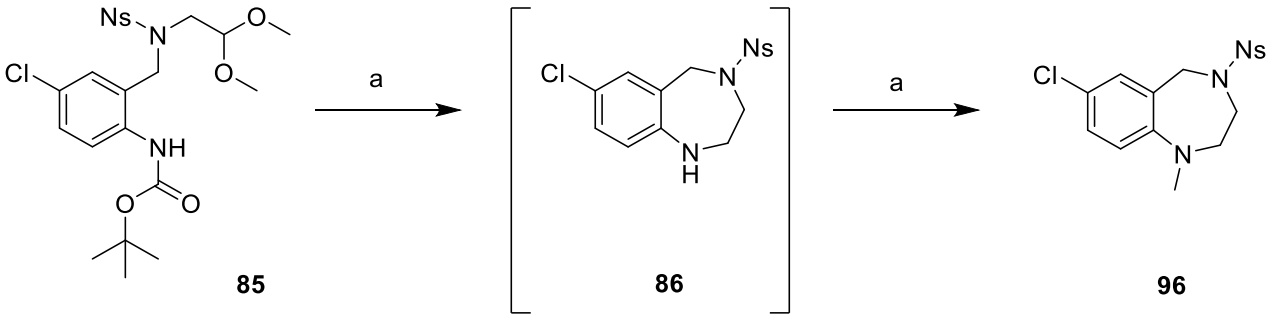
With the one-pot *N*-deprotection/reductive cyclization procedure going so smoothly, we wondered whether an additional reductive amination reaction with the obtained monoprotected 1,4-benzodiazepine was feasible within the same one-pot reaction. An experiment was conducted to benzylate **80** at N-1 in a one-pot reaction, starting with **79** by the known protocol and simply adding benzaldehyde and additional triethylsilane after completed formation of **80**. *N*-alkylations using 4-formylimidazole and triethylsilane-trifluoroacetic acid have been described in literature.[103] However, the desired 1-benzyl-4-nosyl-benzodiazepine **95** was obtained in 11 % only (Scheme 4.14) and 68 % of non-benzylated compound **80** was found in the mixture. At this point it was decided not to make any further experiments and not to answer the question, whether this extended protocol is simply not suitable for the introduction of larger residues at N-1 or whether it could work better with acetals.



Scheme 4.14. Further one-pot functionalization at N-1 following upon the first reductive amination. Reagents and conditions: (a) TFA, Et_3SiH, DCM, rt; then benzaldehyde, TFA, Et_3SiH, DCM, rt, 11 % of **95** and 68 % of **80**.

Instead another idea aroused our interest: The behaviour of the cyclic acetal-type formaldehyde trimer 1,3,5-trioxane in a triethylsilane/trifluoroacetic acid/aniline mixture. As mentioned[68] and demonstrated, acetals undergo reductive aminations aided by the triethylsilane/trifluoroacetic acid mixture. We were keen to learn, whether the combination of triethylsilane, 1,3,5-trioxane, and trifluoroacetic acid (TTT) was applicable for the *N*-methylation of anilines. Upon a number of preliminary experiments we managed to develop and publish this novel TTT system for the chemoselective *N*-methylation of aromatic amines. The development of this method is described in detail in Chapter V. This TTT system could also be applied effectively for the one-pot methylation at N-1 following the conversion of **85** into **86** (Scheme 4.15). This was

accomplished by treating **85** as described above with the mixture of triethylsilane, trifluoroacetic acid, and dichloromethane for 24 hours. After confirmation of the conversion to **86** via TLC, 1,3,5-trioxane and additional triethylsilane was added and the 1-methyl-4-nosyl-1,4-benzodiazepine **96** obtained in 79 % yield.



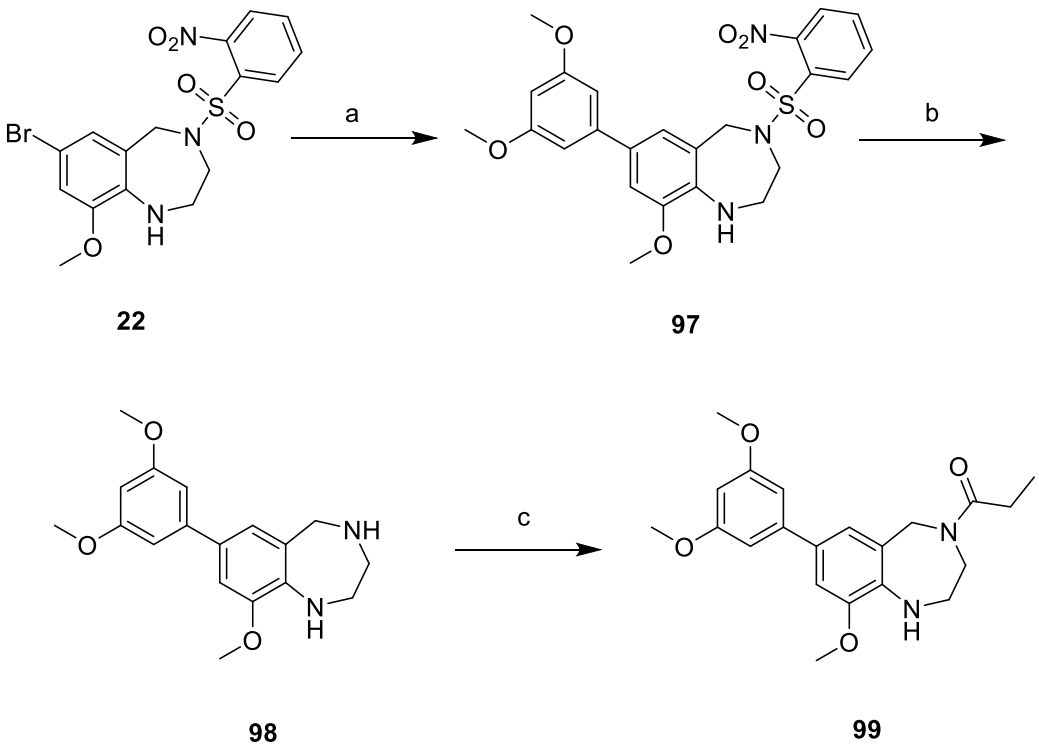
85 **86** **96**

Scheme 4.15. Further one-pot functionalization at N-1 following upon the ring closure by reductive amination. Reagents and conditions: (a) TFA, Et_3SiH, DCM, rt; then 1,3,5-trioxane, TFA, Et_3SiH, DCM, rt, 79 %.

4.3.4 Synthesis of a 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine analogue of the CBP inhibitors

Finally we could now synthesize a 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine compound for comparison with the 2,3,4,5-tetrahydro-1,4-benzoxazepine inhibitors. The oxygen at position 1 of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold is only capable to act as hydrogen bond acceptor. A secondary amine at N-1 is capable to act as both hydrogen bond acceptor and donor, making this structure particularly interesting. Compound **22** was subjected to a standard Suzuki coupling protocol[51b] with the meanwhile most interesting boronic acid, 3,5-dimethoxyphenylboronic acid, to obtain compound **97** (Scheme 4.16). We decided to conduct the Suzuki coupling prior to Nosyl deprotection to facilitate workup and to avoid losses with the very polar, deprotected and uncoupled 1,4-benzodiazepine **18**. The Nosyl group was then cleaved under standard conditions (K_2CO_3 in thiophenol) and replaced by an acyl function to obtain compound **98**. Chemoselective introduction of the propionyl residue at N-4 (and

not at the aromatic amino group N-1)[62d, 62e] proved not to be a problem due to the significantly higher nucleophilicity of the secondary aliphatic amino group compared to the aromatic amino group. Thus compound **99** was obtained.



Scheme 4.16. Preparation of a CBP inhibitor with 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold. Reagents and conditions: (a) 3,5-dimethoxyphenylboronic acid, $Pd(dppf)Cl_2$ * DCM, DIPEA, H_2O, 1,4-dioxane, 95 °C, 68 %; (b) K_2CO_3, thiophenol, MeCN, 50 °C, 75 %; (c) propionyl chloride, DIPEA, DCM, 0 °C - rt, 56 %.

4.3.5 Screening results

The screening results for the 1,4-benzodiazepine **99** were rather disappointing. For CBP a T_m shift of 4.8 °C and an IC_{50} of 1.8 µM was measured. For BRD4(1), IC_{50} was around 25 µM, at least indicating selectivity to some extent. Obviously no additional interaction with the protein through a hydrogen bond was introduced, and consequently no further effort was made on the synthesis of benzodiazepine-type CBP inhibitors.

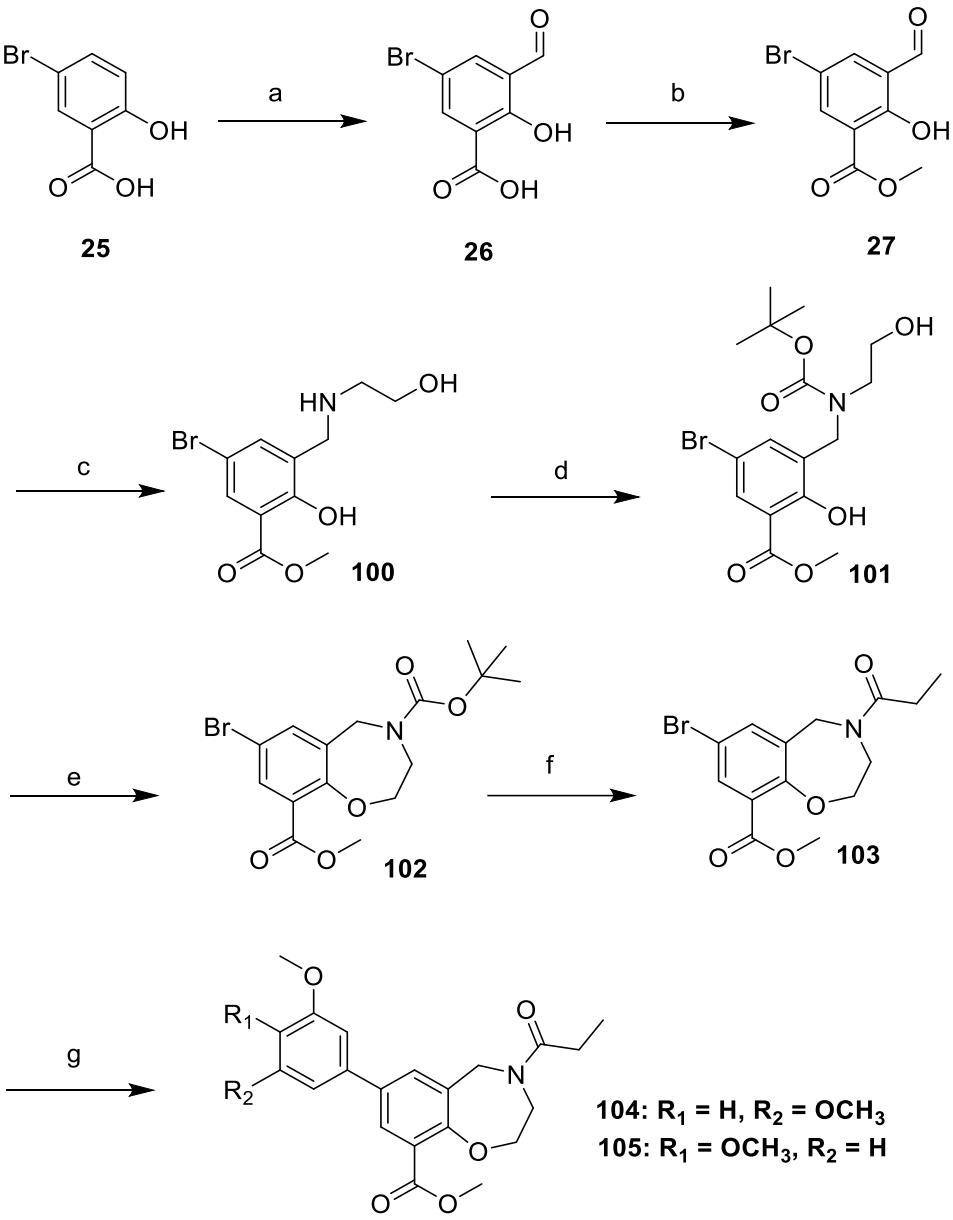
4.4 The 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with ester function at C-9

4.4.1 Synthesis of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with ester function at C-9

The findings so far suggested that a very potent CBP/p300 inhibitor had the following structural elements: A 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with a 3,5-dimethoxyphenyl substituent at position 7, and a propionyl residue at N-4. A 3,4-dimethoxyphenyl moiety was also considered as it is present in **I-CBP112**. Having this in mind we could follow the planned route to a new 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with a versatile methyl ester function at C-9 (Chapter 2.2.4). An ester function was considered as it could allow an additional hydrogen bond compared to **I-CBP112**'s ether function**.** Furthermore a methyl ester function seemed very suitable as it could easily be converted into the carboxylic acid and subsequently into various amides. Theoretically also an aromatic amine function could be introduced at C-9 by Schmidt reaction or Hofmann rearrangement or an urea or carbamate could be introduced by Curtius rearrangement. Moreover, starting from the neutral, uncharged methyl ester, the introduction of acidic and basic (and thus charged) functions seemed swiftly accomplishable. One of the main objectives was the introduction of a basic amino function comparable to that of **I-CBP112**. Finally reactions and workups were expected to be far less troublesome with a methyl ester than with the free carboxylic acid.

This approach was successful: 5-bromosalicylic acid underwent Duff formylation and subsequent esterification as described[69] in good yields (Scheme 4.17). Thus intermediate **27** was available at large scale and we could apply the same protocols as used before for the synthesis of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with methyl ether at C-9: Reductive amination of aromatic aldehyde **27** with 2-aminoethanol, and following *N*-Boc protection were accomplished with good yields and gave phenol **101**. Intramolecular Mitsunobu ring closure reaction of **101** to the 2,3,4,5-tetrahydro-1,4-benzoxazepine **102**, and acidic *N*-Boc deprotection and *N*-acylation with propionyl chloride followed. These four steps with an overall yield of 33 % gave the *N*-propionylbenzoxazepine intermediate **103**. Using **103**, we synthesized two intermediates for two different batches of compounds by a standard Suzuki cross-coupling protocol[51b], namely intermediate **104** for one batch of

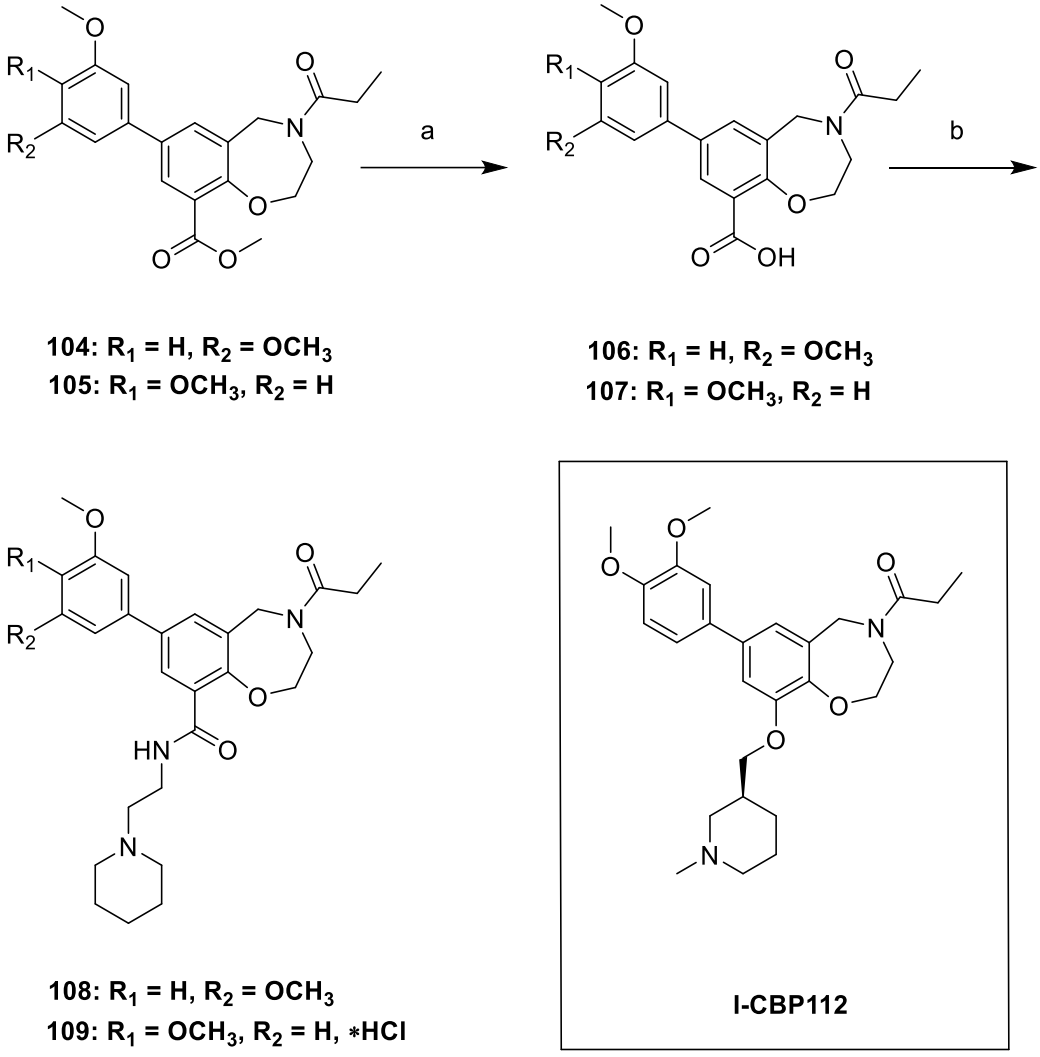
compounds bearing the 3,5-dimethoxyphenyl substituent at C-7, and **105** for the other batch with 3,4-dimethoxyphenyl substituent.



Scheme 4.17. Preparation of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with ester function at C-9. Reagents and conditions: (a) (i) urotropine, TFA, 90 °C, (ii) HCl, rt, 76 % (b) H_2SO_4, MeOH, reflux, 50 %; (c) 2-aminoethanol, $NaBH_4$, MeOH, THF, rt, 93 %; (d) di-*tert*-butyl dicarbonate, EtOAc, $NaHCO_3$ solution, rt, 56 %; (e) PPh_3, DIAD, THF, 0 °C - rt, 85 %; (f) (i) HCl, 1,4-dioxane, rt, (ii) propionyl chloride, DIPEA, DCM, 0 °C - rt, 74 %; (g) 3,5-/3,4-dimethoxyphenylboronic acid, $Pd(dppf)Cl_2$ x DCM, DIPEA, H_2O,1,4-dioxane, 95 °C, 72 % / 80 %;

4.4.2 Preparation of compounds with 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold and different functional groups at C-9

Compounds **104** and **106** bearing an ester group at C-9 were interesting candidates for CBP/p300 inhibition. In contrast to **I-CBP112**, which is positively charged at physiological pH due to its amino group in the side chain at C-9, these can neither be protonated nor deprotonated under physiological pH and will thus be uncharged. Furthermore the ester (and later amide) function may form an additional hydrogen bond and possibly require more space than **I-CBP112**'s ether function. Esters **104** and **105** smoothly underwent alkaline hydrolysis into the corresponding carboxylic acids **106** and **107** (Scheme 4.18). The effects of a negative charge at position 9 resulting from deprotonation of the carboxylic acids at physiological pH were of interest, too. Furthermore EDC-mediated amidation of these carboxylic acids with 1-(2-aminoethyl)piperidine gave the amides **108** and **109** in moderate yields. Those were bearing a basic, tertiary amine in a similar position than **I-CBP112**. At the same time, introduction of a stereocenter was avoided with the diamine building block employed here.

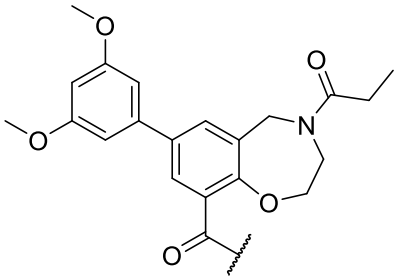


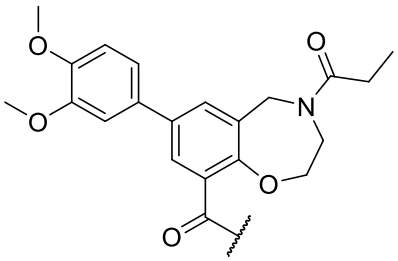
Scheme 4.18. Preparation of two batches of compounds with either 3,5- or 3,4-dimethoxyphenyl moiety at C-7. Reagents and conditions: (a) NaOH, MeOH, THF, 70 °C, 93 % / 87 %; (b) (i) 1-(2-aminoethyl)piperidine, EDC, DMAP, DIPEA, DCM, 0 °C - rt, 31 %, (ii, **109** only) HCl, 1,4-dioxane, rt, 43 %;

4.4.3 Screening results

A DSF screening of these compounds **104** – **109** demonstrated good binding to CBP and no T_m shift for BRD4(1), which was an excellent result (Table 4.4). Merely the potency (by T_m shift) was not on the same level as that of **I-CBP112** (CBP: 7.8 ±0.5 °C, BRD4(1) 2.1 ±0.4 °C). For our compounds there seemed little difference between a neutral, a basic, and an acidic residue at C-9. Despite the assumption that the C-9 moiety – like that of **I-CBP112**[29a] - is rather directed towards the solvent than towards

the binding pocket of the bromodomain, this was still surprising. Although the difference found between the 3,5- and 3,4-dimethoxyphenyl substitution pattern was not huge, the 3,5-pattern was slightly superior. The introduction of a residue containing a carbonyl group at C-9 was recognized as an extremely promising structure variation, especially regarding subtype selectivity.





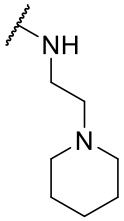
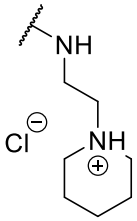
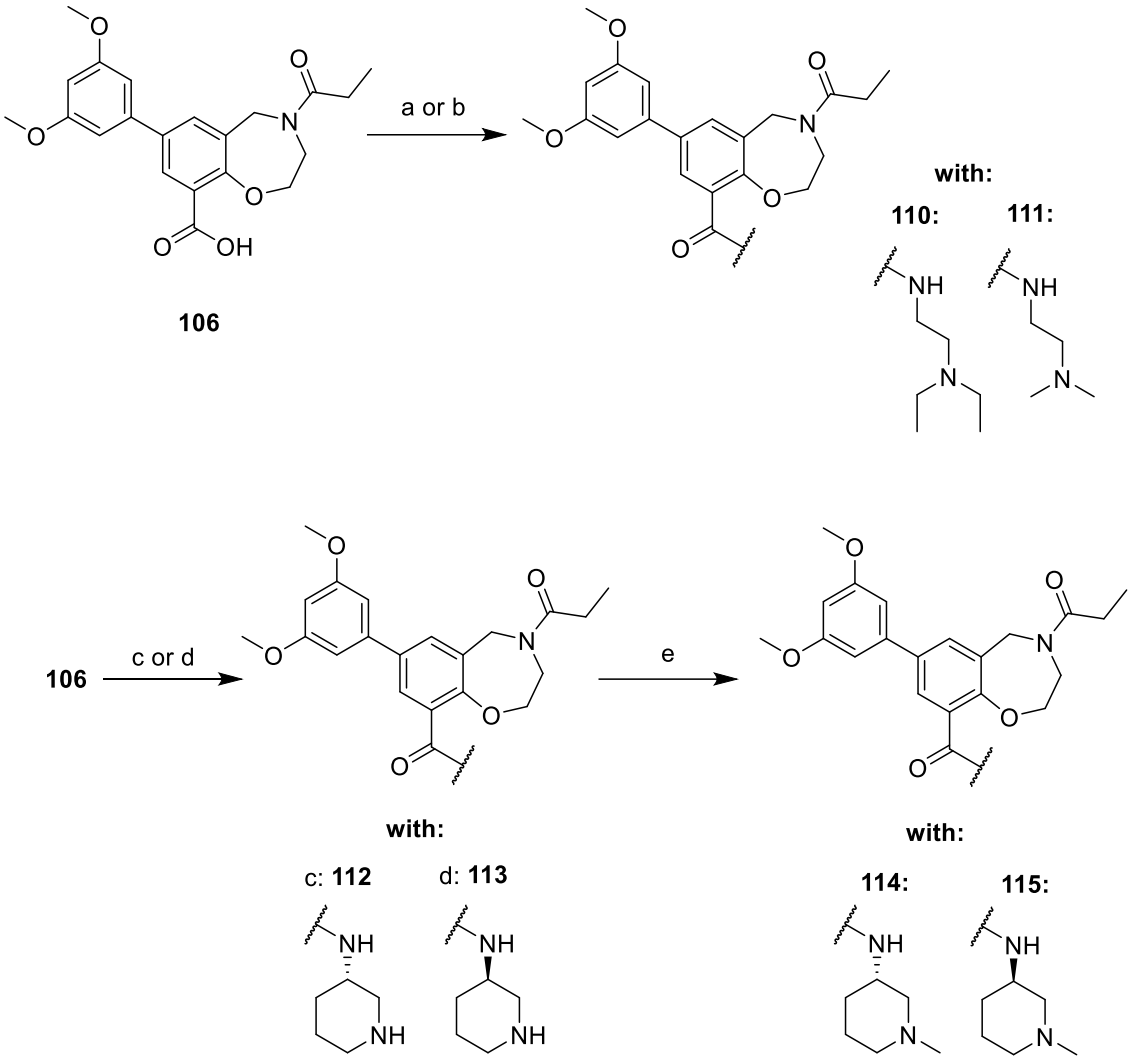
R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)	R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)
	**104**	5.4 (±0.2)	0.5 (±0.3)		**105**	4.7 (±0.8)	1.8 (±0.6)
	**106**	4.9 (±0.6)	0.1 (±0.1)		**107**	4.1 (±1.1)	0.1 (±0.2)
	**108**	6.5 (±0.5)	0.7 (±0.5)		**109**	4.9 (±0.3)	0.6 (±0.6)

Table 4.4. DSF results for compounds with different functional groups at C-9 (n = 3).

4.4.4 Preparation of further compounds with 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold and amide function at C-9

A number of further compounds were synthesized after the preliminary screening of **104** – **109** had indicated interesting selectivity over BRD4(1). The next challenge was to increase potency to a level comparable with **I-CBP112**. With the compounds with 3,5-dimethoxyphenyl substitution pattern being slightly more potent, compound **106** was selected as new lead structure. Compounds **110** and **111** representing open-chain aminoalkyl amides were synthesized from carboxylic acid **106** using the amidation reagent EDC (Scheme 4.19). Likewise chiral compounds **112** and **113** were synthesized using both (*S*)- and (*R*)-configured 3-amino-1-Boc-piperidine as building blocks, and subsequent, acidic *N*-Boc-deprotection. Finally these secondary amines were *N*-methylated using formaldehyde and $NaCNBH_3$[104] yielding *N*-methylpiperidines **114** and **115**, to obtain compounds for direct comparison with **I-CBP112**.



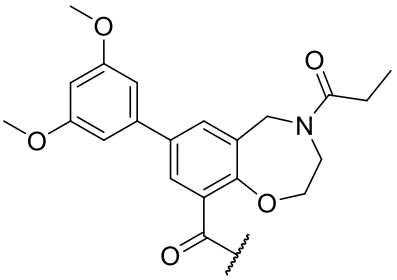
Scheme 4.19. (a) *N*,*N*-dimethylethylenediamine, EDC, DMAP, DIPEA, DCM, 0 °C - rt, 69 %; (b) *N*,*N*-diethylethylenediamine, EDC, DMAP, DIPEA, DCM, 0 °C - rt, 46 %; (c) (i) (*S*)-3-amino-1-Boc-piperidine, EDC, DMAP, DIPEA, DCM, 0 °C - rt; (ii) HCl, 1,4-dioxane, rt, 31 %; (d) (i) (*R*)-3-amino-1-Boc-piperidine, EDC, DMAP, DIPEA, DCM, 0 °C - rt; (ii) HCl, 1,4-dioxane, rt, 55 %; (e) formaldehyde solution, $NaCNBH_3$, AcOH, MeCN, rt, 35 % and 36 %.

4.4.5 Biological evaluation

4.4.5.1 DSF

Compounds **112** – **115** showed a potency at CBP (by DSF, Table 4.5) that was comparable to that of **I-CBP112**[48] (CBP: 7.8 ±0.5 °C, BRD4(1) 2.1 ±0.4 °C). At the same time, the T_m shift for BRD4(1) was extremely low. Configuration of the stereo center did not seem to cause any effect. Also, no decisive difference was observed

between the tertiary amines **114** and **115** and the secondary amines **112** and **113**. The compounds **110** and **111** were far less potent.



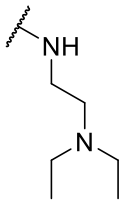
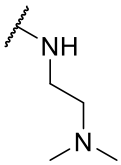
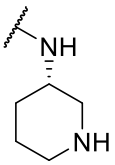
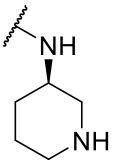
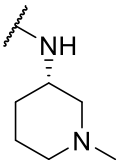
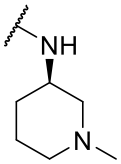
R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)	R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)
	**110**	5.7 (±0.4)	0.4 (±0.3)		**111**	4.4 (±0.5)	0.2 (±0.2)
	**112**	7.2 (±0.4)	0.8 (±0.6)		**113**	6.8 (±0.4)	0.6 (±0.3)
	**114**	7.1 (±0.4)	0.5 (±0.3)		**115**	7.7 (±0.4)	1.2 (±0.2)

Table 4.5. DSF results for compounds with different decoration at C-9 (n = 3).

It was decided to further characterize this novel type of inhibitors on the basis of compounds **112** and **114**, which had shown both promising activity on CBP and exciting selectivity in this screening. First of all a comprehensive DSF screening against 48 bromodomains was done with these two compounds. The T_m shifts of **114** with most of the bromodomains is depicted in Figure 4.3 and shows impressive selectivity. The corresponding table with the specific T_m shifts for each protein with **112** and **114** is shown in Table 4.6. T_m shifts of **I-CBP112** and bromodomains of the BET family are shown in Table 4.7 for comparison.

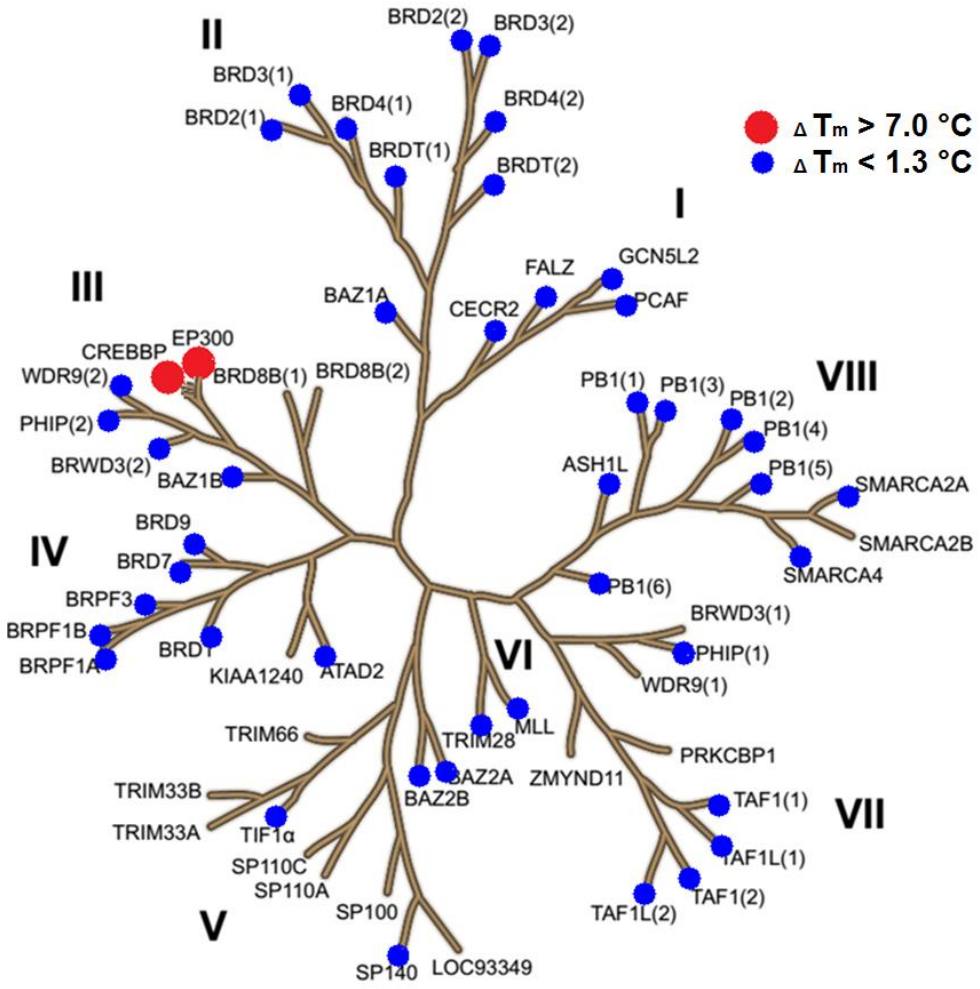


Figure 4.3.* T_m shifts of **114** and bromodomains of all families. T_m shifts with bromodomains without dots were not measured.

* Cutout from figure 1 of: Histone recognition and large-scale structural analysis of the human bromodomain family[27b]. Further modified by removing dots at the bromodomains and placing new dots and a new key. Under public license; Creative Commons Attribution License (CC BY); Elsevier.

Bromodomain	114	112	Bromodomain	114	112
ASH1L	0.09	-0.28	FALZ	-0.03	-0.39
ATAD2	-0.21	-0.02	GCN5L2	-0.97	-0.47
BAZ1A	-0.51	0.25	ATAD2B	-0.26	0.31
BAZ1B	-0.18	-0.42	SP140L	0.0	-0.48
BAZ2A	0.50	0.41	MLL	-1.24	0.53
BAZ2B	0.05	0.28	PB1(1)	-0.91	0.92
BRD1	-0.15	-0.88	PB1(2)	-0.09	-0.53
BRD2(1)	0.43	0.49	PB1(3)	0.35	0.65
BRD2(2)	0.67	0.54	PB1(4)	0.09	0.14
BRD3(1)	-0.35	0.26	PB1(5)	0.07	0.59
BRD3(2)	0.05	0.28	PB1(6)	-0.4	0.09
BRD4(1)	0.76	1.31	PCAF	-0.32	0.62
BRD4(2)	0.42	0.09	PHIP(2)	-1.17	-2.72
BRD7	0.86	1.19	SMARCA2	0.23	-0.05
BRD9	-0.89	1.04	SMARCA4	0.04	0.08
BRDT(1)	-0.44	0.21	SP140	-0.42	-0.44
BRDT(2)	0.32	0.58	TAF1(1)	-0.28	0.27
BRPF1A	0.53	0.67	TAF1(2)	-0.21	0.28
BRPF1B	0.20	-0.67	TAF1L(1)	0.15	-0.58
BRPF3	0.11	-0.86	TAF1L(2)	-0.65	-0.36
BRWD3(2)	1.09	0.24	TIF1-bromo	0.41	-0.12
CECR2	1.08	0.94	TIF1-phd-bromo	-0.19	0.49
CBP	**7.47**	**7.81**	TRIM28	-0.81	0.10
EP300	**8.45**	**8.27**	WDR9(2)	-0.92	1.16

Table 4.6. T_m shifts of different bromodomains with **114** and **112** in °C.

Bromodomain	I-CBP112
BRD1	0.43 ± 0.35
BRD2(1)	1.35 ± 0.48
BRD2(2)	0.87 ± 0.28
BRD3(1)	1.55 ± 0.44
BRD3(2)	0.94 ± 0.25
BRD4(1)	2.09 ± 0.41
BRD4(2)	0.58 ± 0.20
CBP	7.77 ± 0.53
EP300	8.69 ± 0.28

Table 4.7. T_m shifts of **I-CBP112** with BET-bromodomains in °C.[105]

4.4.5.2 ITC

The dissociation constant K_d of an inhibitor can be determined by isothermal titration calorimetry (ITC). This constant is essential for the characterization of an inhibitor. For the established inhibitor **I-CBP112**, this value is 151 ± 6 nM for CBP and 5.6 µM for BRD4(1), which differs by factor 37.[48] A K_d of 134 ± 10 nM was found for **114** and CBP, the titration is displayed in Figure 4.4 (left-hand panel). The K_d for BRD4(1) was determined as 5.02 µM for BRD4(1) by our cooperation partner (right-hand panel), but the determination is not very reliable for such high values. Although **114** is slightly more potent on CBP than **I-CBP112** by ITC, the obtained K_d values for CBP and BRD4(1) differ by a factor of 37 as well, and this data cannot be used to prove the increased selectivity found by DSF.

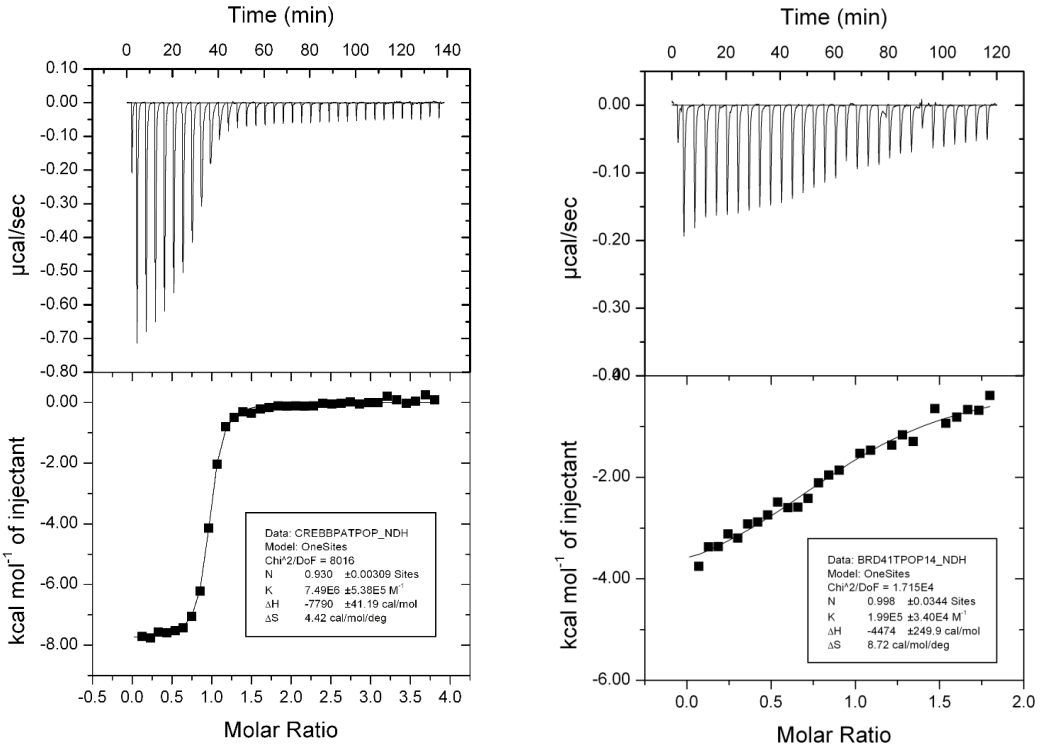


Figure 4.4. Diagram of the ITC experiment of **114**. The titration with CBP is on the left-hand side, the titration with BRD4(1) on the right-hand side.

An binding ratio of CBP and **114** of 1 to 1 can clearly be seen in the titration curve. ΔG^o can be calculated using the Gibbs-equation:

$$\Delta G° = \Delta H° - T\Delta S° = -RT \ln K$$

$$\Delta G° = -7.8\ kcal/mol - (288.15K\ x\ 0.0044 \frac{kcal}{mol\ K})$$

$$\Delta G° = -9.1\ kcal/mol$$

With ΔH^o being - 7.8 kcal/mol and $T\Delta S^o$ being 1.3 kcal/mol, the enthalpy's influence on the binding is larger than entropy's impact. Looking at CBP inhibitors with different scaffolds, CBP30 is certainly the most interesting. This 5-isoxazolyl-benzimidazole compound was published in 2015 and is currently the most potent inhibitor of CBP. CBP30 has been reported to inhibit the production of proinflammatory cytokines in human cells and patient blood samples. Furthermore it is very well characterized, with a K_d of 26 nM of CBP and 890 nM for BRD4(1), which means 34 fold selectivity.[47] It may be difficuilt to develop a more potent inhibitor, but an inhibitor with a different scaffold and a K_d in the µM order for BRD4(1) is a valuable alternative.

4.4.5.3 FRAP assay

To prove efficacy of this compound class in living cells a FRAP experiment was conducted. The results obtained with compound **112** (named E57682a in the experiment) are displayed in Figure 4.5. The diagram on the left-hand side displays the results of five experiments (x-axis), in which the half-recovery times of the fluorescence signal (y-axis) of GFP-tagged CBP under different conditions is displayed. The recovery of the intensity as a function of time is shown on the right-hand side. The first experiment was done with the GFP-tagged wild type CBP bromodomain. Half-recovery time of the fluorescence signal was approximately 1 s. In the next experiment the histone deacetylase inhibitor SAHA was added. This caused global hyperacetylation and increased immobilization of the CBP bromodomain at the chromatin. This reduced migration into the bleached area and half-recovery time increaded to about 2.5 s. Repeating this experiment, but with CBP inhibitor **112** added,

the binding of the GFP-CBP construct to the chromatin was inhibited, CBP migration enabled, and the initial half-recovery time of approximately 1 s was restored. To prove that this effect is due to specific inibition of the bromodomain – K_{ac} interaction, two further experiments were conducted using a mutant form of the GFP-tagged CBP bromodomain. Here asparagine 1168 is replaced by phenylalanine. Asparagine 1168 is vital for the CBP's function as it forms a hydrogen bond to the oxygen of the amide function of *N*-acetyllysine. Using this mutant, K_{ac} – bromodomain binding is impossible. The similar half-recovery times obtained with this mutant GFP-tagged CBP bromodomain with and without SAHA addition, proves the concept of delayed CBP migration due to chromatin binding. Summing the results of this FRAP assay up, **112** is an active inhibitor of the CBP bromodomain in living, human cells.

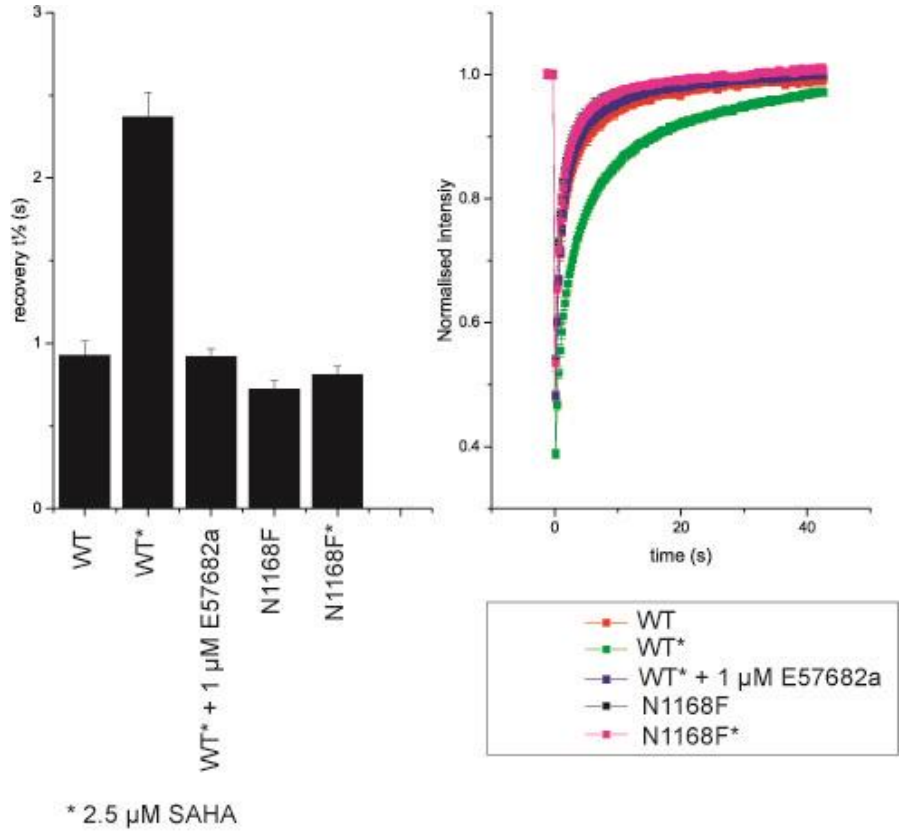
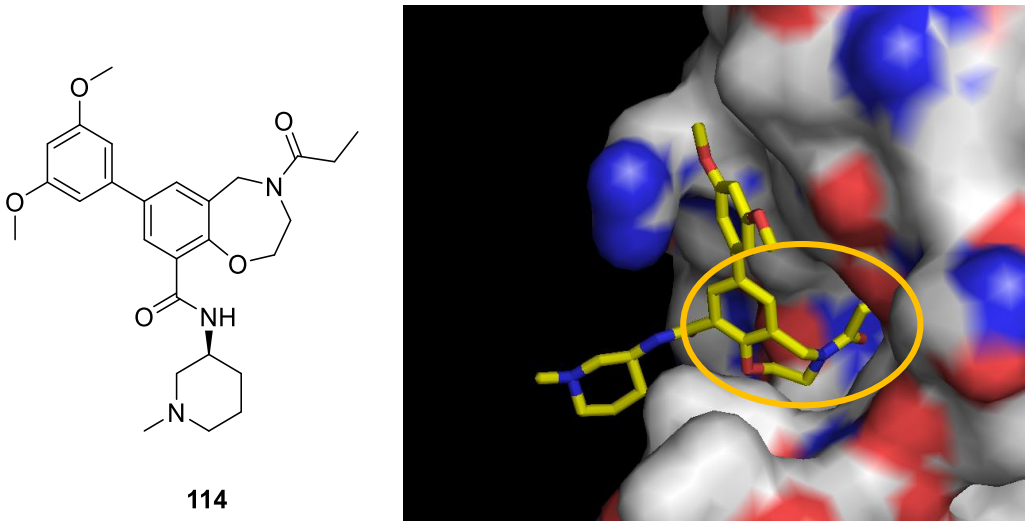


Figure 4.5. Graphs of the results of the FRAP experiment with **112**.

4.4.5.4 Co-crystallization

A co-crystallization of compound **114** and CBP was obtained by our partners at the SGC (Figure 4.6). The 1,4-benzoxazepine core lies in a central position along the entrance to the deep K_{ac} binding pocket, while the *N*-propanoyl moiety immerges into this binding pocket. Accordingly, this benzoxazepine inhibitor and putatively analogous molecules are competitive inhibitors, replacing acetylated lysine residues from the K_{ac}-binding site.



114

Figure 4.6. Compound **114** (left side) and co-crystallization (right side) of **114** (gold) with CBP (grey). Central 1,4-benzoxazepine core and *N*-propanoyl moiety are highlighted by an orange circle. Oxygen atoms are coloured red and nitrogen atoms blue.

Going more into detail and having a closer look at the K_{ac} binding pocket (Figure 4.7), it is clear that the *N*-propanoyl residue acts as an *N*-acetyllysine (K_{ac}) mimic. A hydrogen bond (Figure 4.7, arrow a) is formed between the amide's carbonyl function at N-4 and the NH_2 group of asparagine (N1168, green). The amide group at C-9 also interacts through a water-mediated hydrogen bond (Figure 4.7, arrow b) with this amino acid - here with the oxygen atom of the carboxamide side-chain. Conserved water molecules are found at the deepest point of the pocket (Figure 4.7, arrow c). These water molecules mediate binding to the *N*-acetylated lysine moiety of target proteins and are also typical for bromodomain – inhibitor co-crystallizations.

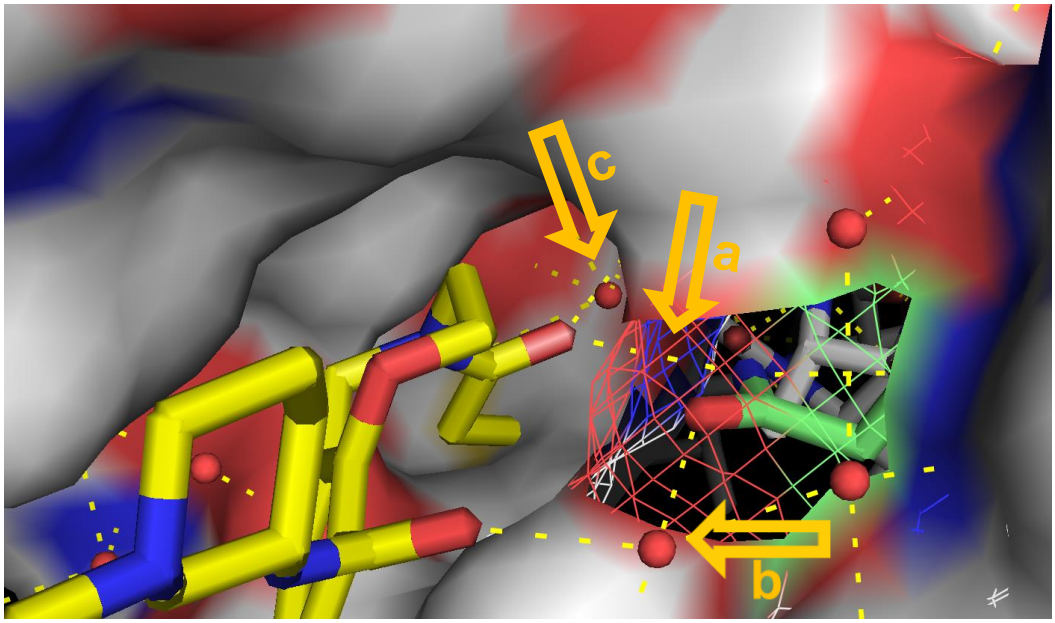


Figure 4.7. K_{ac} binding site with a hydrogen bonds (yellow dotted lines) from asparagine 1168 (green; a, b) and a conserved water molecule (red dot) at the deepest point of the pocket (c).

Indeed one of the conserved water molecules mediates a further hydrogen bond (Figure 4.8) between the *N*-propanoyl residue and tyrosine (Y1125, turquoise), which is a typical interaction between bromodomains and K_{ac} or K_{ac} mimics.

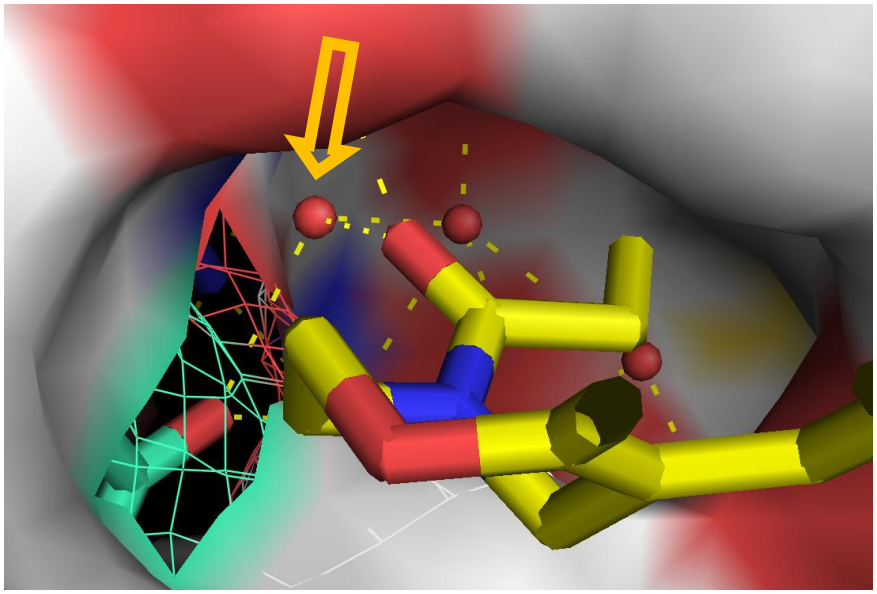


Figure 4.8. K_{ac} binding site with water mediated hydrogen bond between the K_{ac}-mimetic *N*-propanoyl residue of inhibitor **114** and tyrosine 1125.

Moreover the electron rich aromatic moiety at C-7 (Figure 4.9) binds to positively charged arginine (R1173, blue) via π-cation interaction.

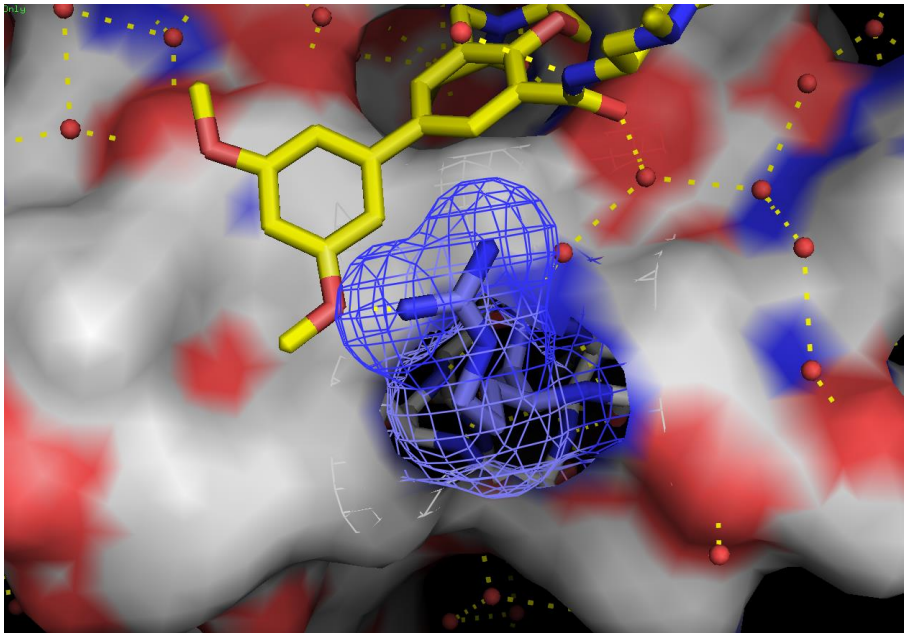


Figure 4.9. Interaction of the electron rich aromatic system at C-7 and arginine 1173.

These interactions are summed up in Figure 4.10. Most of these interactions have also been found for **I-CBP112**[29a], however the water mediated interaction between the novel acyl group at C-9 and asparagine 1168 is an additional interaction, which might contribute positively to both affinity and selectivity of our new chemotype of CBP inhibitors.

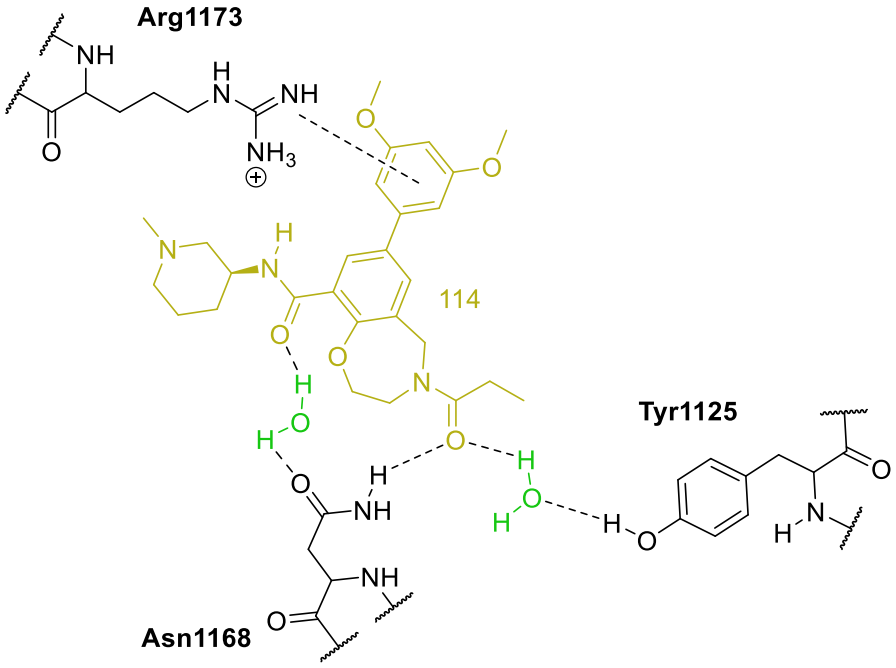


Figure 4.10. Interactions of **114** (gold) and CBP (black). Conserved water molecules are coloured green.

4.5 Results from MTT assay and agar diffusion test

The novel compounds synthesized for the preparation of CBP inhibitors and for the development of the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine synthesis were tested for cell cytotoxicity (MTT assay) and antibacterial and antifungal activity as part of routine measurements. Most of the compounds were not particularly cytotoxic and had a IC_{50} value > 50 µM. This was also the case for the most interesting inhibitors **106** - **115**. This is an important result, because CBP inhibitors are potential drug candidates. Compounds with IC_{50} values < 50 µM are displayed in Table 4.8. Not included are compounds from the master thesis of Edgar Uhl, which were neither particulary effective.

No.	IC_{50} (µM)	No.	IC50 (µM)	No.	IC_{50} (µM)
34	33	**44**	30	**63**	34
37	32	**45**	44	**64**	37
39	28	**46**	13	**65**	34
40	14	**47**	28	**67**	18
41	37	**48**	8	**69**	36
42	40	**51**	14	**102**	45
43	10	**60**	40	**104**	40

Table 4.8. Compounds with IC_{50} values < 50 µM. Reference drug cisplatin shows an IC_{50} value of 5 µM.

Regarding the agar diffusion test, no exciting results were found. Only a few intermediates showed minor antibacterial or antifungal activity. These are listed in Table 4.9.

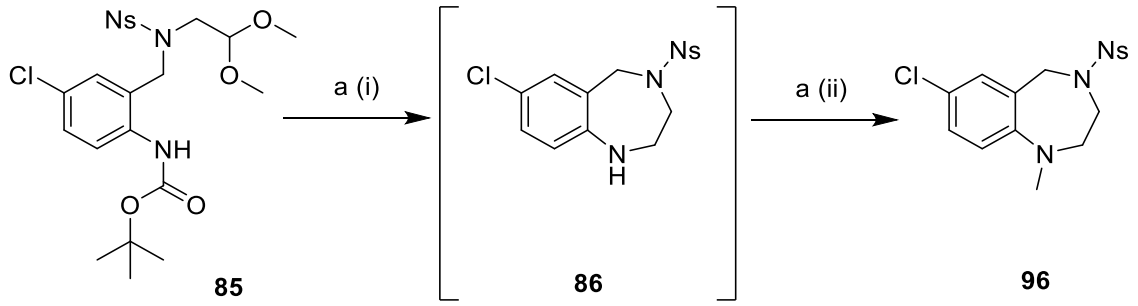
Compound	***Escherichia coli***	***Pseudomonas marginalis***	***Staphylococcus equorum***	***Streptococcus entericus***	***Yarrowia lypolytica***	***Candida glabrata***	***Aspergillus niger***	***Hyphopichia burtonii***
12	-	-	0.3	0.4	-	-	-	-
100	-	-	-	-	0.5	0.4	-	-
102	-	-	-	-	0.4	0.4	-	-
103	-	-	-	-	0.4	0.4	-	-

Table 4.9. Microbiologically active substances. Shown is the quotient of the diameter of growth inhibition of synthesized compound and reference substance (clotrimazole or tetracycline).

Chapter V - *N*-Methylation of aromatic amines and N-heterocycles under acidic conditions with the TTT (1,3,5-trioxane – triethylsilane – trifluoroacetic acid) system

5.1 Introduction

As described in chapter 4.3.3, the reductive alkylation of anilines with aldehydes[100a] and especially acetals[68, 100a] was thoroughly examined as it was essential for the preparation of the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffold (Scheme 5.1, **85** -> **86**). This inspired us to investigate an extension of this methodology and examine the cyclic acetal-type formaldehyde trimer 1,3,5-trioxane for the purpose of reductive *N*-methylations, which had not been desribed at that time. The later application of this methodology in a one-pot *N*-methylation following the intramolecular ring closure reaction of an (at the beginning of the reaction Boc-protected) aniline and a dimethyl acetal group (in **85**) under reductive and acidic conditions is mentioned in chapter 4.3.3 and again displayed in Scheme 5.1:



85 **86** **96**

Scheme 5.1. Addition of 1,3,5-trioxane and additional Et_3SiH to the reaction mixture results in methylation of the aniline. Reagents and conditions: (a) (i) TFA, Et_3SiH, DCM, rt; (ii) 1,3,5-trioxane, TFA, Et_3SiH, DCM, rt, 79 %.

It was decided to examine the potential of a mixture of 1,3,5-trioxane, trifluoroacetic acid, and triethylsilane ("TTT system") for reductive N-methylation of further anilines, but also aliphatic amines and N-heterocycles.

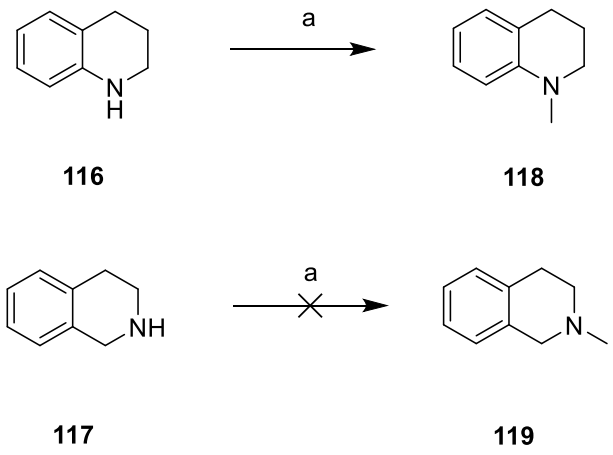
Certainly numerous protocols for *N*-methylations are already available, but one distinct advantage of 1,3,5-trioxane (and also trifluoroacetic acid and triethylsilane) is its formidable solubility in organic solvents[106]. Reagents typically employed for *N*-methylations under mild conditions are paraformaldehyde or aqueous solutions of formaldehyde. However the polymeric formaldehyde paraformaldehyde is not readily soluble in many solvents, and the aqueous formaldehyde solution obviously does not allow working under anhydrous conditions. Along with formaldehyde or paraformaldehyde, reducing agents like formic acid (Eschweiler-Clarke reaction[107]) and complex hydrides (sodium borohydride[108], sodium cyanoborohydride[104] in combination with Lewis acids[109]) are typically employed. Due to the high nucleophilicity of primary and secondary amines, most *N*-methylation protocols applied on primary amines give the *N,N*-dimethylated products directly. However, *N*-monomethylated products are accessible in two steps: The aliphatic nitrogen of tryptamine derivatives for example is *N*-monomethylated via reduction of *N*-formyl derivatives[110] or alkyl carbamates with lithium aluminum hydride[111]. Another publication explicitely describes the *N*-methylation reaction according to the formaldehyde/$NaBH_4$/$ZnCl_2$ protocol to be slower for aromatic amines[112], which is not surprising as these are less nucleophilic than aliphatic amines. Consequently for many aromatic substrates like anilines, pyrroles, azoles, and annulated analogues harsher protocols are used: Either methyl halides or dimethyl sulfate are applied directly on the amide anions or on the substrate in the presence of acid scavengers. These protocols often result in good yields, but volatility and toxicity of the methylation agents are an issue.[113] Furthermore, under the described alkaline conditions overalkylation to the quarternary ammonium salts or *C*-alkylations at acidic postitions may occur.[114] The less toxic dimethyl carbonate may be used as an alternative, but *C*-methylations[113] and *N*-methoxycarbonylations[115] are also reported for this agent.

The stability of the reducing agent triethylsilane in strongly acidic media may be an advantage and may allow simple protocols. Furthermore, the triethylsilane-trifluoroacetic acid mixture was demonstrated to be compatible with a large number of functional and protective groups.[116],[68] We were keen to learn about the scope and

limitations of *N*-methylations with the TTT system and how this protocol would integrate into the existing set of protocols.

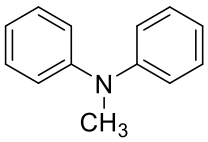
5.2 Scope and limitations

We started with two simple experiments employing the TTT system on the aromatic amine 1,2,3,4-tetrahydroquinoline (**116**) and its regioisomer 1,2,3,4-tetrahydroisoquinoline (**117**) as a secondary aliphatic amine. We found that the aromatic amine did undergo *N*-methylation readily with 64 % yield and without any by-products, while the aliphatic amine did not react at all (Scheme 5.2). This indicated an interesting (and to the best of our knowledge unprecedented) selectivity of the TTT system for aromatic amines and reaction conditions were optimized. Best results were obtained with 3 equivalents of 1,3,5-trioxane and 10 equivalents of triethylsilane in a 1:2 trifluoroacetic acid-dichloromethane mixture. The mixtures were stirred under nitrogen atmosphere at room temperature for either 24 h, or in the case of incomplete conversion (tlc control) for 48 h.

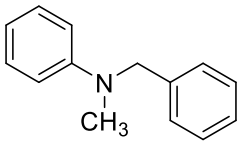


Scheme 5.2. Initial experiments indicate selectivity for aromatic amines. Reagents and conditions: (a) 1,3,5-trioxane, Et_3SiH, TFA, CH_2Cl_2, rt.

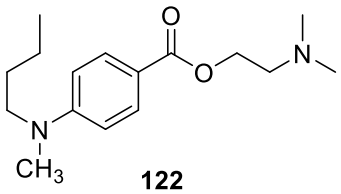
The TTT system was applied to a number of further substances, and the obtained compounds, compound numbers, reaction times, and yields are depicted in Figure 5.1.



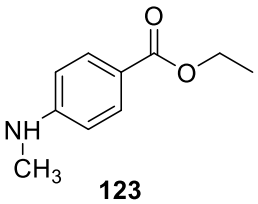
120
(24 h, 89 %)



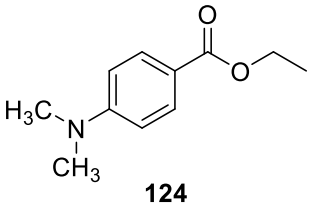
121
(48 h, 51 %)



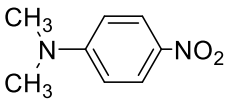
122
(48 h, 51 %)



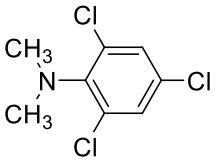
123
(48 h, 37 %)



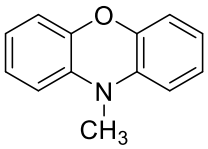
124
(48 h, 57 %)



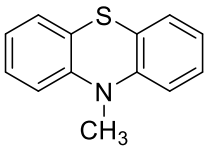
125
(48 h, 99 %)



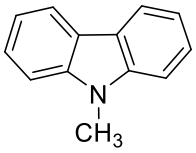
126
(48 h, 93 %)



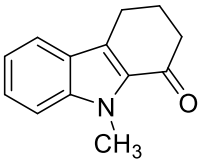
127
(24 h, 99 %)



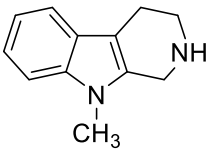
128
(24 h, 93 %)



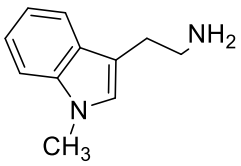
129
(24 h, 61 %)



130
(48 h, 29 %)



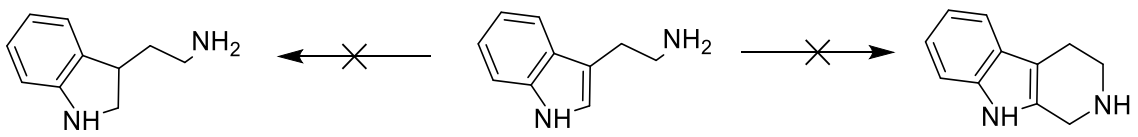
131
(48 h, 67 %)



132
(48 h, 30 %)

Figure 5.1. Products obtained from *N*-methylation with the TTT system, in parentheses reaction times and yields. Introduced methyl groups are labeled "CH_3".

The secondary aromatic amines diphenylamine, *N*-benzylaniline, and tetracaine were converted with good yields and without any by-products into the corresponding *N*-methylated compounds **120**, **121**, and **122**. Not surprisingly, from the primary aromatic amine ethyl 4-aminobenzoate, two compounds were obtained, *N*-monomethylated aniline **123**, and mainly *N,N*-dimethylated aniline **124**. 4-Nitroaniline was quantitatively alkylated to *N,N*-dimethylaniline **125** without reduction of the nitro group. Sterically more hindered and electron poorer 2,4,6-trichloroaniline seemed a particular challenge, since the previous *N,N*-dimethylation was accomplished with only 54 % yield by refluxing with dimethyl sulfate in toluene. The TTT system gave **126** with a yield of 93 % under mild conditions. Likewise heterocyclic substrates phenoxazine and phenothiazine were converted in excellent yields, and *N*-methylcarbazole **129** was prepared with 61 %. 1-Oxo-1,2,3,4-tetrahydrocarbazole was converted into the *N*-methylated product **130** in 29 % yield only, the remainder being educt. This seems disappointing at first glance, but earlier attempts made in our group with the classical reagents sodium hydride and iodomethane only yielded the 2,2,9-trimethyl derivative.[114] The conversion of 1,2,3,4-tetrahydro-β-carboline was particularly exciting, because an aliphatic and an aromatic amine is present. But apart from the educt only one compound was found in the reaction mixture. It was shown to bear a methyl group at N-9 only, and thus compound **131** was isolated with 67 % yield. Likewise tryptamine gave derivative **132** with a methyl group at N-1 exclusively in 33 %. The aliphatic amino side chain was not affected and the remaining percentage was educt. This conversion was also interesting, because neither reduction to the indoline **134**, nor a Pictet-Spengler-type cyclization to tetrahydro-β-carboline **135** was observed (Scheme 5.3). The reduction to indolines is reported for several indoles in TFA/TES mixtures without or with small portions of dichloromethane,[117] and Pictet-Spengler-type cyclization is demonstrated for related arylethylamines, when treated with 1,3,5-trioxane and acid[118].

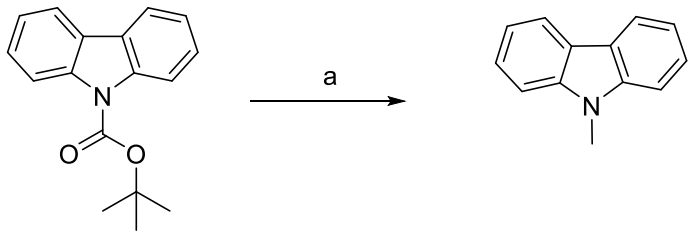


134 **133** **135**

Scheme 5.3. Conceivable side products of the application of the TTT system on tryptamine.

As shown, no conversion of the aliphatic amino group of three substances was observed with the TTT mixture. We assume that the aliphatic, basic amino function is fully protonated under the acidic reaction conditions, and is thus protected from the electrophilic attack of a reactive, cationic intermediate generated from 1,3,5-trioxane. Accordingly this newly developed TTT protocol is complementary to existing protocols such as the standard reductive *N*-methylation protocol with aqueous formaldehyde and sodium cyanoborohydride, which allows conversion of 1,2,3,4-tetrahydro-β-carboline into 2-methyl-1,2,3,4-tetrahydro-β-carboline[119], and of tryptamine into *N',N'*-dimethyltryptamine[110].

The TTT system was also tested for a one-pot acidic deprotection/*N*-methylation on a substrate other than 1,4-benzodiazepine precursor **85**. It is a standard procedure to deprotect *N*-Boc with a mixture of trifluoroacetic acid and dichloromethane. Moreover the triethylsilane employed within the TTT system, is known as a beneficial scavenger for *tert*-butyl cations in deprotections of *tert*-butyl esters and *tert*-butoxycarbonyl residues[117a, 120], so the TTT system seemed an excellent candidate for general one-pot acidic deprotection/*N*-methylation reactions. *N*-Boc-carbazole[121] (**136**) was selected and converted into *N*-methylcarbazole (**129**) with 49 % yield (Scheme 5.4).



136 **129**

Scheme 5.4. One-pot acidic deprotection/*N*-methylation. Reagents and conditions: (a) 1,3,5-trioxane, Et_3SiH, TFA, CH_2Cl_2, 49 %.

Of course, not all conversions were successful. Apart from the aliphatic amines, a number of further compounds showed no *N*-methylation with the TTT system. Acridone (**137**), harmane (**138**), 1-oxo-1,2,3,4-tetrahydro-β-carboline[122] (**139**), and lactam

substrate **140**[123] neither showed conversion (Figure 5.2). We assume that the nucleophilicity of the nitrogen atom is eradicated by a conjugated protonated carbonyl group in acridone (**137**) and lactam (**140**) or a conjugated, protonated pyridine ring (**138**). Likewise nucleophilicity may be eliminated for the basic heteroarenes theophylline (**141**), benzimidazole (**142**), 2-chlorobenzimidazole (**143**), 4-iodopyrazole (**144**), and benzotriazole (**145**). The attempt to extend the scope of this approach to the *N*-ethylation of aromatic amines by replacing 1,3,5-trioxane with paraldehyde (2,4,6-trimethyl-1,3,5-trioxane), the cyclic trimer of acetaldehyde, failed. The application of the corresponding PTT system on diphenylamine resulted in a strongly exothermal reaction with a darkening of the solution within seconds and finally an inseperable mixture of products. Cooling to 0 °C did only slow this process down.

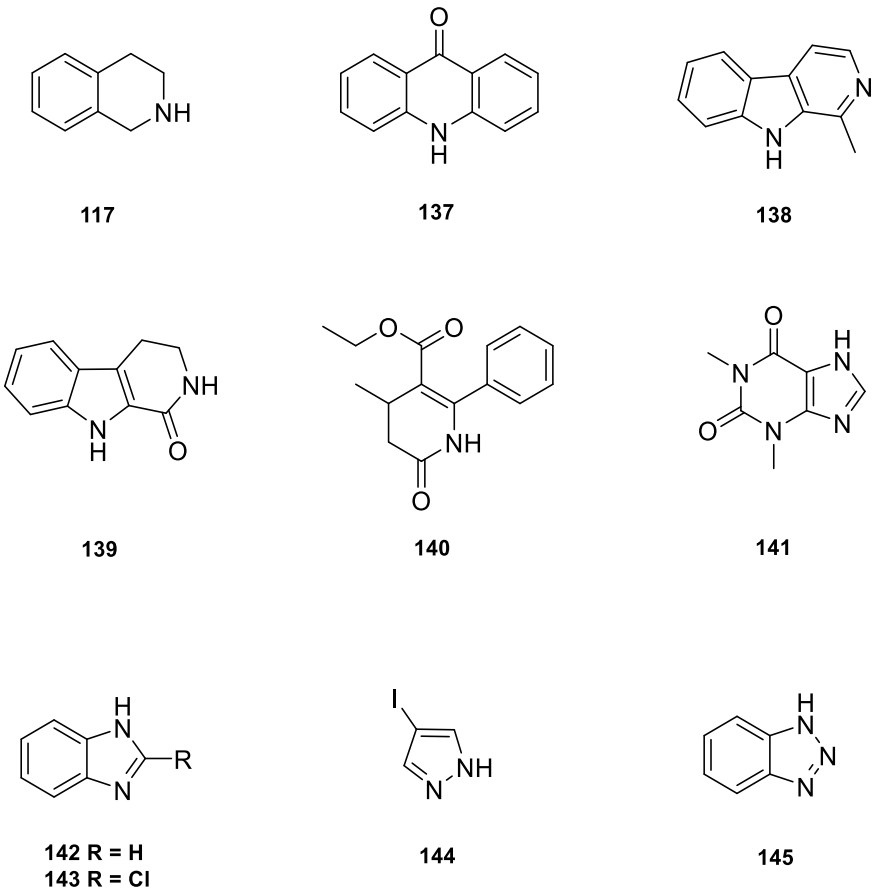


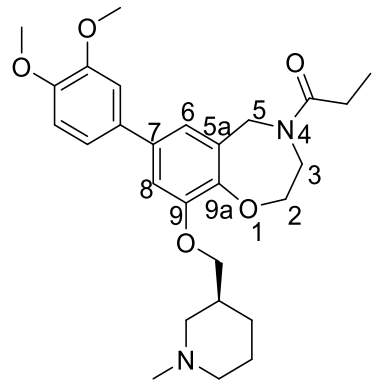
Figure 5.2. Substances inert to the TTT system.

In conclusion we worked out a new protocol for the selective *N*-methylation of aromatic amines and N-heterocycles (indoles and annulated analogues, phenoxazine,

phenothiazine), that was published in Synthesis in 2015.[124] This method is highly chemoselective and inert to the normally more reactive/nucleophilic aliphatic amines. As demonstrated with tryptamine and 1,2,3,4-tetrahydro-β-carboline, this protocol is complementary to the known standard protocols, as merely the less nucleophilic nitrogen is methylated. The yields obtained ranged from 29 – 99 % and were highest for those educts, that were expected to be less reactive. The reaction protocol is simple, and the conditions are mild and compatible with many functional groups such as esters and nitro groups. No side reactions such as C-alkylation, or overalkylation to quarternary ammonium salts were observed. Moreover, this protocol can be combined with other reactions, such as acidic deprotections or intramolecular reductive aminations.

Chapter VI - Summary

Based on the screening of a commercial library and some custom made compounds, the SGC had developed the 1,4-benzoxazepine type CBP/p300 inhibitor **I-CBP112** (Figure 6.1). This inhibitor is very potent and to some extent selective, but several edges, especially the 2,3,4,5-tetrahyro-1,4-benzoxazepine scaffold, the acyl moiety at N-4, the C-7-, and the C-9 moiety were not fully explored. Initially, the objective of this thesis was the further refinement of these moieties of this inhibitor. Difficulties in the preparation of some compounds for inhibitor refinement prompted us to extend the topic of this thesis to the development of a novel and mild approach to 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines. Moreover this approach finally inspired us to develop a new preparative protocol for the chemoselective *N*-methylation of aromatic amines.

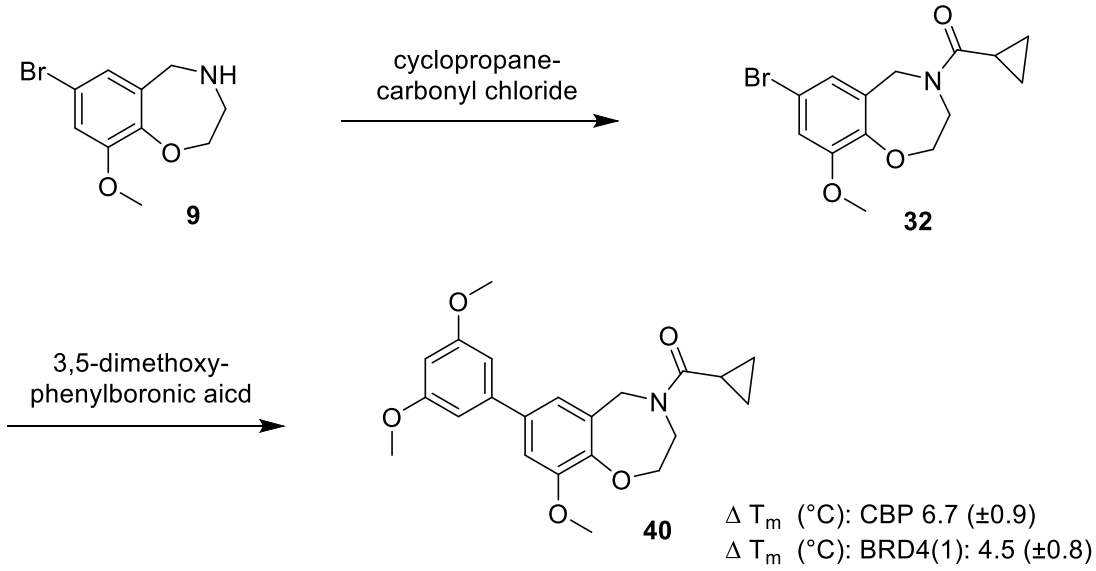


ΔT_m (°C): CBP: 7.8 (±0.5)
ΔT_m (°C): BRD4(1): 2.1 (±0.4)

Figure 6.1. CBP/p300 bromodomain inhibitor **I-CBP112** and T_m shifts.

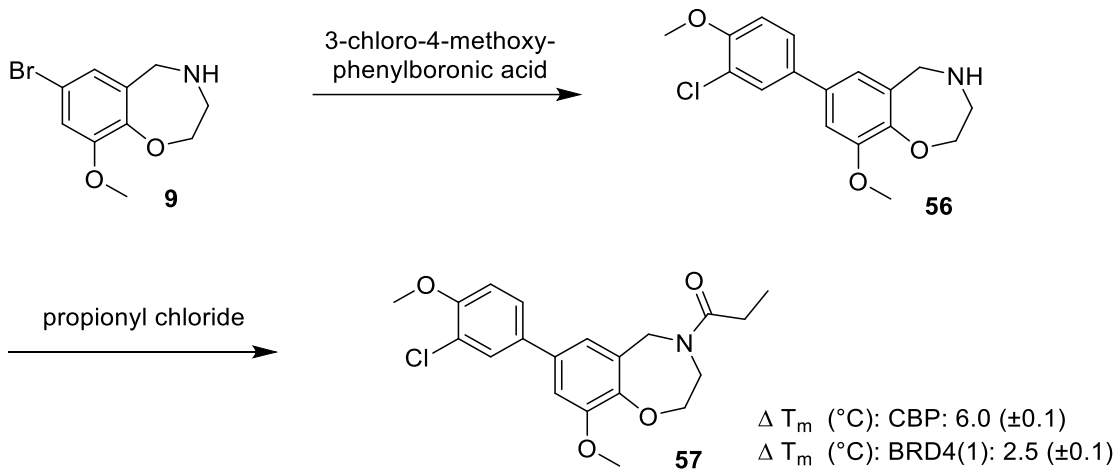
Since co-crystallizations indicated that **I-CBP112**'s bulky ether moiety at C-9 does not interact with CBP, we conducted first experiments on a simplified scaffold. Thanks to standard protocols[51b, 51c] a new 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold **9** with a methyl ether group at C-9 (Scheme 6.1) could be prepared on a large scale. An appropriate number of novel compounds could then be synthesized to obtain a clearer picture of promising substituents at C-7 and N-4. For variations at C-7 secondary amine **9** was first converted into intermediate **32**, bearing a promising acyl moiety at

N-4. Subsequently, palladium-catalyzed cross-coupling reactions were performed to introduce aryl and heteroaryl residues at C-7. Introduction of residues at C-7 bearing a positive or negative charge at physiological pH diminished the affinity to both bromodomains. Generally electron-rich phenyl substituents at C-7 were more potent, although this was not the case for all compounds. Finally, compound **40** with 3,5-dimethoxyphenyl moiety was the most promising in this series. For most compounds, affinity was only determined for CBP and not p300, since it was not expected to obtain selectivity for one of the two. However selectivity over the BET family was important and thus affinity towards BRD4(1) was determined, too.



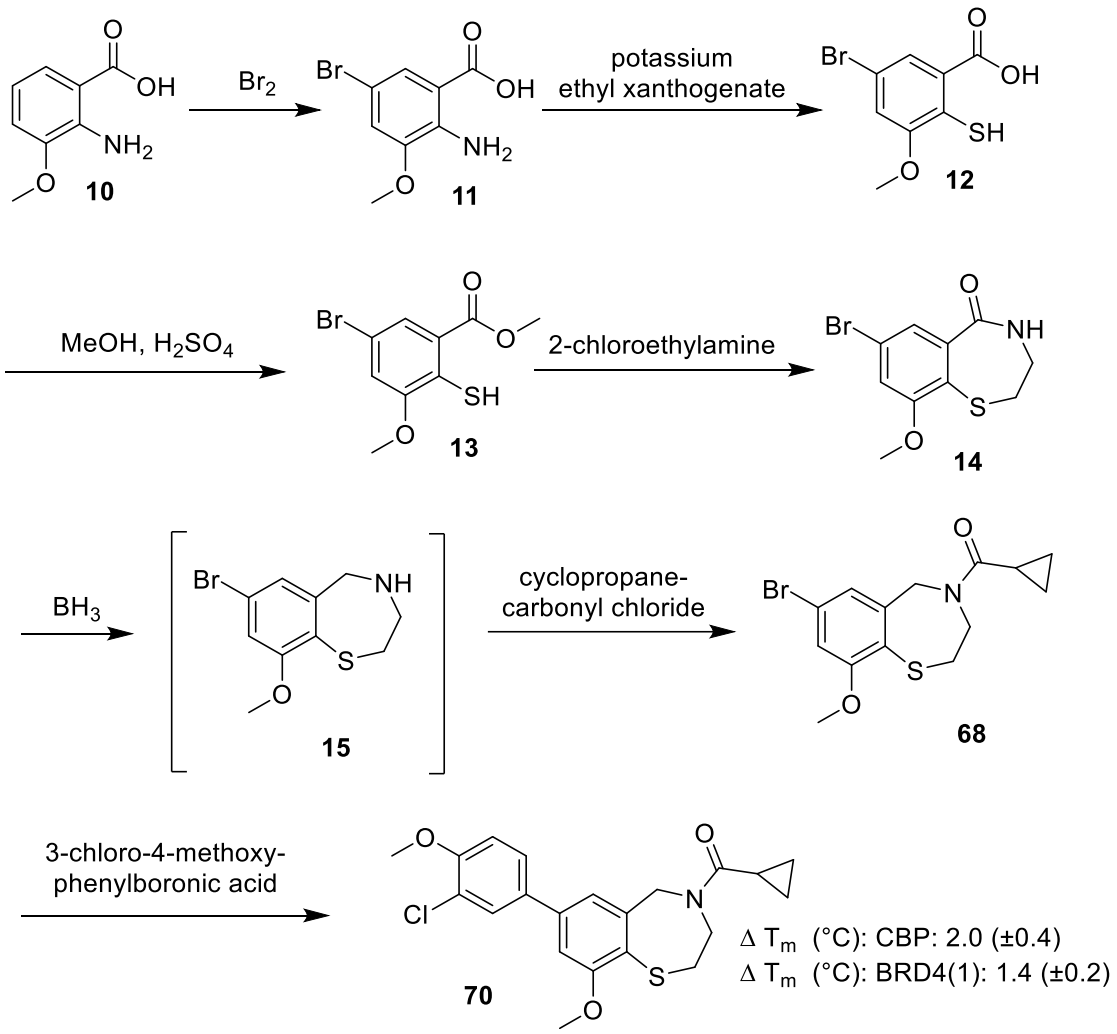
Scheme 6.1. In the process of optimizing residues at C-7, compound **40** showed the best activity in the DSF screening.

The optimization of the moiety at N-4 was conducted simultaneously. Converting bromoaryl scaffold **9** into the biaryl **56** allowed the introduction of eleven different moieties at N-4 to give a library with small, functional groups such as thioamides, carbamates, and (thio)ureas, which had not been tested for CBP inhibition before (Scheme 6.2). The most potent inhibitor in this series was the *N*-propionylamide **57**. Despite thorough examinations of the moieties at C-7 or N-4, no improvement of potency or selectivity over **I-CBP112** could be obtained so far.



Scheme 6.2. DSF screening of eleven compounds with different, small moieties at N-4 revealed that the **I-CBP112**'s *N*-propionyl residue is still the best.

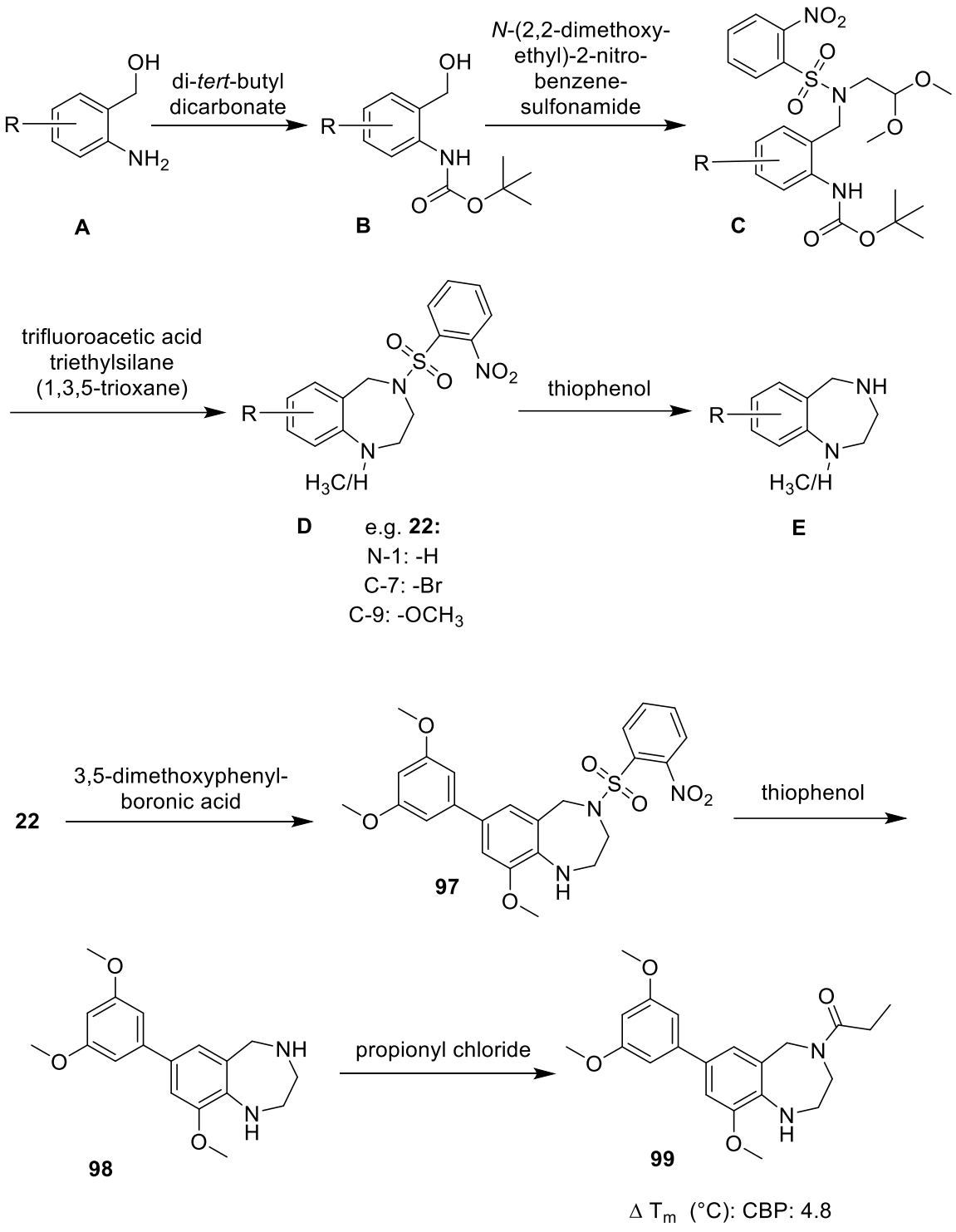
We next turned to the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold itself and aimed at the replacement of oxygen by sulfur (2,3,4,5-tetrahydro-1,4-benzothiazepine) or nitrogen (2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine). The preparation of the novel 2,3,4,5-tetrahydro-1,4-benzothiazepine scaffold **15** went as planned and described for similar compounds with satisfactory yields. Following bromination, the anthranilic acid **11** was converted into the mercaptobenzoic acid **12** (Scheme 6.3). Its methyl ester **13** was *S*-alkylated with 2-chloroethylamine and intramolecular amide formation gave lactam **14**. Reduction of **14** gave the secondary amine **15**, which was then converted into three novel structure analogues (e.g. compound **70**) of the 2,3,4,5-tetrahydro-1,4-benzoxazepine inhibitors. However, these new compounds were neither potent nor selective inhibitors of CBP.



Scheme 6.3. Preparation of the 2,3,4,5-tetrahydro-1,4-benzothiazepine scaffold and exemplarily the synthesis of the 2,3,4,5-tetrahydro-1,4-benzothiazepine analogue **70**.

Likewise the 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine **99** showed poor potency, not even slightly matching the efforts required to accomplish its synthesis. A classical, drastic approach to 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines via an isatoic acid anhydride[62] failed at the last step, and prompted us to experiment with *N*-nosylaziridine[63]. Having learned to supress side reactions and raise yields, the last intramolecular ring closure reaction, a Fukuyama-type[65] Mitsunobu[66] reaction could not be accomplished. Fortunately this failed synthesis gave inspiration for another approach: The desired compound was finally synthesized using the newly developed approach to monoprotected 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines *via* a one-pot *N*-deprotection/reductive cyclization procedure (Scheme 6.4). This approach begins with the *N*-Boc protection of a 2-aminobenzylalcohol **A**. A Fukuyama amine synthesis[65] with obtained *N*-Boc protected compounds of type **B** and *N*-(2,2-

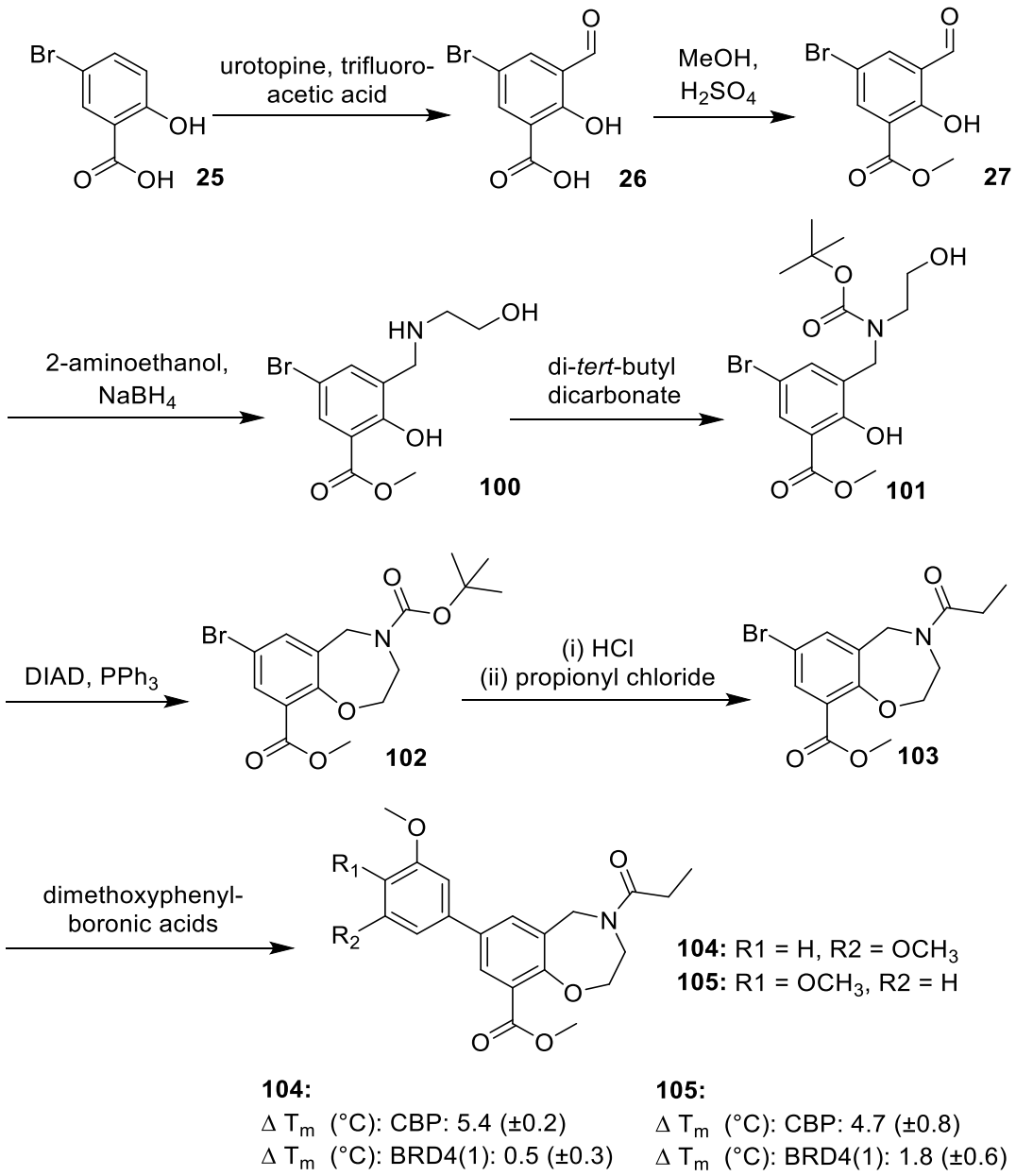
dimethoxy-ethyl)-2-nitrobenzenesulfonamide[67] under Mitsunobu conditions[66] is then conducted. The one-pot *N*-deprotection/reductive cyclization procedure of compounds with structure **C** with triethylsilane, trifluoroacetic acid, and dichloromethane directly gives a protected 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine **D** (e.g. compound **22**), which can be easily deprotected with thiophenol to obtain compounds of type **E**.



Scheme 6.4. Novel, mild approach to various 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine scaffolds and CBP inhibitor **99**.

The general applicability of this approach was demonstrated for five differently substituted 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines and was published by us in Tetrahedron in 2016.[96] The preparation is accomplished under mild conditions (mild reagents and temperature never >50 °C), consists of few steps and results in substances with a monoprotected aliphatic amine at position 4. This is advantageous, because it allows facile and selective conversion of the less nucleophilic nitrogen at position 1. As demonstrated, this conversion of N-1 may even be conducted in a one-pot reaction along with the described ring closure step. Given the importance of the 1,4-benzodiazepine scaffold,[56] we are confident that this approach will be of value for the community.

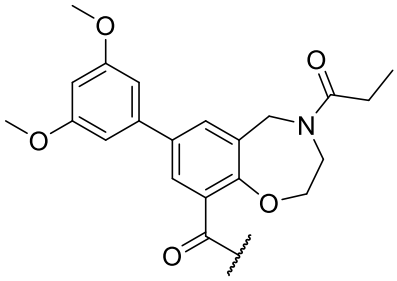
Having analyzed the moieties at C-7 (3,5-dimethoxyphenyl substituent best), and N-4 (propionyl best), and having found the 1,4-benzoxazepine scaffold to be the most suitable backbone, we turned to the ether function at C-9. This edge of the molecule had only been poorly explored by the SGC so far. No moieties other than an ether function with neutral or basic residues had been tested. Starting with Duff formylation of 5-bromosalicylic acid and subsequent esterification, we combined a number of known protocols[51b, 51c, 69] to develop an efficient five-step synthesis of the new 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold **102** with methyl ester group at C-9 (Scheme 6.5). Due to the numerous effects reported for 1,4-benzoxazepines (anti-inflammatory[49], anti-thrombotic[50], anti-tumor[51], and anti-amyloid-beta plaque activity[52]), particularly versatile intermediate **102**, which can be smoothly prepared in large scale, may be of interest for other researchers, too. Three further reactions with high yields (*N*-Boc deprotection, *N*-acylation, Suzuki cross-coupling) gave the methyl esters **104** and **105**, which were interesting as potential inhibitors and suitable intermediates for further syntheses.



Scheme 6.5. Preparation of the 2,3,4,5-tetrahydro-1,4-benzoxazepine scaffold with methyl ester at C-9.

Preparing several compounds using methyl esters **104** and **105** as educts, we found a slight superiority of the 3,5-dimethoxyphenyl substitution pattern over the 3,4-dimethoxyphenyl substitution pattern at C-7 for this scaffold. The uncharged, neutral methyl esters **104** and **105** were converted into the corresponding carboxylic acids and further into amides with protonable, basic amino moieties, thus giving negatively or positively charged side-chains at physiological pH. DSF results for CBP were similar for the three chemotypes (neutral, basic, acidic) with the charge seeming irrelevant. However, DSF screening revealed absolute selectivity over BRD4(1) for five (of six) compounds with carbonyl function at C-9. Further compounds were synthesized and

the introduction of an amide function at C-9 and a stereocenter yielded compounds that were comparable in potency with **I-CBP112**, but showed no affinity to BRD4(1) (**112** - **115**; Table 6.1). The configuration of the stereocenter itself was irrelevant.



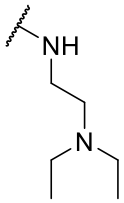
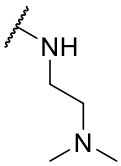
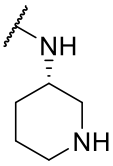
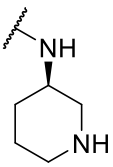
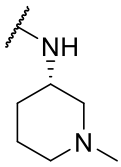
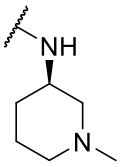
R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)	R	No.	Δ T_m (°C): CBP	Δ T_m (°C): BRD4(1)
	**110**	5.7 (±0.4)	0.4 (±0.3)		**111**	4.4 (±0.5)	0.2 (±0.2)
	**112**	7.2 (±0.4)	0.8 (±0.6)		**113**	6.8 (±0.4)	0.6 (±0.3)
	**114**	7.1 (±0.4)	0.5 (±0.3)		**115**	7.7 (±0.4)	1.2 (±0.2)

Table 6.1. Compounds **112** – **115** are of similar potency as **I-CBP112**, but show no affinity towards BRD4(1).

With absolute selectivity over BRD4(1) being a great result, compounds **112** and **114** were selected as lead structures and screened against a broad panel of 48 bromodomains. The T_m shifts of **114** were <1.3 °C with all bromodomains except CBP and p300 (Figure 6.2). This confirmed selectivity over other bromodomains, most importantly over the BET-family.

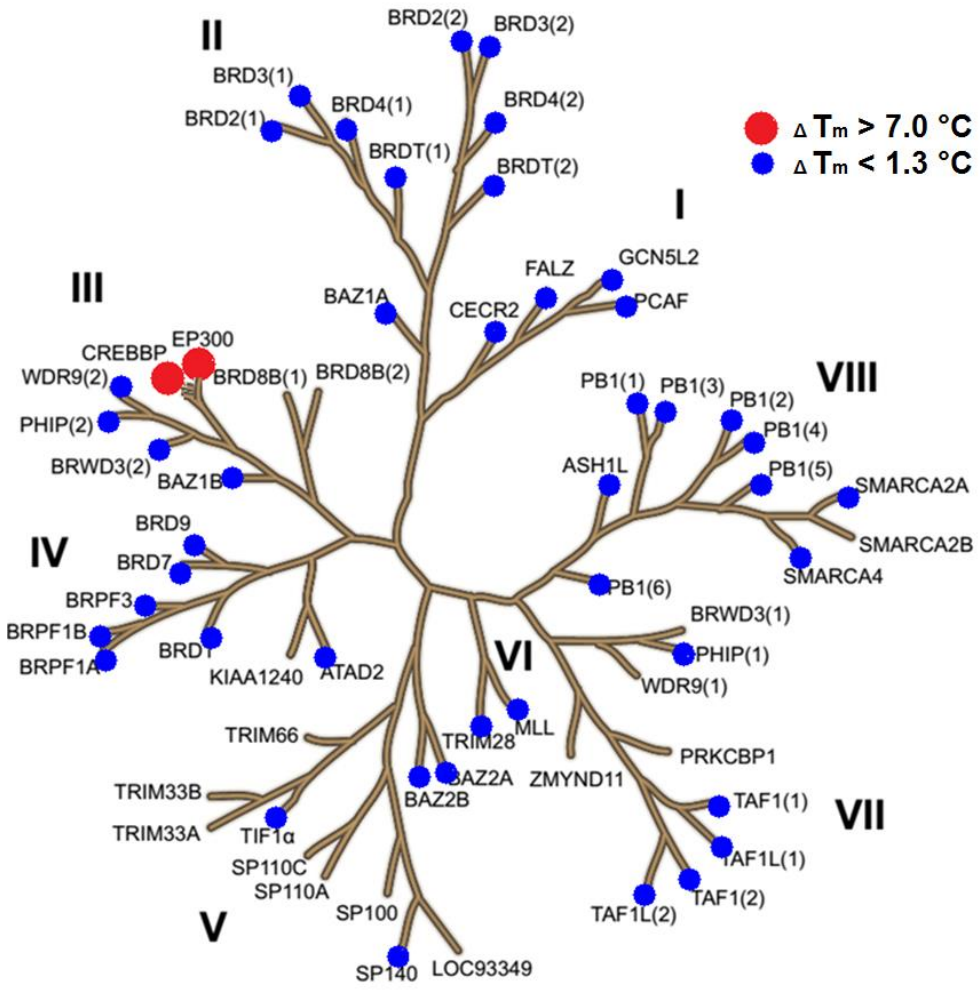
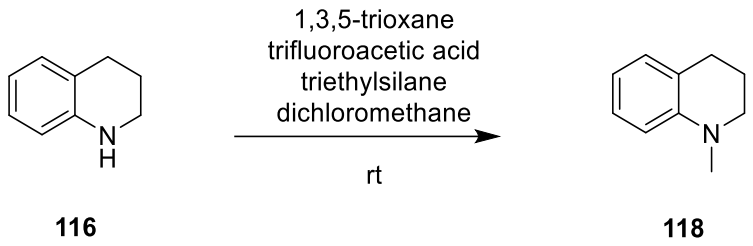


Figure 6.2. Comprehensive DSF screening with **114**. The few bromodomains without red or blue dots were not tested.

The K_d of **114** was determined as 134 nM for CBP, which is slightly lower than that of **I-CBP112** (151 nM). This may results from an additional water-mediated hydrogen bond that is revealed through a co-crystallization of CBP with competitive inhibitor **114**. Moreover a FRAP experiment with **112** demonstrated activity of this more selective generation of 1,4-benzoxazepine-type inhibitors in living, human cells. At the same time no general cytotoxic effects were found for **112**, **114**, and similar compounds in human leukemia cells (HL-60). This is essential when **114** shall be considered as a more selective alternative to preclinical drug candidate **I-CBP112**. Furthermore due to **114**'s greater selectivity compared to **I-CBP112**, **114** may serve as valuable, specific chemical probe for the further elucidation of the exact role of the bromodomains CBP and p300 in human cells.

In the course of this thesis we also developed a new protocol for the chemoselective, reductive *N*-methylation of aromatic amines and N-heterocycles. The protocol uses the new TTT-system (1,3,5-trioxane, trimethylsilane, trifluoroacetic acid, Scheme 6.6.) and was published in Synthesis in 2015.[124]



Scheme 6.6. The TTT-system.

It is a very mild protocol, conducted at room temperature and compatible with many functional groups sensitive to reduction, such as nitro groups or esters. No side reactions such as *C*-alkylation or overalkylation to quarternary ammonium salts, for which harsher protocols are known, were observed. We demonstrated that this protocol can be combined with other reactions, such as acidic deprotections or intramolecular reductive aminations. Yields ranged from 29-99 %, depending on the educt and this is the actual highlight of this protocol: The order of reactivity was reversed: Generally more reactive aliphatic amines are not *N*-methylated at all under our reaction conditions, and those aromatic amines are *N*-methylated with best yields, that are expected to be least nucleophilic. A selection of particularly interesting *N*-methylated products are shown in Figure 6.3. Unreactive nitrogens were *N*-methylated in excellent yields to anilines **120**, **125**, **126** and *N*-methyl phenoxazine **127**, while yield was worse for slightly more nucleophilic *N*-benzylaniline. Products **131** and **132** confirmed inertness of aliphatic amines and proved this protocol to be complementary to standard procedures, with which exclusively the more nucleophilic, aliphatic amine is *N*-methylated.

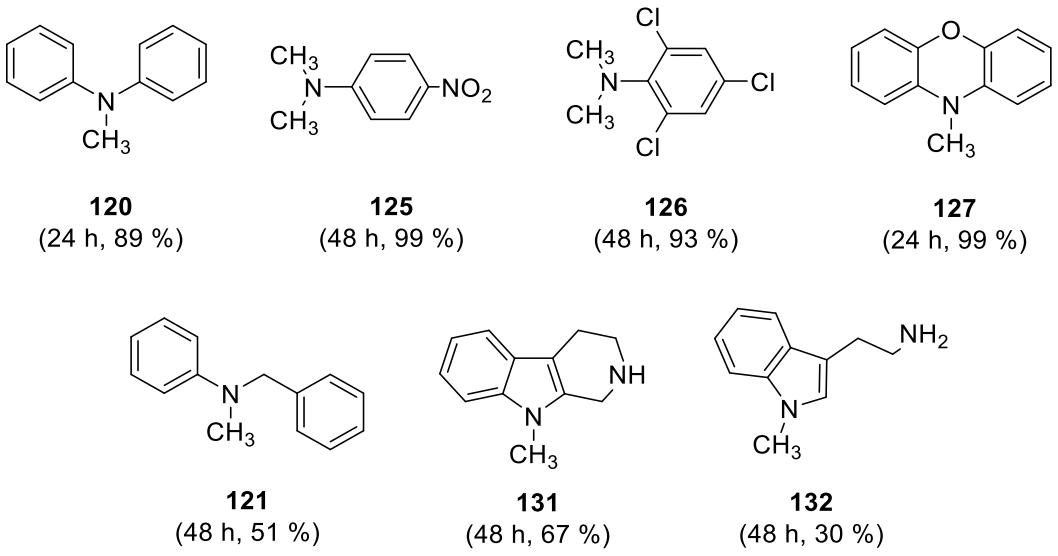


Figure 6.3. Highlighted products of the new *N*-methylation protocol. Reaction times and yields in parentheses.

As a final conclusion we could further enhance the CBP/p300 inhibitor **I-CBP112**, although retrospectively **I-CBP112** was already pretty optimized. However, introduction of the amide function at C-9 resulted in further selectivity over the BET-family (demonstrated by DSF), while slightly improving potency. It will be interesting to see, whether this selectivity yields benefits, and whether inhibitor **114** can replace **I-CBP112** in case its residual affinity towards the BET-family should be problematic. This enhancement of **I-CBP112** could be beneficial for the elucidation of the functions of CBP and p300 and possibly lead to novel therapeutic approaches. Furthermore two novel, valuable synthetic protocols were worked out and published during this thesis. Both protocols use mild reagents, temperatures from room temperature to a maximum of 50 °C, and are outstanding with regard to chemoselectivity: They allow conversion of a generally more unreactive aniline in the presence of an aliphatic amine. For the newly developed *N*-methylation protocol of aromatic amines and N-heterocycles, this is achieved due to the mere inertness of aliphatic amines in the TTT system. The newly published, short and mild approach towards 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines gives a product with nosyl-protected, aliphatic amine, allowing conversion of the aromatic amine prior to deprotection. Although we initially aimed at achieving selectivity in a pharmacological context, the scope of this thesis was soon successfully extended to the accomplishment of selectivity in purely chemical contexts.

Chapter VII - Experimental Section.

7.1 Procedures conducted by our cooperation partners at the University of Oxford

Protein expression

cDNA encoding human bromodomains were cloned, expressed and purified as described by Filippakopoulos *et al.* in 2010.[125]

Thermal shift assay

Thermal melting experiments were carried out using an Mx3005p Real Time PCR machine (Stratagene). Proteins were buffered in 10 mM HEPES pH 7.5, 500 mM NaCl and assayed in a 96-well plate at a final concentration of 2 μM in 20 μL volume. Compounds were added at a final concentration of 10 μM. SYPRO Orange (Molecular Probes) was added as a fluorescence probe at a dilution of 1:1000. Excitation and emission filters for the SYPRO-Orange dye were set to 465 nm and 590 nm, respectively. The temperature was raised with a step of 3 °C per minute from 25 °C to 96 °C and fluorescence readings were taken at each interval.[125]

Isothermal titration calorimetry (ITC)

Experiments were carried out on a VP-ITC microcalorimeter (MicroCal™). All experiments were performed at 15 °C in 50 mM HEPES pH 7.5, 150 mM NaCl. The titrations were conducted using an initial injection of 2 μl followed by 34 identical injections of 8 μl. The dilution heats were measured on separate experiments and were subtracted from the titration data. Thermodynamic parameters were calculated using $\Delta G = \Delta H - T\Delta S = -rtln K_B$, where ΔG, ΔH and ΔS are the changes in free energy,

enthalpy and entropy of binding respectively. In all cases a single binding site model was employed.

Alphascreen assay

Assays were performed as described previously[126] with minor modifications from the manufacturer's protocol (PerkinElmer, USA). All reagents were diluted in 25 mM HEPES, 100 mM NaCl, 0.1 % BSA, pH 7.4 supplemented with 0.05 % CHAPS and allowed to equilibrate to room temperature prior to addition to plates. A 11-point 1:2.5 serial dilution of the ligands was prepared over the range of 5000 – 0 µM and 0.1 µL transferred to low-volume 384-well plates filled with 5 uL of the assay buffer (ProxiPlateTM-384 Plus, PerkinElmer, USA), followed by 7 uL of biotinylated peptide H-ALREIRRYQK(ac) STELLIRKLK(biotin)-OH and His-tagged protein to achieve final assay concentrations of 50 nM. Plates were sealed and incubated for a further 30 minutes, before the addition of 8 µl of the mixture of streptavidin-coated donor beads (12.5 µg/ml) and nickel chelate acceptor beads (12.5 µg/ml) under low light conditions. Plates were foil-sealed to protect from light, incubated at room temperature for 60 minutes and read on a PHERAstar FS plate reader (BMG Labtech, Germany) using an Alphascreen 680 excitation/570 emission filter set. IC_{50} values were calculated in Prism 5 (GraphPad Software, USA) after normalization against corresponding DMSO controls and are given as the final concentration of compound in the 20 µl reaction volume.

Protein crystallization

Aliquots of the purified proteins were set up for crystallization using a mosquito® crystallization robot (TTP Labtech). Coarse screens were typically setup onto Greiner 3-well plates using three different drop ratios of precipitant to protein per condition (100+50 nL, 75+75 nL and 50+100 nL). All crystallizations were carried out using the sitting drop vapour diffusion method at 4°C. CBP crystals with 114 (2 mM final concentration) were grown by mixing 200 nL of the protein (8.6 mg/ml) with 100 µL of

reservoir solution containing 0.10 M $MgCl_2$, 0.1 M MES pH 6.0, 20 % PEG 6K and 10 % ethylene glycol.

Fluorescence recovery after photobleaching (FRAP)

FRAP studies were performed essentially as described.[76] In brief, U2OS cells were transfected (Fugene HD; Roche) with mammalian over-expression constructs a triplicated CBP bromodomain harbouring a nuclear localization sequence. The imaging system consisted of a Zeiss LSM 710 laser-scanning and control system (Zeiss) coupled to an inverted Zeiss Axio Observer.Z1 microscope equipped with a high-numerical-aperture (N. A. 1.3) 40 x oil immersion objective (Zeiss). Samples were placed in an incubator chamber in order to maintaining temperature and humidity. FRAP and GFP fluorescence imaging were both carried out with an argon-ion laser (488 nm) and with a PMT detector set to detect fluorescence between 500-550 nm. Once an initial scan had been taken, a region of interest corresponding to approximately 50 % of the entire GFP positive nucleus was empirically selected for bleaching. A time lapse series was then taken to record GFP recovery using 1% of the power used for bleaching. The image datasets and fluorescence recovery data were exported from ZEN 2009, the microscope control software, into Origin to determine the average half-time for full recovery for 10-20 cells per treatment point. Data were analysed using one-way ANOVA with Dunnetts's multiple comparisons test.

7.2 General procedures for biological characterization conducted by Martina Stadler in the Bracher laboratory of the LMU

MTT assay

The MTT assay was conducted with HL-60 cells. First the number of cells per mL was determined with a hematocyte cell counter (Fuchs-Rosenthal). Then the cell suspension was diluted with medium to 9 x 10^5 cells mL^{-1} on a Petri dish. 99 µL of this cell suspension were filled into each well of a 96-well plate and incubated at 37 °C for 24 h. 10 mM stock solutions were prepared from the synthesized compounds in DMSO. These were diluted with DMSO six times 1 : 2. For negative control 1 µL of DMSO was added to the 99 µL cell medium in the wells. For positive control 1 µL of Triton® X-100 solution with a final concentration of 1 µg/mL was added. Of each dilution of the stock solution 1 µL was added to a well with 99 µL cell culture. The 99-well plate was incubated at 37 °C with 5 % CO_2 for 24 h. Then 10 µL MTT solution (5 mg MTT in 1.0 mL PBS) was added to each well and further incubated for two hours. Then 190 µL DMSO were added and the plate shaked for one hour. Photometric quantification was conducted at a wavelength of 570 nm with an ELISA reader (SLT Spectra, Crailsheim). Statistical analysis and calculation of IC_{50} values was done with Prism 4 Software (GraphPad, USA).

Agar diffusion test

Solutions with 1 % (m/V) compound in DMSO were prepared. Of these solutions 3.0 µL were given on a test platelete (diameter 6 mm, Macherey-Nagel), equivalent to 30 µg substance. The same was done for clotrimazole and tetracycline. Blind control was conducted with mere DMSO. The test platelets were then dried for 24 hours at room temperature. Microorganisms were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig and cultivated according to recommendations in liquid culture. For the agar diffusion test, different agars were required. For *Candida glabrata* (DSM number: 11226), *Hyphopichia burtonii* (DSM number: 70663)*, Yarrowia lipolytica* (DSM number: 1345)*, Escherichia*

coli (DSM number: 426) and *Pseudomonas marginalis* (DSM number: 7527) all-culture agar (AC-agar) of Sigma Aldrich was used. 35.2 g AC-agar and 20 g agar were suspended in 1.0 L water and treated by autoclave. For *Staphylococcus equorum* (DSM number: 20675) and *Streptococcus entericus* (DSM number: 14446) an agar is likewise prepared from 10.0 g caseinpeptone, 5.0 g yeast extract, 5.0 g glucose and 5.0 g sodium chloride in 1.0 L water. For *Aspergillus niger* (DSM number: 1988) 32 g potato dextrose agar and 20 g agar in 1.0 L water were used. After treatment in the autoclave 15 mL of the warm, liquid agar was filled into Petri dishes under aseptic conditions and cooled to 8 °C for one hour. The germs were then brought onto the different agars using cotton swabs. The platelets containing the substances, the reference, and the blind control were put onto the agar. The agar plates were incubated for 36 h at 32 °C (bacteria) or 28 °C (yeasts). Then the diameters of growth inhibiton were measured manually.

7.3 General procedures for compound preparation and chemical characterization conducted in the Bracher laboratory of the LMU

Melting points were determined with a Büchi Melting Point B-540 and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded either with Avance III HD 400 MHz Bruker BioSpin or Avance III HD 500 MHz Bruker BioSpin spectrometers. Chemical shifts (δ) are given in ppm relative to TMS or residual undeuterated solvent, and coupling constants (*J*) are given in hertz (Hz). Splitting patterns are abbreviated as follows: s = singlet; d = doublet; t = triplet; q = quartet; dd = doublet of doublet; m = multiplet, br s = broad singlet. If no temperature is given, measurements were performed at ambient temperature, but some NMR spectra were recorded at elevated temperature to suppress the appearance of double peaks arising from the rotameric isomers. Those occurred in the NMR spectra of almost all CBP inhibitors and arise from the (thio-)amide bond of the aliphatic nitrogen (N-4) of the seven-membered ring. EI mass spectra were recorded at an ionization energy of 70 eV either with a JMS GCmate II Jeol or a JEOL JMS-700 MStation. ESI-Mass spectra were recorded on a Thermo Finnigan LTQ FT at 4 kV. Purification by flash column chromatography (FCC) was performed using Silica Gel 60 from Merck KGaA. For microwave experiments a CEM

Discover was used with power set to 300 W. Optical rotations were determined with a Perkin Elmer 241 polarimeter. HPLC purity analysis was individually performed on an Agilent 1100 Series apparatus with a G1311A QuatPump, and a G1329A ALS autosampler, and a G1316A ColComp column oven, and Agilent ChemStation Rev. B04.02 as software. A G1315A DAD detector was set to 210 nm for detection. Injection volume was 5 or 10 µL of a dilution of 100 µg/mL (sample in mobile phase). Column temperature was 50 °C, flow either 0.3 mL/min, or 0.8 mL/min or 1.0 mL/min. Different solvent mixtures were used as mobile phase, from 50 % to 25 % water and from 50 % to 75 % acetonitrile respectively. The water used for preparation of the mobile phase contained 1 % THF. The following columns were used: Kinetex 2.6 u PFP, 100 A, (100 x 2.10 mm), Agilent Poroshell 120, PFP 2.7 µm, (3.0 x 100 mm), Varian Pursuit UPS 2.4 Diphenyl (50 x 2.0 mm), and Agilent Poroshell 120, EC-C18 2.7 µm, (3.0 x 100 mm). All tested substances showed a purity > 95.0 %. The key to the different methods is shown on the next page.

Methods for the determination of purity by HPLC

Method 1: Agilent Poroshell 120, EC-C18 2.7 µm, (3.0 x 100 mm); flow: 1 mL/min;

a) MeCN – water with 0,1 % NaOH: 75 - 25;

b) MeCN – water with 0,1 % NaOH: 55 - 45;

c) MeCN – water with 0,1 % NaOH: 70 - 30;

d) MeCN – water: 70 - 30;

Method 2: Kinetex 2.6 u PFP, 100 A, (100 x 2.10 mm); flow: 0.3 mL/min;

a) MeCN – water: 50 - 50;

b) MeCN – water with 0,1 % NaOH: 50 - 50;

Method 3: Agilent Poroshell 120, PFP 2.7 µm, (3.0 x 100 mm); flow: 1 mL/min;

a) MeCN – water: 70 - 30;

b) MeCN – water: 50 - 50;

Method 4: Agilent Poroshell 120, EC-C18 2.7 µm, (3,0 x 100 mm); flow: 0.8 mL/min;

a) MeCN – water: 55 - 45;

Method 5: Varian Pursuit UPS 2.4 Diphenyl (50 x 2.0 mm); flow: 0.3 mL/min;

a) MeCN – water: 55 – 45;

Method 6: Agilent Poroshell 120, EC-C18 2,7 µm, (3,0 x 100 mm); flow: 0.8 mL/min;

a) MeCN – water: 80 - 20;

b) MeCN – water with 0,1 % NaOH: 75 - 25;

Method 7: Agilent Poroshell 120, EC-C18 2,7 µm, (3,0 x 100 mm); flow: 1 mL/min;

a) MeCN – water: 75 - 25;

b) MeCN – water: 80 - 20;

Standard synthetic protocols

Standard protocol 1 (*N*-acylation of 2,3,4,5-tetrahydro-1,4-benzoxazepines and 2,3,4,5-tetrahydro-1,4-benzothiazepines): The educt (0.50 mmol) was dissolved in 1.0 mL DCM and 1.5 mmol DIPEA was added at 0 °C. 1.3 mmol acyl chloride was added, the mixture warmed to rt and stirred for 1.5 h. Then 50 mL 2 M NaOH was added and the mixture was extracted with DCM (3 x 50 mL) three times. The combined organic layers were dried over $MgSO_4$, concentrated in vacuo and purified by FCC with EtOAc and hexanes.

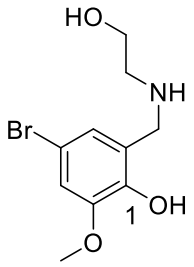
Standard protocol 2 (Suzuki cross-coupling of 7-bromo-2,3,4,5-tetrahydro-1,4-benzoxazepines and 7-bromo-2,3,4,5-tetrahydro-1,4-benzothiazepines with boronic acids and boronic acid pinacol esters): To 0.30 mmol bromoarene, 0.36 mmol boronic acid or boronic acid pinacol ester and 0.03 mmol [1.1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) were added. A mixture of 0.50 mL water, 2.0 mL 1,4-dioxane and 1.2 mmol DIPEA was added under nitrogen atmosphere, and the mixture heated under vigorous stirring to 95 °C for 3.5 h. To this solution was added 20 mL of either water or 0.5 M NaOH for compounds with basic moieties or 0.5 M HCl for compounds with acidic moieties. The mixture was extracted with DCM (3 x 20 mL) three times and the combined organic layers were dried over $MgSO_4$, and concentrated in vacuo, and purified by FCC with EtOAc and hexanes.

Standard protocol 3 (conversion of the carboxylic acids **106** and **107** to carboxamides): 0.29 mmol carboxylic acid and 0.35 mmol EDC-HCl were dissolved in 3.0 mL DCM and cooled to 0 °C. Then 0.29 mmol DIPEA, 0.35 mmol of the required primary amine, and 2 mg DMAP were added. The solution was stirred at rt for 16 h. To this mixture was added 50 mL 1 M NaOH was added and the mixture was extracted with EtOAc (3 x 50 mL) three times. The combined organic layers were dried over $MgSO_4$, concentrated in vacuo, and purified by FCC.

7.4 Description of compounds

4-Bromo-2-{[(2-hydroxyethyl)amino]methyl}

-6-methoxyphenol (6)



MF: $C_{10}H_{14}BrNO_3$ MW: 276.13 g/mol

To a solution of 9.9 g (43 mmol) 5-bromo-3-methoxysalicylaldehyde in 200 mL THF and 20 mL MeOH, 3.3 g (54 mmol) 2-aminoethanol were added and the mixture was stirred for 25 min. Over 1.5 h three equal portions of 1.5 g (40 mmol) $NaBH_4$ were added and the mixture stirred for 16 h. The solvent was evaporated under reduced pressure, and the residue dissolved in 100 mL EtOAc. Upon addition of 200 mL water, the product partially precipitated as white solid and was collected by filtration. This mixture was extracted with EtOAc (3 x 200 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC of precipitate and concentrate of organic phases with EtOAc and MeOH (4:1, Rf 0.3) gave 11 g (40 mmol, 93 %) of **6** as a white solid.

mp: 153 - 154 °C.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 6.96 (d, *J* = 2.2 Hz, 1H, 5-H), 6.88 (d, *J* = 2.2 Hz, 1H, 3-H), 3.80 (s, 2H, 2-CH_2), 3.75 (s, 3H, OCH_3), 3.46 (t, *J* = 5.7 Hz, 2H, CH_2OH), 2.54 (t, *J* = 5.7 Hz, 2H, CH_2NH).

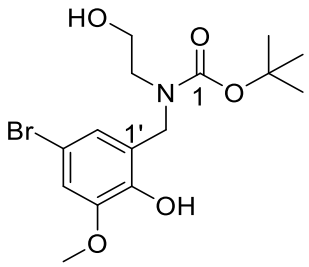
^{13}C NMR (101 MHz, DMSO-d_6): δ = 148.3 (C-6), 145.9 (C-1), 126.3 (C-2), 122.7 (C-3), 113.5 (C-5), 108.8 (C-4), 59.7 (CH_2OH), 55.8 (OCH_3), 50.2 (CH_2NH), 49.5 (2-CH_2).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3178, 2949, 2936, 2880, 2830, 1618, 1556, 1483, 1443, 1321, 1246, 1087, 967, 828, 762.

MS (EI+): m/z (%) = 108 (32), 215 (100), 217 (88), 244 (50), 246 (52), 275 (14) $[M]^{+\bullet}$, 277 (15).

HRMS (EI+): m/z calcd for $C_{10}H_{14}{}^{79}BrNO_3$ 275.0157, found 275.0157.

tert-Butyl (5-bromo-2-hydroxy-3-methoxybenzyl)(2-hydroxyethyl)carbamate (7)



MF: $C_{15}H_{22}BrNO_5$ MW: 376.25 g/mol

To a suspension of 11 g (40 mmol) **6** in 100 mL EtOAc and 58 mL saturated $NaHCO_3$ solution, 12 g (53 mmol) di-*tert*-butyl dicarbonate were added and the mixture was stirred for 16 h. The suspension turned into a clear two phase system and the organic layer was separated. The aqueous phase was extracted with EtOAc (3 x 60 mL) three times, and the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (3:2, Rf 0.5) gave 11 g (28 mmol, 69 %) of **7** as a white solid.

mp: 95 - 96 °C.

^{1}H NMR (70 °C, 400 MHz, DMSO-d_6): δ = 8.75 (s, 1H, 2'-OH), 7.01 (d, J = 2.3 Hz, 1H, 4'-H), 6.81 (d, J = 2.3 Hz, 1H, 6'-H), 4.53 – 4.32 (m, 3H, 1'-CH_2, CH_2OH), 3.81 (s, 3H, OCH_3), 3.53 – 3.43 (m, 2H, CH_2OH), 3.32 – 3.19 (m, 2H, NCH_2), 1.40 (s, 9H, $(CH_3)_3$).

^{13}C NMR (70 °C, 101 MHz, DMSO-d_6): δ = 154.9 (C-1), 148.3 (C-3'), 143.3 (C-2'), 127.1 (C-1'), 122.3 (C-6'), 113.5 (C-4'), 109.4 (C-5'), 78.6 ($C(CH_3)_3$), 58.9 (CH_2OH), 56.1 (OCH_3), 49.0 (NCH_2), 45.2 (1'-CH_2), 27.7 ($(CH_3)_3$).

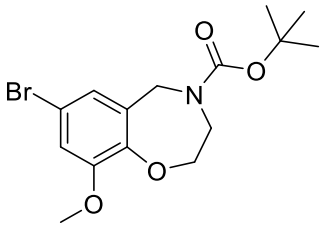
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3447, 3200, 2975, 2943, 1669, 1460, 1415, 1233, 1163, 1055, 955, 830.

MS (EI+): m/z (%) = 244 (100), 246 (91), 319 (15), 321 (10), 375 (8) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{15}H_{22}{}^{79}BrNO_5$ 375.0681, found 375.0685.

Purity (HPLC): 98 % (210 nm; method 5a).

tert-Butyl 7-bromo-9-methoxy-2,3-dihydro-1,4-benzoxazepine-4(5_H_)-carboxylate (8)



MF: $C_{15}H_{20}BrNO_4$ MW: 358.23 g/mol

To a solution of 9.7 g (26 mmol) **7** and 11 g (42 mmol) triphenylphosphine in 260 mL DCM, 8.7 mL (40 mmol) DIAD were added and the mixture was stirred for 16 h. Then 100 mL water was added and the mixture was extracted with EtOAc (3 x 100 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:4, Rf 0.3) gave 8.6 g (24 mmol, 91 %) of **8** as a white solid.

mp: 96 - 97 °C.

^{1}H NMR (70 °C, 400 MHz, DMSO-d_6): δ = 7.09 (d, J = 2.3 Hz, 1H, 8-H), 6.98 (d, J = 2.3 Hz, 1H, 6-H), 4.38 (s, 2H, 5-H), 4.05 – 3.93 (m, 2H, 2-H), 3.78 (s, 3H, OCH_3), 3.74 – 3.67 (m, 2H, 3-H), 1.35 (s, 9H, $C(CH_3)$).

^{13}C NMR (70 °C, 101 MHz, DMSO-d_6): δ = 154.6 (C=O), 152.8 (C-9), 148.2 (C-9a), 134.9 (C-5a), 124.4 (C-6), 116.0 (C-8), 114.7 (C-7), 79.8 ($C(CH_3)$), 72.3 (C-2), 56.9 (OCH_3), 49.9 (C-3), 49.3 (C-5), 28.5 ($C(CH_3)$).

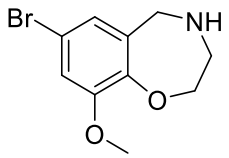
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3432, 2975, 2933, 2867, 2839, 1686, 1578, 1486, 1455, 1399, 1297, 1188, 1082, 1023, 834, 721.

MS (EI+): m/z (%) = 134 (41), 214 (62), 216 (54), 257 (100), 259 (80), 300 (84), 302 (88), 357 (59) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{15}H_{20}{}^{79}BrNO_4$ 357.0576, found 357.0575.

Purity (HPLC): > 99 % (210 nm; method 5a).

7-Bromo-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepine (9)



MF: $C_{10}H_{12}BrNO_2$ MW: 258.12 g/mol

To a suspension of 10 g (28 mmol) **8** in 120 mL MeOH was added a mixture of 80 mL 36 % HCl and 120 mL 1,4-dioxane. The mixture was refluxed for 2 h and then concentrated in vacuo. Then 200 mL saturated Na_2CO_3 solution was carefully added and the mixture was extracted with DCM (3 x 200 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give 6.7 g (26 mmol, 94 %) of **9** as a white solid.

mp: 119 - 120 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.05 (d, *J* = 2.4 Hz, 1H, 8-H), 6.98 (d, *J* = 2.4 Hz, 1H, 6-H), 3.93 – 3.83 (m, 2H, 2-H), 3.75 (s, 3H, OCH_3), 3.73 (s, 2H, 5-H), 3.06 – 2.94 (m, 2H, 3-H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 153.1 (C-9), 149.0 (C-9a), 140.0 (C-5a), 124.1 (C-6), 115.2 (C-8), 115.1 (C-7), 75.8 (C-2), 56.9 (OCH_3), 52.9 (C-5), 52.8 (C-3).

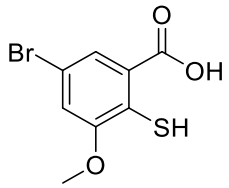
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3433, 3324, 3085, 2978, 2951, 2930, 2904, 1736, 1690, 1590, 1574, 1485, 1289, 1266, 1205, 1079, 985, 834.

MS (EI+): m/z (%) = 134 (30), 214 (38), 216 (32), 257 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{10}H_{12}{}^{79}BrNO_2$ 257.0051, found 257.0052.

Purity (HPLC): > 99 % (210 nm; method 5a).

5-Bromo-2-mercapto-3-methoxybenzoic acid (12)



MF: $C_8H_5O_2BrS$ MW: 263.11 g/mol

A suspension of 9.6 g (30 mmol) 4-bromo-2-carboxy-6-methoxybenzenaminium bromide[54], 2.4 g (60 mmol) NaOH, and 2.1 g (30 mmol) $NaNO_2$ in 60 mL water was added over 0.5 h to a mixture of 20 mL conc. HCl with ice and the temperature was kept at 0 °C by the addition of ice. After 0.5 h at 0 °C, potassium acetate was used to adjust to neutral pH. The resulting yellow solution was added to a stirred solution of 23 g (140 mmol) of potassium ethyl xanthate in 40 mL water at 90 °C. After 0.5 h at 90 °C the solution was cooled to 0 °C. Conc. HCl was added until acidic pH. The resulting precipitate was collected by filtration and dissolved in 100 mL 10 % NaOH. The solution was heated to 85 °C for 2 h. Then 3.1 g (30 mmol) $NaHSO_3$ were added and 85 °C were maintained for 0.25 h. The solution was filtrated, cooled to 0 °C, and acidified with conc. HCl. The precipitate was separated by filtration and dissolved in 300 mL of a mixture of EtOAc and THF (1:1). This organic layer was washed with 100 mL saturated NaCl solution twice, dried over $MgSO_4$ and concentrated in vacuo. Purification by FCC with DCM with 4 % EtOH and 5 % AcOH (Rf 0.2) gave 4.9 g (19 mmol, 62 %) of **12** as a white solid.

mp: 208 – 209 °C.

^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.90 (d, *J* = 2.0 Hz, 6-H), 7.20 (d, *J* = 2.0 Hz, 4-H), 5.28 (br s, 1H, SH), 3.96 (s, 3 H, OCH_3).

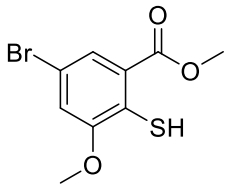
^{13}C NMR (101 MHz, CD_2Cl_2): δ = 169.2 (C=O), 155.3 (C-3), 129.0 (C-2), 127.1 (C-6), 127.0 (C-1), 118.0 (C-4), 117.3 (C-5), 57.4 (OCH_3).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3445, 3066, 3007, 2973, 2941, 2855, 2622, 1695, 1449, 1316, 1255, 1058, 856.

MS (EI+): m/z (%) = 109 (17), 186 (20), 188 (20), 216 (30), 218 (29), 244 (100) $[M]^{+\bullet}$, 246 (91), 262 (39), 264 (35).

HRMS (EI+): m/z calcd for $C_8H_5O_2{}^{79}BrS$ 243.9193, found 243.9195.

Methyl 5-bromo-2-mercapto-3-methoxybenzoate (13)



MF: $C_9H_9O_3BrS$ MW: 277.13 g/mol

A solution of 3.0 g (12 mmol) **12** in 25 mL anhydrous MeOH and 1.0 mL 96 % sulfuric acid was refluxed under N_2 for 12 h and then concentrated in vacuo. Ice was added and the mixture was extracted with EtOAc (3 x 100 mL) three times. After drying over $MgSO_4$ and concentration in vacuo, FCC with hexanes and EtOAc (9:1, Rf 0.4) gave 2.3 g (8.6 mmol, 72 %) of **13** as a yellow solid.

mp: 60 - 61 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.69 – 7.65 (m, 1H, 6-H), 7.45 – 7.42 (m, 1H, 4-H), 5.42 – 5.39 (m, 1H, SH), 3.95 (s, 3H, 5-OCH_3), 3.85 (s, 3H, O=$COCH_3$).

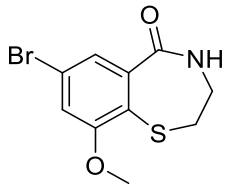
^{13}C NMR (126 MHz, DMSO-d_6): δ = 165.2 (C=O), 154.8 (C-3), 127.7 (C-2), 126.3 (C-1), 125.1 (C-6), 117.3 (C-4), 116.7 (C-5), 57.3 (5-OCH_3), 52.6 (O=CO*C*H_3).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3435, 2971, 2951, 2939, 1715, 1448, 1427, 1315, 1258, 1059, 838, 777, 624.

MS (EI+): m/z (%) = 276 (100) $[M]^{+\bullet}$, 277 (11), 278 (97), 279 (11).

HRMS (EI+): m/z calcd for $C_9H_9O_3{}^{79}BrS$ 275.9456, found 275.9452.

7-Bromo-9-methoxy-3,4-dihydro-1,4-benzothiazepin-5(2*H*)-one (14)



MF: $C_{10}H_{10}BrNO_2S$ MW: 286.96 g/mol

To a solution of 2.3 g (8.3 mmol) **13** and 1.0 g (9.0 mmol) 2-chloroethylamine hydrochloride in 17 mL anhydrous DMF at 0 °C under N_2, 1.0 g (19 mmol) NaOMe was added and the mixture stirred for 12 h. Water was added, the pH adjusted with 2 M NaOH to 12 and the mixture extracted with DCM three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in 60 mL anhydrous THF and cooled to 0 °C. 7.2 g (64 mmol) *t*-BuOK was added and the mixture stirred at 45 °C for 1 h. Saturated ammonium chloride solution was added and the mixture extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with DCM with 4 % MeOH (Rf 0.3) gave 1.5 g (5.2 mmol, 63 %) of **14** as a yellow solid.

mp: 185 - 186 °C.

^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.33 (d, *J* = 2.0 Hz, 1H, 6-H), 7.16 (d, *J* = 2.0 Hz, 1H, 8-H), 7.02 (br s, 1H, NH), 3.87 (s, 3H, OCH_3), 3.32 – 3.26 (m, 2H, 3-H), 3.10 – 3.05 (m, 2H, 2-H).

^{13}C NMR (101 MHz, CD_2Cl_2): δ = 170.9 (C-5), 160.3 (C-9), 144.1 (C-5a), 124.4 (C-6), 123.7 (C-7), 117.1 (C-8), 116.9 (C-9a), 57.0 (OCH_3), 40.2 (C-3), 37.4 (C-2).

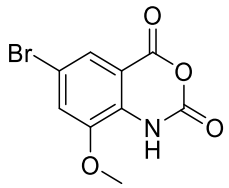
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3290, 3189, 3075, 2939, 1661, 1559, 1423, 1379, 1253, 1049, 888, 834, 809, 645, 609.

MS (ESI+): m/z (%) = 210 (13), 288 (98) $[M + H]^+$, 290 (100).

HRMS (ESI+): m/z calcd for $[C_{10}H_{11}{}^{79}BrNO_2S]^+$ 287.9694, found 287.9688.

Purity (HPLC): > 99 % (210 nm; method 5a).

6-bromo-8-methoxy-3,1-benzoxazine-2,4(1*H*)-dione (16)



MF: $C_9H_6BrNO_4$ MW: 272,05 g/mol

4.5 g (14 mmol) **11** was suspended in 240 mL anhydrous THF under nitrogen atmosphere. After addition of 2.4 mL DIPEA (25 mmol) a solution was obtained, which was refluxed. A solution of 1.8 g (6.1 mmol) triphosgene in 30 mL anhydrous THF was added and the mixture was refluxed for 0.5 h. The mixture was then cooled to rt, and 300 mL saturated $NaHCO_3$ solution was added. The mixture was extracted with DCM (3 x 300 mL) three times and the combined organic layers were concentrated in vacuo. The obtained precipitate was washed with DCM to obtain 3.0 g (11 mmol, 79 %) of **16** as a white solid.

mp: 208 °C (decomposition).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.45 (s, 1H, NH), 7.58 – 7.51 (m, 2H, 5-H, 7-H), 3.91 (s, 3H, OCH_3).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 158.8 (C-4), 147.3 (C-8), 146.5, 131.3, 121.2 (C-5/C-7), 120.0 (C-5/C-7), 114.6, 112.3, 56.9 (OCH_3).

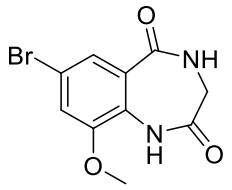
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3391, 3197, 3074, 2946, 1789, 1721, 1502, 1336, 1251, 1001.

MS (ESI-): m/z (%) = 226 (21), 228 (21), 270 (87) $[M - H]^-$, 272 (100).

HRMS (ESI-): m/z calcd for $C_9H_5Br^{79}NO_4$ 269.9407, found 269.9403.

Purity (HPLC): > 99 % (210 nm; method 5).

7-bromo-9-methoxy-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione (17)



MF: $C_{10}H_9BrN_2O_3$ MW: 285,10 g/mol

0.20 g (0.74 mmol) **16** and 0.11 g (1.5 mmol) glycine were suspended in 1 mL anhydrous DMF and 1 mL glacial acetic acid. This mixture was refluxed for 18 h. The mixture was then cooled to room temperature and 50 mL saturated $NaHCO_3$ solution was added. The mixture was extracted with EtOAc (3 x 50 mL) three times and the combined organic layers were dried over $MgSO_4$. After concentration in vacuo, the obtained solid was washed with DCM to give 0.15 g (0.53 mmol, 72 %) of **17** as a white solid.

mp: 243 – 244 °C.

^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.85 (br s, 1H, NH), 7.63 (d, J = 2.1 Hz, 1H, 6-H), 7.19 (d, J = 2.1 Hz, 1H, 8-H), 6.77 (br s, 1H, NH), 3.91 (s, 3H, OCH_3), 3.81 (d, J = 0.7 Hz, 1H, 3-H), 3.80 (d, J = 0.7 Hz, 1H, 3-H).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 170.2 (C-2), 167.7 (C-5), 149.7 (C-9), 126.5 (C-5a/C-7), 126.1 (C-9a), 125.5 (C-6), 117.6 (C-5a/C-7), 117.2 (C-8), 57.1 (OCH_3), 45.4 (C-3).

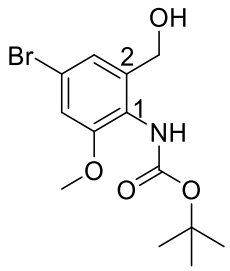
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3218, 3081, 2918, 1689, 1624, 1482, 1457, 1370, 1244, 1056.

MS (ESI-): m/z (%) = 283 (91) $[M - H]^-$, 285 (100).

HRMS (ESI-): m/z calcd for $C_{10}H_8Br^{79}N_2O_3$ 282.9724, found 282.9719.

Purity (HPLC): > 99 % (210 nm; method 5).

tert-Butyl [4-bromo-2-(hydroxymethyl)-6-methoxyphenyl]carbamate[a] (23)



MF: $C_{13}H_{18}BrNO_4$ MW: 332.19 g/mol

To a solution of 2.3 g (11 mmol) di-tert-butyl dicarbonate in 20 mL anhydrous THF under N_2 atmosphere, 2.3 g (9.9 mmol) (2-amino-5-bromo-3-methoxyphenyl)methanol[127] was added and the resulting solution was stirred at 40 °C for 2 d. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:5, Rf 0.2) gave 1.6 g (4.8 mmol, 48 %) of **23** as a white solid.

mp: 135 - 137 °C.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.20 (d, *J* = 2.1 Hz, 1H, 3-H), 6.89 (d, *J* = 2.1 Hz, 1H, 5-H), 6.19 (br s, 1H, NH), 4.42 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3), 1.44 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (rt, 126 MHz, $CDCl_3$) δ = 155.8 (C=O), 153.6 (C-6), 139.3 (C-2), 125.8 (C-3), 123.5 (C-1), 119.9 (C-4), 113.7 (C-5), 81.6 ($C(CH_3)_3$), 61.8 (CH_2), 56.2 (OCH_3), 28.3 ($C(CH_3)_3$).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3462, 3327, 3009, 2981, 2944, 1695, 1509, 1275, 1164, 1043.

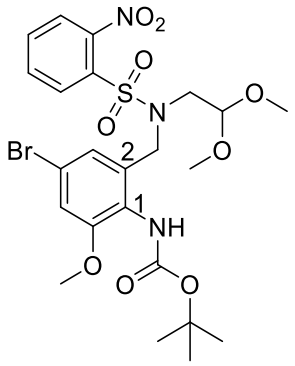
MS (EI+): m/z (%) = 185 (40), 187 (38), 213 (53), 215 (60), 231 (100), 233 (81), 257 (25), 259 (24), 275 (12), 277 (15), 331 (15) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{13}H_{18}{}^{79}BrNO_4$ 331.0419, found 331.0431.

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

[a]prepared and characterized by Edgar Uhl for his master thesis.

tert-Butyl [4-bromo-2-({[*N*-(2,2-dimethoxyethyl)-2-nitrophenyl]sulfonylamino}methyl)-6-methoxyphenyl]carbamate[a] (24)



MF: $C_{23}H_{30}BrN_3O_4S$ MW: 604.47 g/mol

To a vigorously stirred solution of 0.55 g (2.1 mmol) triphenylphosphine in 2.0 mL anhydrous THF under N_2 atmosphere, 0.33 mL (1.7 mmol) DIAD was added. When a homogenous white precipitate formed, 0.48 g (1.7 mmol) *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide[67] was added and the reaction mixture was treated in an ultrasonic bath. After 10 min 0.50 g (1.5 mmol) **23** was added and the suspension was sonicated until a clear solution was obtained. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:2, Rf 0.4) followed by a second FCC with pure CH_2Cl_2 gave 0.46 g (0.76 mmol, 36 %) of **24** as a colorless oil.

^{1}H NMR (500 MHz, CD_2Cl_2) δ = 7.93 (dd, *J* = 7.9, 1.3 Hz, 1H, Ar-H_{Nosyl}), 7.76 – 7.57 (m, 3H, Ar-H_{Nosyl}), 7.02 – 6.95 (m, 1H, 3-H), 6.92 (d, *J* = 2.1 Hz, 1H, 5-H), 6.26 (br s, 1H, NH), 4.62 (s, 2H, 2-CH_2-N), 4.35 (t, *J* = 5.2 Hz, 1H, C*H*$(OCH_3)_2$), 3.80 (s, 3H, 6-OCH_3), 3.35 (d, *J* = 5.2 Hz, 2H, N-C*H*$_2$-CH), 3.27 (s, 6H, CH(OC*H*$_3$)$_2$), 1.47 (s, 9H, C(*C*H_3)$_3$).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 154.9 (C-6), 154.3 (C=O), 148.3 (quart. C_{Nosyl}), 135.7 (C-2), 134.3 (CH_{Nosyl}), 134.0 (quart. C_{Nosyl}), 132.2 (CH_{Nosyl}), 131.3 (CH_{Nosyl}), 124.7 (C-1), 124.7 (CH_{Nosyl}), 123.4 (C-3), 120.3 (C-4), 114.1 (C-5), 104.2 (*C*H$(OCH_3)_2$), 81.1 (*C*$(CH_3)_3$), 56.7 (6-OCH_3), 55.3 (CH(O*C*H_3)$_2$), 50.4 (N-*C*H_2-CH), 49.1 (2-CH_2-N), 28.5 (C(*C*H_3)$_3$).

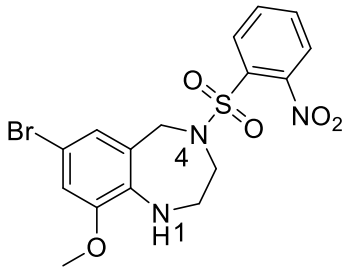
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3095, 2936, 2837, 1720, 1544, 1368, 1161, 1070, 779.

MS (EI+): m/z (%) = 385 (20), 387 (26), 503 (93), 505 (100), 603 (65) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{23}H_{30}{}^{79}BrN_3O_4S$ 603.0886, found 603.0881.

[a]prepared and characterized by Edgar Uhl for his master thesis.

7-Bromo-9-methoxy-4-[(2-nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine[a] (25)



MF: $C_{16}H_{16}BrN_3O_5S$ MW: 442.28 g/mol

To a solution of 0.85 g (1.4 mmol) **24** in 3.4 mL CH_2Cl_2 under N_2 atmosphere, 1.7 mL trifluoroacetic acid and 0.56 mL (3.5 mmol) triethylsilane were added in rapid succession. After 48 h 2 M NaOH was added and the mixture extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:1, Rf 0.4) gave 0.51 g (1.2 mmol, 86 %) of **25** as a yellow solid.

mp: 68 – 69 °C.

^{1}H NMR (500 MHz, CD_2Cl_2) δ = 7.89 (dd, *J* = 7.8, 1.5 Hz, 1H, Ar-H_{Nosyl}), 7.71 – 7.57 (m, 3H, Ar-H_{Nosyl}), 6.97 (d, *J* = 2.1 Hz, 1H, 6-H), 6.88 (d, *J* = 2.1 Hz, 1H, 8-H), 4.70 (br s, NH), 4.39 (s, 2H, 5-H), 3.80 (s, 3H, OCH_3), 3.64 – 3.59 (m, 2H, 3-H), 3.25 – 3.20 (m, 2H, 2-H).

^{13}C NMR (126 MHz, CD_2Cl_2) δ = 150.2 (C-9), 148.4 (quart. C_{Nosyl}), 138.9 (C-9a), 134.0 (CH_{Nosyl}), 133.3 (quart. C_{Nosyl}), 132.0 (CH_{Nosyl}), 130.9 (CH_{Nosyl}), 129.1 (C-5a), 124.7 (C-6), 124.3 (CH_{Nosyl}), 113.5 (C-8), 111.7 (C-7), 56.5 (OCH_3), 52.0 (C-5), 51.9 (C-3), 48.2 (C-2).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3420, 3092, 2936, 1629, 1542, 1488, 1372, 1341, 1162, 1029.

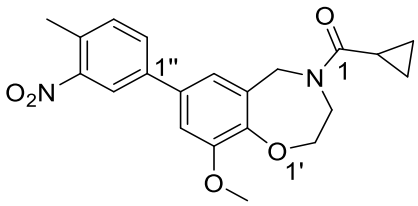
MS (EI+): m/z (%) = 169 (100), 181 (90), 441 (25) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{16}H_{16}{}^{79}BrN_3O_5S$ 440.9994, found 440.9998.

Purity (HPLC): 90 % (210 nm; method 1b).

[a]prepared and characterized by Edgar Uhl for his master thesis.

1-Cyclopropyl-1-[9-methoxy-7-(4-methyl-3-nitrophenyl)-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]methanone (30)



MF: $C_{21}H_{22}N_2O_5$ MW: 382.42 g/mol

Standard protocol 2 with 0.80 g (2.5 mmol) **32** and 0.51 g (2.8 mmol) 4-methyl-3-nitrophenylboronic acid. FCC with DCM with 1 % MeOH (Rf 0.3) gave 0.56 g (1.5 mmol, 60 %) of **30** as a yellow solid.

mp: 141 - 142 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 8.04 (d, *J* = 2.0 Hz, 1H, 2''-H), 7.63 (dd, *J* = 8.0, 2.0 Hz, 1H, 6''-H), 7.35 (d, *J* = 8.0 Hz, 1H, 5''-H), 7.06 – 7.00 (m, 2H, 6'-H, 8'-H), 4.73 (s, 2H, 5'-H), 4.22 – 4.13 (m, 2H, 2'-H), 4.04 – 3.97 (m, 2H, 3'-H), 3.89 (s, 3H, OCH_3), 2.58 (s, 3H, 4''-CH_3), 1.80 – 1.68 (m, 1H, C*H*-CH_2), 0.95 – 0.87 (m, 2H, CH-C*H*$_2$), 0.78 – 0.68 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 172.2 (C-1), 152.2 (C-9'), 149.9 (C-3''), 149.1 (C-9a'), 139.7 (C-1''), 133.9 (C-7'), 132.8 (C-5''), 132.5 (C-5a'), 131.6 (C-4''), 130.7 (C-6''), 122.3 (C-2''), 120.0 (C-6'), 112.2 (C-8'), 72.2 (C-2'), 56.9 (OCH_3), 49.7 (C-5'), 49.4 (C-3'), 19.1 (4''-CH_3), 11.6 (*C*H-CH_2), 7.0 (CH-*C*H_2).

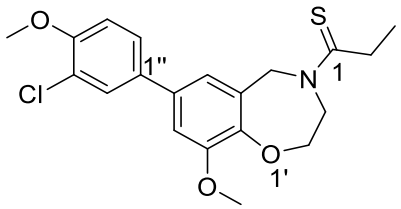
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3439, 3057, 2987, 2926, 2875, 2345, 1634, 1620, 1589, 1528, 1504, 1481, 1469, 1345, 1203, 1183, 1089, 826, 732.

MS (ESI+): m/z (%) = 383 (100) $[M + H]^+$, 405 (29), 765 (15), 787 (14).

HRMS (ESI+): m/z calcd for $[C_{21}H_{23}N_2O_5]^+$ 383.1607, found 383.1600.

Purity (HPLC): 99 % (210 nm; method 2a).

1-[7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]propane-1-thione (31)



MF: $C_{20}H_{22}ClNO_3S$ MW: 391.91 g/mol

A solution of 0.052 g (0.13 mmol) **57** and 0.073 g (0.18 mmol) Lawesson's reagent in 1.0 mL anhydrous THF was stirred at rt for 72 h. To this solution was added 20 mL water and this mixture extracted with DCM (3 x 20 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (3:1, Rf 0.7) gave 0.051 g (0.13 mmol, 96 %) of **31** as a white solid.

mp: 82 – 83 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, $C_2D_2Cl_4$): δ = 7.58 (d, *J* = 2.3 Hz, 0.5H, 2''-H), 7.54 (d, *J* = 2.3 Hz, 0.5H, 2''-H), 7.45 (dd, *J* = 8.6, 2.3 Hz, 0.5H, 6''-H), 7.40 (dd, *J* = 8.5, 2.3 Hz, 0.5H, 6''-H), 7.25 (d, *J* = 2.1 Hz, 0.5H, 6'-H), 7.04 – 6.96 (m, 2H, 8'-H, 5''-H), 6.92 (d, *J* = 2.1 Hz, 0.5H, 6'-H), 5.29 (s, 1.0H, 5'-H), 4.86 (s, 1.0H, 5'-H), 4.76 – 4.67 (m, 1.0H, 3'-H), 4.31 – 4.25 (m, 1.0H, 2'-H), 4.25 – 4.17 (m, 1.0H, 2'-H), 4.17 – 4.09 (m, 1.0H, 3'-H), 3.93 (s, 3H, 4''-OCH_3), 3.90 (s, 3H, 9'-OCH_3), 2.87 (q, *J* = 7.4 Hz, 1.0H, 2-H), 2.77 (q, *J* = 7.4 Hz, 1.0H, 2-H), 1.32 (t, *J* = 7.4 Hz, 1.5H, 3-H), 1.25 (t, *J* = 7.4 Hz, 1.5H, 3-H).

^{13}C NMR (mixture of rotamers, 101 MHz, $C_2D_2Cl_4$): δ = 206.4 (C-1), 205.1 (C-1), 154.3 (C-4''), 154.2 (C-4''), 151.9 (C-9'), 151.3 (C-9'), 147.24 (C-9a'), 147.19 (C-9a'), 135.4 (C-7'), 134.8 (C-7'), 133.6 (C-1''), 133.5 (C-1''), 129.4 (C-5a'), 129.0 (C-5a'), 128.5 (C-2''), 126.2 (C-6''), 122.5 (C-3''), 122.4 (C-3''), 121.6 (C-6'), 119.2 (C-6'), 112.3 (C-5''), 112.2 (C-5''), 111.1 (C-8'), 110.4 (C-8'), 71.5 (C-2'), 70.8 (C-2'), 56.4 (C-3'), 56.3 (OCH_3), 56.3 (OCH_3), 56.2 (OCH_3), 54.9 (C-5'), 54.4 (C-3'), 54.1 (C-5'), 37.0 (C-2), 35.9 (C-2), 13.9 (C-3), 13.5 (C-3).

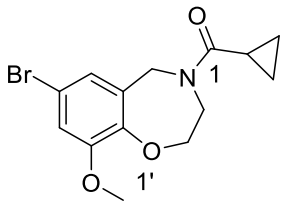
IR (Film): $\tilde{\nu}$ (cm^{-1}) = 3384, 2965, 2935, 2839, 1587, 1485, 1441, 1343, 1290, 1256, 1200, 1063, 1023, 967, 811, 752.

MS (EI+): m/z (%) = 131 (85), 169 (87), 181 (71), 219 (60), 281 (38), 331 (30), 391 (4) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{20}H_{22}{}^{35}ClNO_3S$ 391.1009, found 391.0988.

Purity (HPLC): 98 % (210 nm; method 1c).

1-(7-Bromo-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl)-1-(cyclopropyl)methanone (32)



MF: $C_{14}H_{16}BrNO_3$ MW: 326.19 g/mol

Standard protocol 1 with 7.9 g (31 mmol) **9** and 3.6 mL (40 mmol) cyclopropanecarbonyl chloride. FCC with EtOAc and hexanes (1:2, Rf 0.1) gave 7.4 g (22 mmol, 71 %) of **32** as a white solid.

mp: 85 - 86 °C.

^{1}H NMR (mixture of rotamers, 500 MHz, DMSO-d_6): δ = 7.29 (d, *J* = 2.1 Hz, 0.6H, 6'-H), 7.12 (d, *J* = 2.1 Hz, 0.6H, 8'-H), 7.08 (d, *J* = 2.1 Hz, 0.4H, 8'-H), 6.97 (d, *J* = 2.1 Hz, 0.4H, 6'-H), 4.77 (s, 1.2H, 5'-H), 4.51 (s, 0.8H, 5'-H), 4.10 – 4.06 (m, 1.6H, 2'-H, 3'-H), 3.99 – 3.94 (m, 1.2H, 2'-H), 3.87 – 3.81 (m, 1.2H, 3'-H), 3.76 (s, 3H, OCH_3), 2.14 – 2.08 (m, 0.6H, C*H*-CH_2), 1.96 – 1.89 (m, 0.4H, C*H*-CH_2), 0.71 – 0.63 (m, 4H, CH-C*H*$_2$).

^{13}C NMR (mixture of rotamers, 126 MHz, DMSO-d_6): δ = 173.1 (C-1), 172.6 (C-1), 153.1 (C-9'), 152.9 (C-9'), 148.3 (C-9a'), 148.2 (C-9a'), 134.9 (C-5a'), 134.7 (C-5a'), 124.9 (C-6'), 124.0 (C-6'), 116.1 (C-8'), 115.7 (C-8'), 115.5 (C-7'), 115.2 (C-7'), 73.2 (C-2'), 72.5 (C-2'), 57.0 (OCH_3), 51.3 (C-3'), 49.8 (C-5'), 49.0 (C-3'), 48.2 (C-5'), 11.6 (*C*H-CH_2), 8.4 (CH-*C*H_2), 8.0 (CH-*C*H_2).

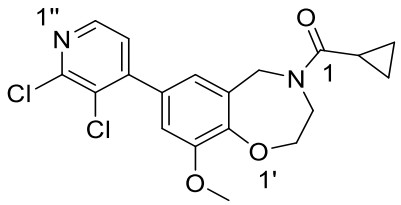
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3424, 3092, 3013, 3002, 2980, 2939, 2870, 1637, 1590, 1573, 1482, 1412, 1291, 1208, 1082, 1048, 980, 840, 782, 657.

MS (EI+): m/z (%) = 159 (16), 238 (37), 240 (40), 325 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{14}H_{16}{}^{79}BrNO_3$ 325.0314, found 325.0316.

Purity (HPLC): > 99 % (210 nm; method 5a).

1-Cyclopropyl-1-[7-(2,3-dichloropyridin-4-yl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]methanone (33)



MF: $C_{19}H_{18}Cl_2N_2O_3$ MW: 393.26 g/mol

Standard protocol 2 with 0.098 g (0.30 mmol) **32** and 0.069 g (0.36 mmol) 2,3-dichloropyridine-4-boronic acid. FCC with EtOAc and hexanes (3:1, Rf 0.2) gave 0.091 g (0.23 mmol, 77 %) of **33** as an orange solid.

mp: 186 - 187 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, CD_2Cl_2): δ = 8.34 – 8.23 (m, 1H, 6‘‘-H), 7.29 – 7.20 (m, 1H, 5‘‘-H), 7.04 – 6.89 (m, 2H, 6‘-H, 8‘-H), 4.77 (s, 1.2H, 5‘-H), 4.67 (s, 0.8H, 5‘-H), 4.28 – 4.21 (m, 0.8H, 2‘-H), 4.14 – 4.08 (m, 2H, 2‘-H, 3‘-H), 4.03 – 3.97 (m, 1.2H, 3‘-H), 3.88 – 3.82 (m, 3H, OCH_3), 1.91 – 1.81 (m, 0.6H, C*H*-CH_2), 1.78 – 1.65 (m, 0.4H, C*H*-CH_2), 0.89 – 0.82 (m, 2H, CH-C*H*$_2$), 0.78 – 0.69 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (mixture of rotamers, 126 MHz, CD_2Cl_2): δ = 172.6 (C-1), 172.1 (C-1), 152.0 (C-9‘), 151.2 (C-9‘), 150.4 (C-2‘‘), 150.4 (C-4‘‘), 149.2 (C-9a‘), 149.1 (C-9a‘), 146.8 (C-6‘‘), 146.7 (C-6‘‘), 132.7 (C-5a‘), 132.4 (C-5a‘), 132.0 (C-3‘‘), 131.6 (C-3‘‘), 128.6 (C-7‘), 128.5 (C-7‘), 124.9 (C-5‘‘), 124.7 (C-5‘‘), 122.6 (C-6‘), 121.1 (C-6‘), 113.0 (C-8‘), 112.5 (C-8‘), 72.6 (C-2‘), 56.4 (OCH_3), 51.1 (C-3‘), 50.9 (C-5‘), 48.8 (C-3‘), 48.2 (C-5‘), 11.4 (*C*H-CH_2), 11.3 (*C*H-CH_2), 7.4 (CH-*C*H$_2$), 7.2 (CH-*C*H$_2$).

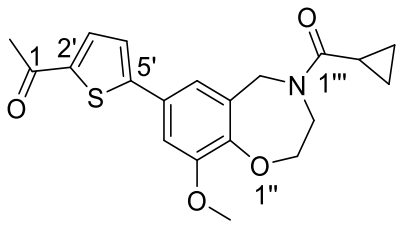
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3441, 2993, 2941, 2866, 2841, 1633, 1574, 1490, 1463, 1441, 1354, 1213, 1089, 1046.

MS (EI+): m/z (%) = 293 (42), 295 (49), 305 (63), 307 (49), 323 (70), 392 (100) $[M]^{+\bullet}$, 393 (36), 394 (67), 395 (16), 396 (12).

HRMS (EI+): m/z calcd for $C_{19}H_{18}{}^{35}Cl_2N_2O_3$ 392.0694, found 392.0699.

Purity (HPLC): 96 % (210 nm; method 1a).

1-{5-[4-(Cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]thiophen-2-yl}ethan-1-one (34)



MF: $C_{20}H_{21}NO_4S$ MW: 371.45 g/mol

Standard protocol 2 with 0.098 g (0.30 mmol) **32** and 0.061 g (0.36 mmol) 5-acetyl-2-thienylboronic acid. FCC with EtOAc and hexanes (2:1, Rf 0.2) gave 0.079 g (0.21 mmol, 71 %) of **34** as an orange solid.

mp: 85 - 87 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.58 (d, *J* = 3.9 Hz, 1H, 3‘-H), 7.20 (d, *J* = 3.9 Hz, 1H, 4‘-H), 7.15 – 7.03 (m, 2H, 6‘‘-H, 8‘‘-H), 4.70 (s, 2H, 5‘‘-H), 4.21 – 4.09 (m, 2H, 2‘‘-H), 4.04 – 3.92 (m, 2H, 3‘‘-H), 3.88 (s, 3H, OCH_3), 2.49 (s, 3H, 2-H), 1.81 – 1.64 (m, 1H, C*H*-CH_2), 0.98 – 0.84 (m, 2H, CH-C*H*$_2$), 0.79 – 0.64 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 189.7 (C-1), 172.4 (C-1‘‘‘), 152.4 (C-9‘‘), 151.9 (C-5a), 149.8 (C-9a‘‘), 143.3 (C-2‘), 133.0 (C-3‘), 132.7 (C-7‘‘), 129.1 (C-5a‘‘), 123.9 (4‘-H), 119.8 (C-6‘‘), 111.8 (C-8‘‘), 72.5 (C-2‘‘), 57.1 (OCH_3), 49.8 (C-5‘‘), 49.6 (C-3‘‘), 26.4 (C-2), 11.8 (*C*H-CH_2), 7.3 (CH-*C*H_2).

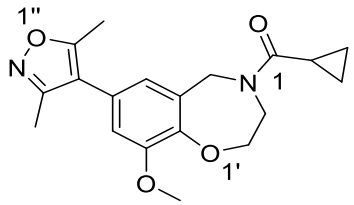
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3427, 2985, 2960, 2938, 1652, 1632, 1441, 1421, 1277, 1209, 1085, 1051, 991.

MS (EI+): m/z (%) = 260 (54), 274 (35), 284 (63), 302 (43), 371 (100) $[M]^{+\bullet}$, 372 (24).

HRMS (EI+): m/z calcd for $C_{20}H_{21}NO_4S$ 371.1191, found 371.1192.

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

1-Cyclopropyl-1-[7-(3,5-dimethylisoxazol-4-yl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]methanone (35)



MF: $C_{19}H_{22}N_2O_4$ MW: 342.40 g/mol

Standard protocol 2 with 0.098 g (0.30 mmol) **32** and 0.080 g (0.36 mmol) 3,5-dimethylisoxazole-4-boronic acid pinacol ester. FCC with EtOAc and hexanes (3:1, Rf 0.2) gave 0.089 g (0.26 mmol, 87 %) of **35** as a white solid.

mp: 175 - 176 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 6.74 – 6.66 (m, 2H, 6‘-H, 8‘-H), 4.69 (s, 2H, 5‘-H), 4.20 – 4.10 (m, 2H, 2‘-H), 4.05 – 3.95 (m, 2H, 3‘-H), 3.84 (s, 3H, OCH_3), 2.35 (s, 3H, (isoxazole-CH_3)), 2.21 (s, 3H, (isoxazole-CH_3)), 1.80 – 1.64 (m, 1H, C*H*-CH_2), 0.96 – 0.87 (m, 2H, CH-C*H*$_2$), 0.76 – 0.66 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 172.4 (C-1), 165.0 (C-3’’/C-5’’), 158.4 (C-3’’/C-5’’), 152.2 (C-9’), 148.4 (C-9a’), 132.7 (C-5a’), 126.0 (C-7’), 122.4 (C-6’), 116.3 (C-4’’), 114.7 (C-8’), 72.4 (C-2’), 57.1 (OCH_3), 50.1 (C-5’), 49.6 (C-3’), 11.7 (*C*H-CH_2), 11.4 (isoxazole-CH_3), 10.6 (isoxazole -CH_3), 7.3 (CH-*C*H_2).

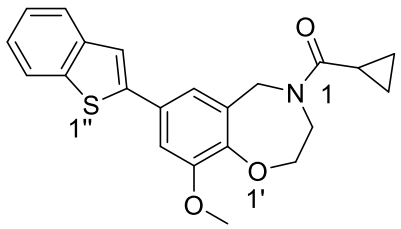
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3427, 2991, 2969, 2935, 1646, 1585, 1467, 1452, 1415, 1324, 1257, 1201, 1178, 1104, 990.

MS (EI+): m/z (%) = 119 (54), 169 (41), 255 (37), 273 (45), 342 (86) $[M]^{+\bullet}$, 343 (25), 344 (48).

HRMS (EI+): m/z calcd for $C_{19}H_{22}N_2O_4$ 342.1580, found 342.1572.

Purity (HPLC): > 99 % (210 nm; method 1c).

1-[7-(Benzo[*b*]thiophen-2-yl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-1-(cyclopropyl)methanone (36)



MF: $C_{22}H_{21}NO_3S$ MW: 379.47 g/mol

Standard protocol 2 with 0.12 g (0.36 mmol) **32** and 0.080 g (0.43 mmol) benzo[*b*]thien-2-ylboronic acid. FCC with EtOAc and hexanes (2:1, Rf 0.2) gave 0.13 g (0.34 mmol, 94 %) of **36** as a white solid.

mp: 83 - 84 °C.

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 7.95 – 7.87 (m, 1H, Ar-H), 7.87 – 7.79 (m, 1H, 4‘‘-H), 7.79 – 7.70 (m, 1H, 3‘‘-H), 7.43 – 7.25 (m, 4H, 6‘-H, 8‘-H, Ar-H), 4.79 (s, 2H, 5‘-H), 4.20 – 4.08 (m, 2H, 2‘-H), 4.06 – 3.92 (m, 2H, 3‘-H), 3.89 (s, 3H, OCH_3), 2.12 – 1.95 (m, 1H, C*H*-CH_2), 0.78 – 0.66 (m, 4H, CH-C*H*$_2$).

^{13}C NMR (100 °C, 101 MHz, DMSO-d_6): δ = 171.4 (C-1), 151.2 (C-9‘), 148.3 (C-9a‘), 142.5 (C-2‘‘/C-3a‘‘), 140.0 (C-2‘‘/C-3a‘‘), 138.2 (C-7a‘‘), 131.9 (C-5a‘), 128.2 (C-7‘), 124.1 (aromat. CH), 123.8 (aromat. CH), 122.9 (C-4‘‘), 121.6 (aromat. CH), 119.2 (C-3‘‘), 119.1 (C-6‘), 110.6 (aromat. CH), 71.2 (C-2‘), 56.1 (OCH_3), 48.4 (C-3‘, C-5’), 10.5 (*C*H-CH_2), 6.3 (CH-*C*H$_2$).

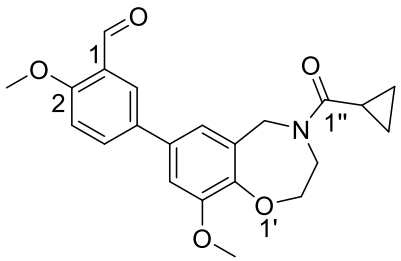
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3441, 3004, 2931, 1640, 1583, 1487, 1460, 1434, 1296, 1226, 1166, 1102, 1048.

MS (EI+): m/z (%) = 268 (63), 281 (35), 291 (53), 308 (32), 379 (100) $[M]^{+\bullet}$, 380 (24).

HRMS (EI+): m/z calcd for $C_{22}H_{21}NO_3S$ 379.1242, found 379.1240.

Purity (HPLC): 97 % (210 nm; method 1a).

5-[4-(Cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]-2-methoxybenzaldehyde (37)



MF: $C_{22}H_{23}NO_5$ MW: 381.43 g/mol

Standard protocol 2 with 1.5 g (4.6 mmol) **32** and with 0.96 g (5.4 mmol) 3-formyl-4-methoxyphenylboronic acid. FCC with EtOAc and hexanes (3:1, Rf 0.2) gave 1.4 g of **37** as ayellow solid (3.7 mmol, 80 %).

mp: 152 – 153 °C.

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 10.42 (s, 1H, HC=O), 7.97 – 7.88 (m, 2H, 6-H, 4-H), 7.32 – 7.26 (m, 1H, 3-H), 7.20 (br s, 1H, 6'-H), 7.16 (d, *J* = 2.2 Hz, 1H, 8'-H), 4.78 (s, 2H, 5'-H), 4.14 – 4.06 (m, 2H, 2'-H), 4.01 – 3.94 (m, 5H, H'-3, 2-OCH_3), 3.87 (s, 3H, 9'-OCH_3), 2.11 – 1.95 (m, 1H, C*H*-CH_2), 0.75 – 0.66 (m, 4H, CH-C*H*$_2$).

^{13}C NMR (100 °C, 101 MHz, DMSO-d_6): δ = 188.5 (HC=O), 171.4 (C-1''), 160.4 (C-2), 151.2 (C-9'), 147.4 (C-9a'), 133.6 (C-4), 133.5 (C-7'), 132.1 (C-5), 131.9 (C-5a'), 125.2 (C-6), 124.4 (C-1), 119.0 (C-6'), 113.1 (C-3), 111.0 (C-8'), 71.2 (C-2'), 56.1 (9'-OCH_3), 55.9 (2-OCH_3), 48.5 (C-5'), 48.4 (C-3'), 10.4 (*C*H-CH_2), 6.3 (CH-*C*H$_2$).

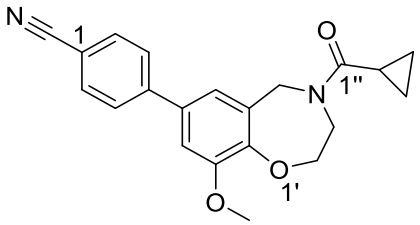
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3448, 3080, 3005, 2938, 2864, 1734, 1682, 1638, 1608, 1485, 1464, 1431, 1390, 1293, 1249, 1206, 1074, 821.

MS (ESI+): m/z (%) = 382 (100) $[M + H]^+$, 404 (35), 763 (11).

HRMS (ESI+): m/z calcd for $[C_{22}H_{24}NO_5]^+$ 382.1654, found 382.1647.

Purity (HPLC): 99 % (210 nm; method 1c).

4-[4-(Cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]benzonitrile (38)



MF: $C_{21}H_{20}N_2O_3$ MW: 348.40 g/mol

Standard protocol 2 with 0.098 g (0.30 mmol) **32** and 0.053 g (0.36 mmol) 4-cyanophenylboronic acid. FCC with EtOAc and hexanes (2:1, Rf 0.2) gave 0.091 g (0.26 mmol, 87 %) of **38** as an orange solid.

mp: 157 - 158 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, CD_2Cl_2): δ = 7.80 – 7.62 (m, 4H, 2-H, 3-H, 5-H, 6-H), 7.20 – 7.02 (m, 2H, 6‘-H, 8‘-H), 4.78 (s, 1.1H, 5‘-H), 4.68 (s, 0.9H, 5‘-H), 4.26 – 4.17 (m, 0.9H, 2‘-H), 4.17 – 4.04 (m, 2H, 2‘-H, 3‘-H), 4.04 – 3.96 (m, 1.1H, 3‘-H), 3.92 – 3.86 (m, 3H, OCH_3), 1.95 – 1.83 (m, 0.6H, C*H*-CH_2), 1.75 – 1.65 (m, 0.4H, C*H*-CH_2), 0.89 – 0.80 (m, 2H, CH-C*H*$_2$), 0.80 – 0.67 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (mixture of rotamers, 126 MHz, CD_2Cl_2): δ = 172.9 (C-1‘‘), 172.4 (C-1‘‘), 152.8 (C-9‘), 152.3 (C-9‘), 149.5 (C-9a‘), 149.3 (C-9a‘), 145.4 (C-4), 145.3 (C-4), 135.2 (C-7‘), 134.8 (C-7‘), 133.4 (C-5a‘), 133.3 (C-5a‘), 133.0 (C-2, C-6), 132.9 (C-2, C-6), 128.0 (C-3, C-5), 127.9 (C-3, C-5), 121.3 (C-6‘), 119.8 (C-6‘), 119.3 (C-1/CN), 119.2 (C-1/CN), 111.6 (C-8‘), 111.3 (C-1/CN), 111.1 (C-1/CN), 110.9 (C-8‘), 73.2 (C-2‘), 73.0 (C-2‘), 56.7 (OCH_3), 51.5 (C-3‘), 51.3 (C-5‘), 49.1 (C-3‘), 48.8 (C-5‘), 11.8 (*C*H-CH_2), 11.6 (*C*H-CH_2), 7.7 (CH-*C*H$_2$), 7.5 (CH-*C*H$_2$).

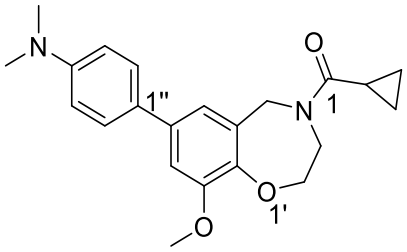
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3427, 3003, 2986, 2927, 2222, 1644, 1604, 1587, 1486, 1463, 1330, 1294, 1206, 1177, 1089.

MS (EI+): m/z (%) = 41 (100), 140 (28), 249 (22), 261 (36), 279 (37), 348 (27) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{21}H_{20}N_2O_3$ 348.1474, found 348.1473.

Purity (HPLC): > 99 % (210 nm; method 1c).

1-Cyclopropyl-1-{7-[4-(dimethylamino)phenyl]-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl}methanone (39)



MF: $C_{22}H_{26}N_2O_3$ MW: 366.46 g/mol

Standard protocol 2 with 0.30 g (0.92 mmol) **32** and 0.23 g (1.4 mmol) 4-(dimethylamino)phenylboronic acid. FCC with EtOAc and hexanes (1:2, Rf 0.1) gave 0.17 g (0.46 mmol, 50 %) of **39** as a white solid.

mp: 79 - 80 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, $C_2D_2Cl_4$): δ = 7.52 – 7.39 (m, 2H, 2''-H, 6''-H), 7.09 – 6.93 (m, 2H, 6'-H, 8'-H), 6.78 (d, *J* = 8.2 Hz, 2H, 3''-H, 5''-H), 4.73 (s, 1.4H, 5'-H), 4.67 (s, 0.6H, 5'-H), 4.28 – 3.93 (m, 4H), 3.88 (s, 3H, OCH_3), 3.03 – 2.93 (m, 6H, $N(CH_3)_2$), 1.91 – 1.80 (m, 0.7H, C*H*-CH_2), 1.69 – 1.62 (m, 0.3H, C*H*-CH_2), 0.96 – 0.87 (m, 2H, CH-C*H*$_2$), 0.81 – 0.69 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (mixture of rotamers, 101 MHz, $C_2D_2Cl_4$): δ = 172.6 (C-1), 172.0 (C-1), 151.6 (C-9'), 151.1 (C-9'), 149.6 (C-4''), 146.6 (C-9a'), 146.5 (C-9a'), 136.8 (C-7'), 136.4 (C-7') , 131.9 (C-5a'), 131.7 (C-5a'), 128.1 (C-1''), 127.5 (C-2'', C-6''), 127.4 (C-2'', C-6''), 119.4 (C-6'), 118.1 (C-6'), 112.5 (C-3'', C-5''), 110.2 (C-8'), 109.5 (C-8'), 72.4 (C-2'), 56.1 (OCH_3), 51.0 (C-5'), 50.9 (C-3'), 48.6 (C-5'), 48.4 (C-3'), 40.4 ($N(CH_3)_2$), 11.6 (*C*H-CH_2), 11.4 (*C*H-CH_2), 7.6 (CH-*C*H_2), 7.4 (CH-*C*H_2).

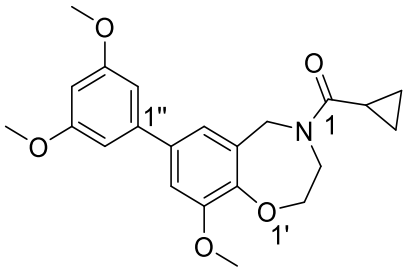
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3432, 2923, 2363, 2344, 1638, 1611, 1586, 1527, 1490, 1459, 1444, 1344, 1207, 1180, 1102, 1045, 816.

MS (ESI+): m/z (%) = 367 (100) $[M + H]^+$, 389 (37).

HRMS (ESI+): m/z calcd for $[C_{22}H_{27}N_2O_3]^+$ 367.2021, found 367.2017.

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

1-Cyclopropyl-1-[7-(3,5-dimethoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]methanone (40)



MF: $C_{22}H_{25}NO_5$ MW: 383.44 g/mol

Standard protocol 2 with 0.12 g (0.37 mmol) **32** and 0.078 g (0.43 mmol) 3,5-dimethoxyphenylboronic acid. FCC with EtOAc and hexanes (2:1, Rf 0.4) gave 0.090 g (0.23 mmol, 62 %) of **40** as a white solid.

mp: 50 - 51 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$) δ = 7.10 – 6.96 (m, 2H, 6‘-H, 8‘-H), 6.66 (d, *J* = 2.2 Hz, 2H, 3‘‘-H, 5‘‘-H), 6.44 (t, *J* = 2.2 Hz, 1H, 4’’-H), 4.72 (s, 2H, 5‘-H), 4.20 – 4.09 (m, 2H, 2‘-H), 4.04 – 3.95 (m, 2H, 3‘-H), 3.87 (s, 3H, 9‘-OCH_3), 3.81 (s, 6H, 3’’-OCH_3 and 5‘‘-OCH_3), 1.85 – 1.70 (m, 1H, C*H*-CH_2), 0.99 – 0.85 (m, 2H, CH-C*H*$_2$), 0.79 – 0.66 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (ambient temperature, mixture of rotamers, 101 MHz, $C_2D_2Cl_4$) δ = 172.6 (C-1), 172.1 (C-1), 160.8 (C-3‘‘, C-5‘‘), 151.6 (C-9‘), 151.1 (C-9‘), 147.8 (C-9a‘), 147.7 (C-9a‘), 142.6 (C-1‘‘), 142.3 (C-1‘‘), 136.5 (C-7‘), 136.0 (C-7‘), 131.9 (C-5a‘), 131.5 (C-5a‘), 120.4 (C-6‘), 119.1 (C-6‘), 111.0 (C-8‘), 110.4 (C-8‘), 105.5 (C-2‘‘, C-6‘‘), 105.1 (C-2‘‘, C-6‘‘), 99.1 (C-4‘‘), 98.6 (C-4‘‘), 72.3 (C-2‘), 72.2 (C-2‘), 56.2 (9‘-OCH_3), 55.4 (3‘‘-OCH_3, 5‘‘-OCH_3), 50.9 (C-3‘, C-5‘), 48.5 (C-3‘), 48.2 (C-5‘), 11.6 (*C*H-CH_2), 11.4 (*C*H-CH_2), 7.6 (CH-*C*H_2), 7.5 (CH-*C*H_2).

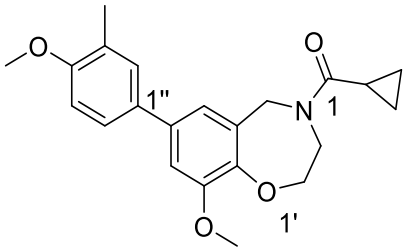
IR (Film): $\tilde{\nu}$ (cm^{-1}) = 3475, 3002, 2957, 2934, 2843, 1638, 1581, 1460, 1402, 1275, 1204, 1154, 1089, 1044, 856, 834, 693.

MS (EI+): m/z (%) = 159 (42), 296 (53), 383 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{22}H_{25}NO_5$ 383.1733, found 383.1724.

Purity (HPLC): 98 % (210 nm; method 3a).

1-Cyclopropyl-1-[9-methoxy-7-(4-methoxy-3-methylphenyl)-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]methanone (41)



MF: $C_{22}H_{25}NO_4$ MW: 367.45 g/mol

Standard protocol 2 with 0.50 g (1.5 mmol) **32** and 0.28 g (1.7 mmol) 4-methoxy-3-methylphenylboronic acid. FCC with EtOAc and hexanes (3:2, Rf 0.3) gave 0.38 g (1.0 mmol, 68 %) of **41** as a pale brown solid.

mp: 109 - 110 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.32 – 7.27 (m, 2H, 2''-H, 6''-H), 7.02 – 6.96 (m, 2H, 6'-H, 8'-H), 6.87 – 6.82 (m, 1H, 5''-H), 4.71 (s, 2H, 5'-H), 4.15 – 4.10 (m, 2H, 2'-H), 4.00 – 3.95 (m, 2H, 3'-H), 3.87 (s, 3H, 9'-OCH_3), 3.83 (s, 3H, 4''-OCH_3), 2.26 (s, 3H, 3''-CH_3), 1.81 – 1.69 (m, 1H, C*H*-CH_2), 0.94 – 0.87 (m, 2H, CH-C*H*$_2$), 0.75 – 0.68 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (ambient temperature, mixture of rotamers, 101 MHz, $C_2D_2Cl_4$): δ = 172.6 (C-1), 172.0 (C-1), 157.22 (C-4''), 157.17 (C-4''), 151.6 (C-9'), 151.1 (C-9'), 147.0 (C-9a'), 146.9 (C-9a'), 136.6 (C-7'), 136.2 (C-7'), 132.4 (C-1''), 132.1 (C-1''), 131.9 (5a'), 131.7 (5a'), 129.2, 129.1, 126.9 (C-3''), 126.8 (C-3''), 125.2 (C-2''/C-6''), 125.1 (C-2''/C-6''), 120.0 (C-6'), 118.6 (C-6'), 110.7 (C-8'), 110.1 (C-5''), 110.0 (C-8'), 72.4 (C-2'), 72.3 (C-2'), 56.2 (9'-OCH_3), 55.4 (4''-OCH_3), 50.9 (C-3', C-5'), 48.6 (C-3'), 48.4 (C-5'), 16.4 (3''-CH_3), 16.3 (3''-CH_3), 11.6 (*C*H-CH_2), 11.4 (*C*H-CH_2), 7.6 (CH-*C*H$_2$), 7.4 (CH-*C*H$_2$).

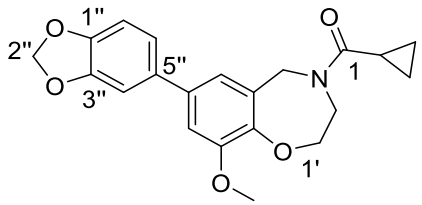
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3448, 3002, 2922, 2836, 1639, 1487, 1464, 1293, 1249, 1207, 1079, 812, 612.

MS (EI+): m/z (%) = 256 (76), 270 (46), 280 (64), 298 (50), 367 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{22}H_{25}NO_4$ 367.1784, found 367.1784.

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

1-[7-(Benzo[*d*][1,3]dioxol-5-yl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-1-(cyclopropyl)methanone (42)



MF: $C_{21}H_{21}NO_5$ MW: 367.40 g/mol

Standard protocol 2 with 0.20 g (0.61 mmol) **32** and 0.11 g (0.67 mmol) 3,4-(methylenedioxy)phenylboronic acid. FCC with EtOAc and hexanes (3:2, Rf 0.4) gave 0.21 g (0.58 mmol, 94 %) of **42** as a pale brown solid.

mp: 127 - 128 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.01 – 6.94 (m, 4H, 6‘-H, 8‘-H, 4‘‘-H, 7‘‘-H), 6.82 (dd, *J* = 7.7, 0.8 Hz, 1H, 6‘‘-H), 5.94 (s, 2H, 2‘‘-H), 4.71 (s, 2H, 5‘-H), 4.15 – 4.10 (m, 2H, 2‘-H), 4.01 – 3.96 (m, 2H, 3‘-H), 3.86 (s, 3H, OCH_3), 1.81 – 1.70 (m, 1H, C*H*-CH_2), 0.95 – 0.87 (m, 2H, CH-C*H*$_2$), 0.76 – 0.69 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 172.6 (C-1), 152.1 (C-9’), 148.3 (C-1’’), 148.2 (C-9a’), 147.2 (C-3’’), 136.7 (C-5’’), 135.1 (C-7’), 132.3 (C-5a’), 120.5 (C-4’’/C-7’’), 120.0 (C-6’), 112.5 (C-4’’/C-7’’), 108.6 (C-6’’), 107.6 (C-8’), 101.2 (C-2’’), 72.5 (C-2’), 57.0 (OCH_3), 50.2 (C-5’), 49.6 (C-3’), 11.9 (*C*H-CH_2), 7.2 (CH-*C*H_2).

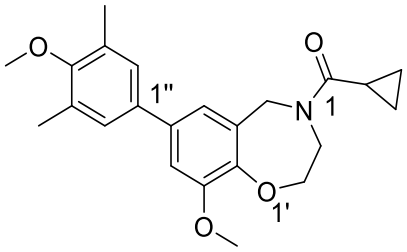
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3447, 3072, 2991, 2929, 2898, 2838, 1628, 1584, 1482, 1419, 1295, 1243, 1197, 1041, 806, 733.

MS (EI+): m/z (%) = 112 (11), 178 (23), 247 (40), 256 (40), 280 (33), 367 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{21}H_{21}NO_5$ 367.1420, found 367.1433.

Purity (HPLC): 97 % (210 nm; method 2a).

1-Cyclopropyl-1-[9-methoxy-7-(4-methoxy-3,5-dimethylphenyl)-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]methanone (43)



MF: $C_{23}H_{27}NO_4$ MW: 381.47 g/mol

Standard protocol 2 with 0.20 g (0.61 mmol) **32** and 0.12 g (0.68 mmol) 3,5-dimethyl-4-methoxyphenylboronic acid. FCC with EtOAc and hexanes (3:2, Rf 0.4) gave 0.071 g (0.19 mmol, 31 %) of **43** as a pale brown solid.

mp: 146 – 147 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.16 – 7.12 (m, 2H, 2‘‘-H, 6‘‘-H), 7.02 – 6.97 (m, 2H, 6‘-H, 8‘-H), 4.71 (s, 2H, 5‘-H), 4.16 – 4.10 (m, 2H, 2‘-H), 4.01 – 3.96 (m, 2H, 3‘-H), 3.87 (s, 3H, 9‘-OCH_3), 3.73 (s, 3H, 4‘‘-OCH_3), 2.31 (s, 6H, 3‘‘-CH_3, 5‘‘-CH_3), 1.81 – 1.70 (m, 1H, C*H*-CH_2), 0.94 – 0.88 (m, 2H, CH-C*H*$_2$), 0.75 – 0.68 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 172.6 (C-1), 156.9 (C-4‘‘), 152.0 (C-9‘), 148.1 (C-9a‘), 136.8 (C-7‘), 135.9 (C-3’‘, C-5’‘), 132.2 (C-5a‘), 131.0 (C-1‘‘), 127.4 (C-2‘‘, C-6‘‘), 120.2 (C-6‘), 112.6 (C-8‘), 72.5 (C-2‘), 59.7 (4‘‘-OCH_3), 57.1 (9‘-OCH_3), 50.2 (C-5‘), 49.6 (C-3‘), 16.2 (3‘‘-CH_3, 5‘‘-CH_3), 11.9 (*C*H-CH_2), 7.2 (CH-*C*H$_2$).

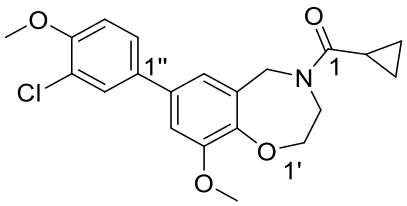
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3432, 2997, 2973, 2926, 2875, 1642, 1580, 1479, 1467, 1416, 1271, 1210, 1091, 1046, 851, 741.

MS (EI+): m/z (%) = 119 (22), 169 (29), 270 (98), 284 (55), 294 (78), 312 (64), 381 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{23}H_{27}NO_4$ 381.1940, found 381.1940.

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

1-[7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-1-(cyclopropyl)methanone (44)



MF: $C_{21}H_{22}ClNO_4$ MW: 387.86 g/mol

Standard protocol 2 with 0.20 g (0.61 mmol) **32** and 0.13 g (0.67 mmol) 3-chloro-4-methoxyphenylboronic acid. FCC with EtOAc and hexanes (3:2, Rf 0.3) gave 0.16 g (0.42 mmol, 69 %) of **44** as a pale brown solid.

mp: 130 – 131 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.52 (d, *J* = 2.3 Hz, 1H, 2‘‘-H), 7.36 (dd, *J* = 8.5, 2.3 Hz, 1H, 6‘‘-H), 7.00 – 6.93 (m, 3H, 6‘-H, 8‘-H, 5‘‘-H), 4.71 (s, 2H, 5‘-H), 4.16 – 4.11 (m, 2H, 2‘-H), 4.01 – 3.96 (m, 2H, 3‘-H), 3.89 (s, 3H, 4‘‘-OCH_3), 3.87 (s, 3H, 9‘-OCH_3), 1.79 – 1.69 (m, 1H, C*H*-CH_2), 0.94 – 0.88 (m, 2H, CH-C*H*$_2$), 0.76 – 0.68 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 172.3 (C-1), 154.9 (C-4‘‘), 152.2 (C-9‘), 148.5 (C-9a‘), 135.3 (C-7‘), 134.5 (C-1‘‘), 132.5 (C-5a‘), 128.8 (C-2‘‘), 126.2 (C-6‘‘), 123.6 (C-3‘‘), 120.0 (C-6‘), 113.4 (C-5‘‘), 112.4 (C-8‘), 72.5 (C-2‘), 57.1 (9‘-OCH_3), 56.7 (4‘‘-OCH_3), 50.1 (C-5‘), 49.6 (C-3‘), 11.8 (*C*H-CH_2), 7.3 (CH-*C*H$_2$).

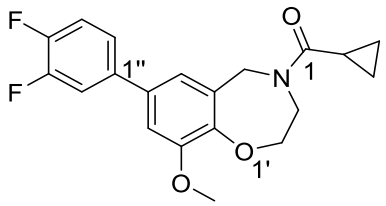
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3621, 3439, 3087, 3003, 2972, 2929, 2840, 1634, 1587, 1486, 1463, 1345, 1259, 1183, 1061, 1042, 807, 700.

MS (ESI+): m/z (%) = 388 (100) $[M + H]^+$, 390 (37).

HRMS (ESI+): m/z calcd for $[C_{21}H_{23}{}^{35}ClNO_4]^+$ 388.1316, found 388.1317.

Purity (HPLC): 99 % (210 nm; method 2a).

1-Cyclopropyl-1-[7-(3,4-difluorophenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]methanone (45)



MF: $C_{20}H_{19}F_2NO_3$ MW: 359.37 g/mol

Standard protocol 2 with 0.12 g (0.37 mmol) **32** and 0.068 g (0.43 mmol) 3,4-difluorophenylboronic acid. FCC with EtOAc and hexanes (2:1, Rf 0.4) gave 0.090 g (0.25 mmol, 68 %) of **45** as a white solid.

mp: 136 - 137 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.34 – 7.26 (m, 1H, 2‘‘-H), 7.26 – 7.09 (m, 2H, 5‘‘-H, 6‘‘-H), 7.04 – 6.91 (m, 2H, 6‘-H, 8‘-H), 4.72 (s, 2H, 5‘-H), 4.22 – 4.10 (m, 2H, 2‘-H), 4.04 – 3.94 (m, 2H, 3‘-H), 3.88 (s, 3H, OCH_3), 1.83 – 1.66 (m, 1H, C*H*-CH_2), 0.95 – 0.86 (m, 2H, CH-C*H*$_2$), 0.79 – 0.66 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 172.4 (C-1), 152.3 (C-9‘), 150.6 (dd, $^1J_{CF}$ = 248.6 Hz, $^2J_{CF}$ =12.8 Hz, C-4’’), 150.0 (dd, $^1J_{CF}$ = 248.9 Hz, $^2J_{CF}$ = 12.6 Hz, C-3’’), 149.0 (C-9a‘), 137.9 (C-1‘‘), 134.8 (C-7‘), 132.6 (C-5a‘), 122.9 (dd, $^3J_{CF}$ = 5.5, $^4J_{CF}$ = 3.4 Hz, C-6’’), 120.2 (C-6‘), 117.5 (d, $^2J_{CF}$ = 17.4 Hz, C-5’’), 115.9 (d, $^2J_{CF}$ = 17.8 Hz, C-2’’), 112.5 (C-8‘), 72.5 (C-2‘), 57.1 (OCH_3), 50.0 (C-5‘), 49.6 (C-3‘), 11.8 (*C*H-CH_2), 7.3 (CH-*C*H_2).

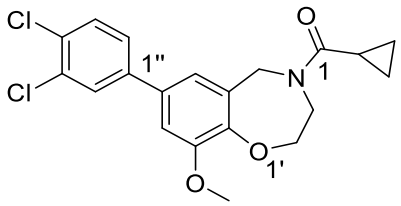
IR (Film): $\tilde{\nu}$ (cm^{-1}) = 3442, 3006, 2956, 2924, 1639, 1584, 1489, 1463, 1272, 1206, 1161, 1103, 992, 854, 770.

MS (EI+): m/z (%) = 131 (100), 169 (83), 181 (62), 219 (51), 251 (53), 280 (34), 359 (6) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{20}H_{19}F_2NO_3$ 359.1333, found 359.1335.

Purity (HPLC): 99 % (210 nm; method 1c).

1-Cyclopropyl-1-[7-(3,4-dichlorophenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]methanone (46)



MF: $C_{20}H_{19}F_2NO_3$ MW: 392.28 g/mol

Standard protocol 2 with 0.098 g (0.30 mmol) **32** and 0.068 g (0.36 mmol) 3,4-dichlorophenylboronic acid. FCC with EtOAc and hexanes (2:1, Rf 0.4) gave 0.095 g (0.24 mmol, 81 %) of **46** as a pale brown solid.

mp: 115 - 116 °C.

^{1}H NMR (mixture of rotamers, 500 MHz, CD_2Cl_2): δ = 7.75 – 7.60 (m, 1H, 2''-H), 7.59 – 7.46 (m, 1H, 5''-H), 7.46 – 7.36 (m, 1H, 6''-H), 7.17 – 6.96 (m, 2H, 6'-H, 8'-H), 4.77 (s, 1.2H, 5'-H), 4.66 (s, 0.8H, 5'-H), 4.23 – 4.16 (m, 0.8H, 2'-H), 4.12 – 4.05 (m, 2H, 2'-H, 3'-H), 4.01 – 3.95 (m, 1.2H, 3'-H), 3.93 – 3.84 (m, 3H, OCH_3), 1.94 – 1.82 (m, 0.6H, C*H*-CH_2), 1.74 – 1.68 (m, 0.4H, C*H*-CH_2), 0.89 – 0.82 (m, 2H, CH-C*H*$_2$), 0.79 – 0.67 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (mixture of rotamers, 101 MHz, CD_2Cl_2): δ = 172.9 (C-1), 172.4 (C-1), 152.7 (C-9'), 152.2 (C-9'), 149.1 (C-9a'), 149.0 (C-9a'), 141.2 (C-1''), 141.1 (C-1''), 134.6 (C-7'), 134.3 (C-7'), 133.4 (C-5a'), 133.3 (C-5a'), 133.0 (C-3''), 131.6 (C-4''), 131.4 (C-4''), 131.1 (C-5''), 131.0 (C-5''), 129.1 (C-2''), 129.0 (C-2''), 126.8 (C-6''), 120.9 (C-6'), 119.5 (C-6'), 111.3 (C-8'), 110.7 (C-8'), 73.2 (C-2'), 72.9 (C-2'), 56.7 (OCH_3), 51.6 (C-3'), 51.3 (C-5'), 49.1 (C-3'), 48.8 (C-5'), 11.8 (*C*H-CH_2), 11.6 (*C*H-CH_2), 7.7 (CH-*C*H$_2$), 7.5 (CH-*C*H$_2$).

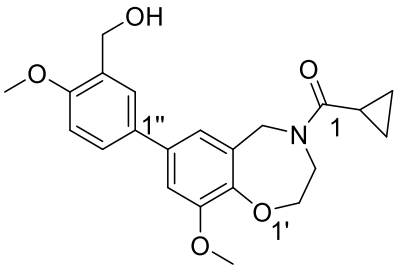
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3433, 3061, 3004, 2930, 1638, 1586, 1469, 1421, 1369, 1203, 1134, 1078, 1044, 852, 819, 737, 679, 530.

MS (EI+): m/z (%) = 169 (52), 181 (43), 219 (27), 294 (46), 304 (64), 306 (44), 322 (56), 324 (25), 325 (11), 391 (2) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{20}H_{19}NO_3{}^{35}Cl_2$ 391.0742, found 391.0749.

Purity (HPLC): 98 % (210 nm; method 3b).

1-Cyclopropyl-1-{7-[3-(hydroxymethyl)-4-methoxyphenyl]-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl}methanone (47)



MF: $C_{22}H_{25}NO_5$ MW: 383.44 g/mol

To a suspension of 0.078 g (2.0 mmol) sodium borohydride in a mixture of 7.5 mL DCM and 2.5 mL MeOH were added 0.43 g (1.1 mmol) **37** and the mixture was stirred for 2 h. 20 mL 2 M HCl was added and after 15 min of stirring, the mixture was extracted with EtOAc (3 x 20 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give 0.42 g (1.9 mmol, 97 %) of **47** as a white solid.

mp: 74 – 75 °C.

^{1}H-NMR (mixture of rotamers, 500 MHz, $CDCl_3$): δ = 7.52 – 7.39 (m, 2H, 2''-H, 6''-H), 7.14 – 6.87 (m, 3H, 6'-H, 8'-H, 5''-H), 4.82 – 4.66 (m, 4H, 5'-H, C*H*$_2$OH), 4.29 – 4.21 (m, 0.7H, 2'-H), 4.19 – 4.00 (m, 3.3H, 2'-H, 3'-H), 3.95 – 3.86 (m, 6H, OCH_3), 2.35 (br s, 1H, OH), 1.92 – 1.85 (m, 0.7H, C*H*-CH_2), 1.73 – 1.67 (m, 0.3H, C*H*-CH_2), 1.01 – 0.90 (m, 2H, CH-C*H*$_2$), 0.82 – 0.69 (m, 2H, CH-C*H*$_2$).

^{13}C-NMR (mixture of rotamers, 101 MHz, $CDCl_3$): δ = 172.8 (C-1), 172.1 (C-1), 157.0 (C-4''), 156.9 (C-4''), 152.0 (C-9'), 151.3 (C-9'), 147.5 (C-9a'), 147.1 (C-9a'), 136.7 (C-7'), 136.4 (C-7'), 133.2 (C-1''), 132.4 (C-5a'), 132.0 (C-5a'), 129.5 (C-3''), 129.3 (C-3''), 127.43 (C-2'', C-6''), 127.39 (C-2''/C-6''), 127.30 (C-2''/C-6''), 120.5 (C-6'), 118.9 (C-6'), 110.8 (C-5''), 110.5 (C-8'), 110.5 (C-5''), 110.1 (C-8'), 72.81 (C-2'), 72.75 (C-2'), 62.1 (3''-CH_2OH), 62.0 (CH_2OH), 56.3 (OCH_3), 56.2 (OCH_3), 55.5 (OCH_3), 51.2 (C-5'), 51.2 (C-3'), 48.9 (C-3'), 48.6 (C-5'), 11.6 (*C*H-CH_2), 11.5 (*C*H-CH_2), 7.7 (CH-*C*H_2), 7.5 (CH-*C*H_2).

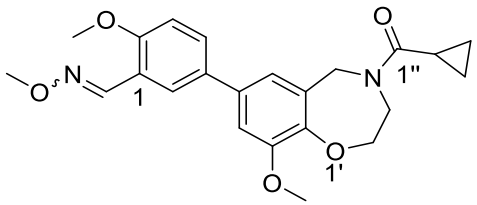
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3406, 3002, 2933, 2865, 2836, 1637, 1486, 1464, 1368, 1344, 1292, 1244, 1044, 813.

MS (ESI+): m/z (%) = 298 (3), 366 (7), 384 (100) $[M + H]^+$, 406 (25).

HRMS (ESI+): m/z calcd for $[C_{22}H_{26}NO_5]^+$ 384.1811, found 384.1806.

Purity (HPLC): > 99 % (210 nm; method 2a).

5-[4-(Cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]-2-methoxybenzaldehyde *O*-methyl oxime (48)



MF: $C_{23}H_{26}N_2O_5$ MW: 410.47 g/mol

To a suspension of 0.15 g (0.39 mmol) **37** in 6 mL EtOH were added 0.13 g (1.5 mmol) *O*-methylhydroxylamine hydrochloride and 0.21 g (1.5 mmol) K_2CO_3. After 12 h the mixture was concentrated in vacuo, treated with 40 mL EtOAc and 40 mL saturated NaCl solution, and extracted with EtOAc (3 x 40 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with DCM with 2 % MeOH (Rf 0.3) gave 0.16 g (0.39 mmol, 99 %) of **48** as a white solid.

mp: 78 – 79 °C.

^{1}H-NMR (mixture of rotamers, 500 MHz, CD_2Cl_2): δ = 8.45 (s, 1H, N=CH), 7.95 (d, *J* = 2.4 Hz, 1H, 6-H), 7.60 – 7.50 (m, 1H, 4-H), 7.13 – 6.95 (m, 3H, 3-H, 6'-H, 8'-H), 4.77 (s, 1.3H, 5'-H), 4.67 (s, 0.7H, 5'-H), 4.23 – 4.13 (m, 0.7H, 2'-H), 4.10 – 4.04 (m, 2H, 2'-H, 3'-H), 4.00 – 3.95 (m, 4.3H, 3'-H, OCH_3), 3.90 – 3.86 (m, 6H, OCH_3), 1.96 – 1.88 (m, 0.7H, C*H*-CH_2), 1.73 – 1.67 (m, 0.3H, C*H*-CH_2), 0.91 – 0.80 (m, 2H, CH-C*H*$_2$), 0.80 – 0.67 (m, 2H, CH-C*H*$_2$).

^{13}C-NMR (mixture of rotamers, 126 MHz, CD_2Cl_2): δ = 172.9 (C-1''), 172.3 (C-1''), 157.5 (C-2), 152.5 (C-9'), 152.0 (C-9'), 148.2 (C-9a'), 148.0 (C-9a'), 144.74 (N=CH), 144.65 (N=CH), 136.6 (C-7'), 136.1 (C-7'), 133.7 (C-5), 133.5 (C-5), 133.2 (C-5a'), 133.1 (C-5a'), 129.9 (C-4), 124.9 (C-6), 124.7 (C-6), 121.4 (C-1), 120.6 (C-6'), 119.3 (C-6'), 111.9 (C-3), 111.3 (C-8'), 110.7 (C-8'), 73.2 (C-2'), 73.0 (C-2'), 62.2 (OCH_3), 56.7 (OCH_3), 56.2 (OCH_3), 51.6 (C-3'), 51.4 (C-5'), 49.2 (C-3'), 49.0 (C-5'), 11.8 (*C*H-CH_2), 11.6 (*C*H-CH_2), 7.7 (CH-*C*H_2), 7.5 (CH-*C*H_2).

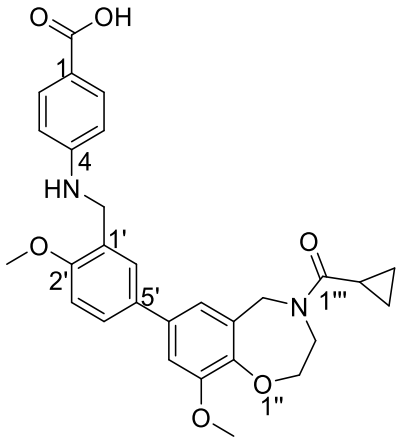
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3447, 3004, 2936, 2838, 2345, 1641, 1587, 1485, 1463, 1431, 1292, 1256, 1205, 1051, 815.

MS (ESI+): m/z (%) = 411 (100) $[M+H]^+$, 412 (16).

HRMS (ESI+): m/z calcd for $[C_{23}H_{27}N_2O_5]^+$ 411.1920, found 411.1912.

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

4-({5-[4-(Cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]-2-methoxybenzyl}amino)benzoic acid (49)



MF: $C_{29}H_{30}N_2O_6$ MW: 502.57 g/mol

0.25 g (0.67 mmol) **37** and 0.29 g (2.1 mmol) 4-aminobenzoic acid were dissolved in 3 mL DCM and 3 mL MeOH. 0.15 g (2.4 mmol) $NaCNBH_3$ were added and stirred for 72 h. To this mixture was added 70 mL of 1 M HCl and it was extracted with DCM (5 x 70 mL) five times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (3:1, Rf 0.2) with 1 % AcOH gave 0.27 g (0.54 mmol, 80%) of **49** as a white solid.

mp: 235 °C (decomposition).

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 7.73 – 7.65 (m, 2H, 2-H, 6-H), 7.53 – 7.48 (m, 2H, 3‘-H, 6‘-H), 7.11 – 7.05 (m, 2H, 4‘-H, 6‘‘-H), 7.02 (d, *J* = 2.1 Hz, 1H, 8‘‘-H), 6.70 – 6.63 (m, 2H, 3-H, 5-H), 6.45 (br s, 1H, NH), 4.73 (s, 2H, 5’’-H), 4.45 – 4.33 (m, 2H, CH_2NH), 4.10 – 4.04 (m, 2H, 2’’-H), 4.00 – 3.93 (m, 2H, 3’’-H), 3.90 (s, 3H, 2’-OCH_3), 3.80 (s, 3H, 9’’-OCH_3), 1.98 – 1.91 (m, 1H, C*H*-CH_2), 0.72 – 0.67 (m, 4H, CH-C*H*$_2$).

^{13}C NMR (100 °C, 101 MHz, DMSO-d_6): δ = 171.3 (C-1‘‘), 166.7 (O=CO), 156.2 (C-2‘), 152.1 (C-4), 151.0 (C-9‘‘), 147.0 (C-9a‘‘), 134.8 (C-7‘‘), 131.7 (C-5‘, C-5a‘‘), 130.4 (C-2, C-6), 126.9 (C-1‘), 126.2 (C-3‘/C-6‘), 125.7 (C-3‘/C-6‘), 118.8 (C-6‘‘), 117.4 (C-1), 111.1 (C-4‘), 110.9 (C-8‘‘), 110.8 (C-3, C-5), 71.2 (C-2‘‘), 56.0 (2’-OCH_3), 55.4 (9’’-OCH_3), 48.6 (C-5‘‘), 48.3 (C-3‘‘), 40.7 (CH_2NH), 10.5 (*C*H-CH_2), 6.3 (CH-*C*H$_2$).

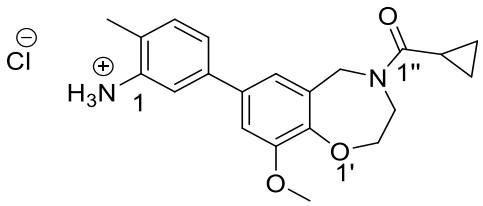
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3366, 2923, 1641, 1602, 1426, 1291, 1220, 817.

MS (EI+): m/z (%) = 225 (59), 255 (69), 296 (72), 364 (34), 366 (58), 388 (80), 456 (100), 459 (74), 502 (100) $[M]^{+\bullet}$, 503 (33).

HRMS (EI+): m/z calcd for $C_{29}H_{30}N_2O_6$ 502.2104, found 502.2098.

Purity (HPLC): 98 % (210 nm; method 1c).

5-[4-(Cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]-2-methylbenzenaminium chloride (50)



MF: $C_{21}H_{24}N_2O_3$ MW: 388.89 g/mol

To a suspension of 6 g Raney nickel in 60 mL water was added 9.2 g NaOH. Upon complete activation after 15 minutes this suspension was washed with three 50 mL portions water and then with three 50 mL portions EtOH. Seperately 0.45 g (1.2 mmol) **30** were dissolved in 35 mL EtOH and then 2.2 mL (44 mmol) hydrazine monohydrate were added. The activated Raney-nickel suspension was then added to this solution and the mixture was refluxed for 40 min. The suspension was filtrated and the filtrate dissolved in a mixture of 30 mL 2 M NaOH and 30 mL EtOAc. The mixture was extracted with EtOAc (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$, and concentrated in vacuo. They were redissolved in 40 mL ethyl ether. Precipitation with 4 M HCl in 1,4-dioxane gave 0.18 g (0.46 mmol, 38 %) of **50** as a purple solid.

mp: 221 °C (decomposition).

^{1}H NMR (mixture of rotamers, 500 MHz, DMSO-d_6): δ = 10.21 (br s, 3H, NH_3^+), 7.81 – 7.63 (m, 1H, 6-H), 7.59 – 7.51 (m, 1H, 4-H), 7.36 (d, *J* = 7.8 Hz, 1H, 3-H), 7.28 (d, *J* = 2.1 Hz, 0.6H, 6'-H), 7.15 – 7.12 (m, 1H, 8'-H), 7.03 (d, *J* = 2.1 Hz, 0.4H, 6'-H), 4.84 (s, 1.2H, 5'-H), 4.61 (s, 0.8H, 5'-H), 4.20 – 4.12 (m, 0.8H, 2'-H), 4.12 – 4.07 (m, 0.8H, 3'-H), 4.05 – 3.96 (m, 1.2H, 2'-H), 3.91 – 3.85 (m, 1.2H, 3'-H), 3.83 (s, 3H, OCH_3), 2.37 (s, 3H, 2-CH_3), 2.21 – 2.12 (m, 1H, 0.6H, C*H*-CH_2), 1.99 – 1.90 (m, 0.4H, C*H*-CH_2), 0.81 – 0.59 (m, 4H, CH-CH_2).

^{13}C NMR (mixture of rotamers, 126 MHz, DMSO-d_6): δ = 172.7 (C-1''), 172.0 (C-1''), 152.0 (C-9'), 151.8 (C-9'), 148.2 (C-9a'), 139.1 (C-5), 138.9 (C-5), 134.7 (C-2), 134.1 (C-2), 133.2 (C-5a'), 132.8 (C-5a'), 132.5 (C-1), 132.2 (C-3), 132.1 (C-3), 130.9 (C-7'), 130.7 (C-7'), 126.1 (C-4), 125.9 (C-4), 121.6 (C-6), 121.4 (C-6), 120.1 (C-6'), 119.4 (C-6'), 110.9 (C-8'), 110.4 (C-8'), 72.7 (C-2'), 72.1 (C-2'), 56.3 (OCH_3), 50.9 (C-3'), 50.1

(C-5‘), 48.7 (C-3‘), 48.3 (C-5‘), 17.3 (2-CH_3), 11.2 (*C*H-CH_2), 11.1 (*C*H-CH_2), 7.9 (CH-*C*H_2), 7.6 (CH-*C*H_2).

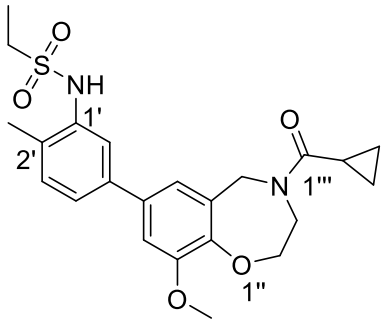
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3425, 3000, 2880, 2757, 2575, 1636, 1583, 1486, 1468, 1208, 1105, 1091, 1051, 992, 829, 584, 551.

MS (ESI+): m/z (%) = 223 (20), 285 (25), 331 (73), 353 (100) $[M + H]^+$.

HRMS (ESI+): m/z calcd for $C_{21}H_{25}N_2O_3$ 353.1860, found 353.1859.

Purity (HPLC): 95 % (210 nm; method 1d).

_N_-{5-[4-(cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]-2-methylphenyl}ethanesulfonamide (51)



MF: $C_{23}H_{28}N_2O_5S$ MW: 444.55 g/mol

To a solution of 0.058 g (0.15 mmol) **50** and 5 mg (0.04 mmol) DMAP in 2.0 mL pyridine were added 0.015 mL (0.16 mmol) ethanesulfonyl chloride at 0 °C. The mixture was warmed to rt and stirred for 16 h. To this mixture was added 20 mL 1 M HCl and the mixture was extracted with EtOAc (3 x 20 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (2:1, Rf 0.3) gave 0.040 g (0.090 mmol, 60 %) of **51** as a white solid.

mp: 155 - 156 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.59 (d, *J* = 1.9 Hz, 1H, 6‘-H), 7.28 (dd, *J* = 7.9, 1.9 Hz, 1H, 4‘-H), 7.23 (d, *J* = 7.9 Hz, 1H, 3‘-H), 7.04 – 6.99 (m, 2H, 6‘‘-H, 8‘‘-H), 6.00 (br s, 1H, NH), 4.72 (s, 2H, 5‘‘-H), 4.19 – 4.09 (m, 2H, 3’’-H), 4.03 – 3.95 (m, 2H, 2’’-H), 3.88 (s, 3H, OCH_3), 3.14 (q, *J* = 7.4 Hz, 2H, C*H*$_2$-CH_3), 2.32 (s, 3H, 2‘-CH_3), 1.83 – 1.69 (m, 1H, C*H*-CH_2), 1.37 (t, *J* = 7.4 Hz, 3H, CH_2-C*H*$_3$), 0.94 – 0.86 (m, 2H, CH-C*H*$_2$), 0.78 – 0.68 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (ambient temperature, mixture of rotamers, 126 MHz, $C_2D_2Cl_4$): δ = 172.7 (C-1‘‘), 172.1 (C-1‘‘), 151.7 (C-9‘‘), 151.2 (C-9‘‘), 147.8 (C-9a‘‘), 147.6 (C-9a‘‘), 139.7 (C-5‘), 135.7 (C-7‘‘), 135.3 (C-7‘‘), 135.1 (C-1‘), 135.0 (C-1‘), 132.1 (C-5a‘‘), 131.7 (C-5a‘‘), 131.5 (C-3‘), 128.5 (C-2‘), 124.3 (C-4‘), 124.2 (C-4‘), 120.4 (C-6‘), 120.2 (C-6‘‘), 119.0 (C-6‘‘), 111.0 (C-8‘‘), 110.4 (C-8‘‘), 72.4 (C-2‘‘), 72.2 (C-2‘‘), 56.2 (OCH_3), 51.6 (C-5‘‘), 50.8 (C-3‘‘), 48.5 (C-3‘‘), 48.2 (C-5‘‘), 46.7 (*C*H_2-CH_3), 46.6 (*C*H_2-CH_3), 17.6 (2‘-CH_3), 11.6 (*C*H-CH_2), 11.4 (*C*H-CH_2), 8.2 (CH_2-*C*H_3), 7.7 (CH-*C*H_2), 7.5 (CH-*C*H_2).

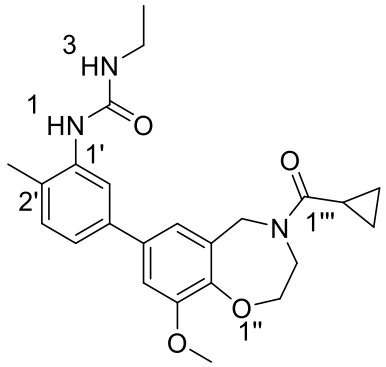
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3438, 3165, 2939, 1617, 1569, 1484, 1315, 1137, 1124, 1046, 993, 914, 809, 726, 568.

MS (EI+): m/z (%) = 240 (28), 357 (47), 444 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{23}H_{28}N_2O_5S$ 444.1719, found 444.1727.

Purity (HPLC): > 99 % (210 nm; method 3a).

1-{5-[4-(Cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]-2-methylphenyl}-3-ethylurea (52)



MF: $C_{24}H_{29}N_3O_4$ MW: 423.51 g/mol

To a solution of 0.058 g (0.15 mmol) **50** in 1.0 mL DCM were added 0.30 mL (1.7 mmol) DIPEA and 0.080 mL (1.3 mmol) ethyl isocyanate and the mixture was stirred for 72 h. To this mixture was added 15 mL 0.1 M HCl and the mixture was extracted with EtOAc (3 x 15 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (2:1, Rf 0.3) gave 0.030 g (0.071 mmol, 47 %) of **52** as a white solid.

mp: 190 - 191 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.63 (d, *J* = 1.9 Hz, 1H, 6'-H), 7.31 – 7.16 (m, 2H, 3'-H, 4'-H), 7.09 – 6.98 (m, 2H, 6''-H, 8''-H), 5.99 (br s, 1H, NH), 4.71 (s, 2H, 5''-H), 4.59 (br s, 1H, NH), 4.17 – 4.08 (m, 2H, 2''-H), 4.03 – 3.95 (m, 2H, 3''-H), 3.87 (s, 3H, OCH_3), 3.24 (q, *J* = 7.2 Hz, 2H, 4-H), 2.25 (s, 3H, 2'-CH_3), 1.81 – 1.69 (m, 1H, C*H*-CH_2), 1.12 (t, *J* = 7.2 Hz, 3H, 5-H), 0.94 – 0.86 (m, 2H, CH-C*H*$_2$), 0.79 – 0.68 (m, 2H, CH-C*H*$_2$).

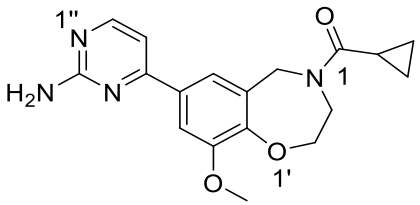
^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 172.4 (C-1'''), 156.0 (C-2), 152.1 (C-9''), 148.5 (C-9a''), 139.6 (C-5'), 137.1 (C-1'), 136.2 (C-7''), 132.3 (C-5a''), 131.2 (C-3'), 130.8 (C-2'), 123.8 (C-4'), 123.4 (C-6'), 120.2 (C-6''), 112.6 (C-8''), 72.4 (C-2''), 57.1 (OCH_3), 50.2 (C-5''), 49.7 (C-3''), 35.5 (C-4), 17.3 (2'-CH_3), 15.4 (C-5), 11.8 (*C*H-CH_2), 7.3 (CH-*C*H_2).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3329, 3093, 2969, 2930, 1644, 1560, 1460, 1398, 1293, 1226, 1171, 1092, 1044, 813, 730, 657.

MS (EI+): m/z (%) = 116 (27), 241 (40), 281 (54), 291 (70), 309 (59), 352 (67), 378 (100), 423 (20) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{24}H_{29}N_3O_4$ 423.2158, found 423.2157.

1-[7-(2-Aminopyrimidin-4-yl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-1-(cyclopropyl)methanone (53)



MF: $C_{18}H_{20}N_4O_3$ MW: 340.39 g/mol

A solution of 0.20 g (0.69 mmol) **55** in 5 mL anhydrous DMF was heated to 160 °C under N_2, then 0.52 mL (2.5 mmol) Bredereck's reagent was added and heating continued for 1 h. Then 0.66 g (3.7 mmol) guanidinium carbonate and 0.34 g (2.5 mmol) K_2CO_3 were added and the mixture was heated to 160 °C for further 4 h. After cooling 40 mL saturated $NaHCO_3$ solution was added and the mixture was extracted with DCM (3 x 40 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with DCM with 5 % MeOH (Rf 0.1) gave 0.13 g (0.39 mmol, 57 %) of **53** as a yellow solid.

mp: 136 – 137 °C.

^{1}H NMR (mixture of rotamers, 500 MHz, CD_2Cl_2): δ = 8.34 – 8.26 (m, 1H, 6''-H), 7.63 – 7.47 (m, 2H, 6'-H, 8'-H), 7.06 – 6.98 (m, 1H, 5''-H), 5.25 – 5.13 (m, 2H, NH_2), 4.79 (s, 1.1H, H-5'), 4.68 (s, 0.9H, H-5'), 4.24 – 4.18 (m, 0.9H, 2'-H), 4.13 – 4.06 (m, 2H, 2'-H, 3'H), 4.01 – 3.95 (m, 1.1H, 3'-H), 3.92 – 3.88 (m, 3H, OCH_3), 1.92 – 1.83 (m, 0.6H, C*H*-CH_2), 1.74 – 1.68 (m, 0.4H, C*H*-CH_2), 0.89 – 0.81 (m, 2H, CH-C*H*$_2$), 0.77 – 0.69 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (mixture of rotamers, 126 MHz, CD_2Cl_2): δ = 173.0 (C-1), 172.5 (C-1), 164.62 (C-4''), 164.57 (C-4''), 163.8 (C-2''), 159.2 (C-6''), 159.1 (C-6''), 152.6 (C-9'), 152.1 (C-9'), 151.1 (C-9a'), 150.9 (C-9a'), 132.9 (C-7'), 132.7 (C-5a'), 121.3 (C-6'), 119.8 (C-6'), 111.1 (C-8'), 110.6 (C-8'), 107.6 (C-5''), 107.5 (C-5''), 73.1 (C-2'), 72.8 (C-2'), 56.64 (OCH_3), 56.58 (OCH_3), 51.5 (C-3'), 51.3 (C-5'), 49.0 (C-3'), 48.8 (C-5'), 11.8 (*C*H-CH_2), 11.6 (*C*H-CH_2), 7.7 (CH-*C*H$_2$), 7.6 (CH-*C*H$_2$).

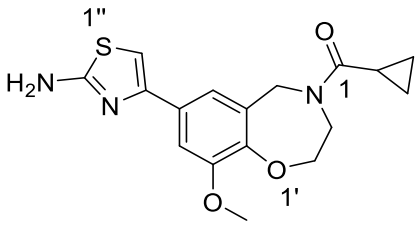
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3423, 3331, 3173, 3002, 2920, 2873, 1655, 1636, 1562, 1460, 1446, 1290, 1215, 1104, 1044, 888, 814, 740.

MS (EI+): m/z (%) = 242 (26), 253 (45), 271 (46), 340 (100) $[M]^{+\bullet}$, 341 (22).

HRMS (EI+): m/z calcd for $C_{18}H_{20}N_4O_3$ 340.1535, found 340.1538.

Purity (HPLC): 99 % (210 nm; method 2a).

1-[7-(2-Aminothiazol-4-yl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-1-(cyclopropyl)methanone (54)



MF: $C_{17}H_{19}N_3O_3S$ MW: 345.42 g/mol

To a solution of 1.0 g (3.1 mmol) **32** and 0.11 g (0.16 mmol) $Pd(PPh_3)_2Cl_2$ in 12 mL anhydrous 1,4-dioxane under N_2 were added 1.4 mL (4.0 mmol) tributyl(1-ethoxyvinyl)tin. The mixture was heated to 140 °C under microwave irradiation with 300 W for 40 minutes. After cooling 50 mL water was added and the mixture extracted with DCM (3 x 50 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (2:1, Rf 0.3) gave 1.0 g of the crude enol ether. This intermediate was dissolved in a mixture of 10 mL THF and 10 mL water and treated at 0 °C with 0.56 g (2.9 mmol) *N*-bromosuccinimide. After one hour at rt the mixture was extracted with EtOAc (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to obtain 0.47 g of the crude α–bromo-ketone. A portion of 0.20 g (0.54 mmol) of this residue and 0.20 g (2.6 mmol) thiourea were dissolved in 5 mL anhydrous DMF and stirred for 16 h. After the addition of 30 mL water the mixture was extracted with DCM (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with DCM with 3 % MeOH (Rf 0.2) gave 0.090 g (0.26 mmol, 20 %) of **54** as a yellow solid.

mp: 103 – 104 °C.

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 7.41 – 7.30 (m, 2H, 6‘-H, 8’-H), 6.84 (s, 1H, 5’’-H), 6.65 (s, 2H, NH_2), 4.71 (s, 2H, 5‘-H), 4.13 – 4.04 (m, 2H, 2‘-H), 4.04 – 3.91 (m, 2H, 3‘-H), 3.82 (s, 3H, OCH_3), 2.06 – 1.88 (m, 1H, C*H*-CH_2), 0.75 – 0.63 (m, 4H, CH-C*H*$_2$).

^{13}C NMR (100 °C, 101 MHz, DMSO-d_6): δ = 171.2 (C-1), 167.5 (C-2‘‘), 150.7 (C-9‘), 149.2 (C-9a‘), 147.2 (C-4‘‘), 131.3 (C-5a‘), 129.8 (C-7‘), 118.5 (C-6‘), 110.3 (C-8‘),

100.5 (C-5‘‘), 71.2 (C-2‘), 55.9 (OCH_3), 48.6 (C-5‘), 48.4 (C-3‘), 10.5 (*C*H-CH_2), 6.2 (CH-*C*H_2).

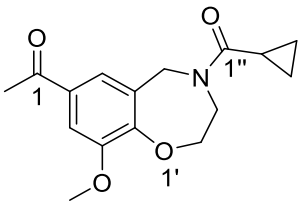
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3357, 3155, 3037, 3004, 2962, 2933, 2869, 2361, 2343, 2231, 2220, 1624, 1590, 1540, 1485, 1461, 1421, 1345, 1222, 1105, 1065, 740.

MS (ESI+): m/z (%) = 278 (7), 346 (100), $[M + H]^+$, 368 (32).

HRMS (ESI+): m/z calcd for $[C_{17}H_{20}N_3O_3S]^+$ 346.1225, found 346.1219.

Purity (HPLC): 97 % (210 nm; method 1b).

1-[4-(Cyclopropanecarbonyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl]ethan-1-one (55)



MF: $C_{16}H_{19}NO_4$ MW: 289.33 g/mol

To a solution of 0.49 g (1.5 mmol) **32** and 0.053 g (0.075 mmol) $Pd(PPh_3)_2Cl_2$ in 6.0 mL anhydrous 1,4-dioxane under N_2 were added 0.66 mL (2.0 mmol) tributyl(1-ethoxyvinyl)tin. The mixture was heated to 140 °C under microwave irradiation with 300 W for 40 minutes. After cooling 30 mL 10 % aqueous HCl was added and the mixture extracted with DCM (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (4:1, Rf 0.5) gave 0.29 g (1.0 mmol, 67 %) of **55** as a white solid.

mp: 76 - 77 °C.

^{1}H-NMR (80 °C, 500 MHz, $C_2D_2Cl_4$): δ = 7.47 – 7.38 (m, 2H, 6'-H, 8'-H), 4.73 (s, 2H, 5'-H), 4.30 – 4.16 (m, 2H, 2'-H), 4.08 – 3.95 (m, 2H, 3'-H), 3.87 (s, 3H, OCH_3), 2.53 (s, 3H, 2-H), 1.81 – 1.63 (m, 1H, C*H*-CH_2), 0.93 – 0.87 (m, 2H, CH-C*H*$_2$), 0.79 – 0.70 (m, 2H, CH-C*H*$_2$).

^{13}C-NMR (ambient temperature, mixture of rotamers, 126 MHz, $C_2D_2Cl_4$): δ = 196.9 (C-1), 196.7 (C-1), 172.8 (C-1''), 172.4 (C-1''), 152.4 (C-9a), 152.1 (C-9a), 151.5 (C-9'), 151.1 (C-9'), 132.2 (C-7'), 132.1 (C-7'), 130.8 (C-5a), 130.2 (C-5a), 123.5 (C-6'), 121.3 (C-6'), 111.4 (C-8'), 110.2 (C-8'), 72.1 (C-2'), 71.7 (C-2'), 56.3 (OCH_3), 56.2 (OCH_3), 50.4 (C-3', C-5'), 48.1 (C-3'), 47.6 (C-5'), 26.5 (C-2), 26.4 (C-2), 11.5 (*C*H-CH_2), 11.4 (*C*H-CH_2), 7.7 (CH-*C*H_2), 7.6 (CH-*C*H_2).

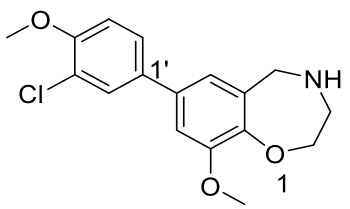
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3433, 3084, 3009, 2934, 2362, 2345, 2234, 2220, 1666, 1629, 1583, 1465, 1422, 1370, 1305, 1223, 1202, 1092, 1042, 870, 744.

MS (ESI+): m/z (%) = 112 (10), 222 (12), 290 (100), $[M + H]^+$, 312 (88).

HRMS (ESI+): m/z calcd for $[C_{16}H_{20}NO_4]^+$ 290.1392, found 290.1386.

Purity (HPLC): 98 % (210 nm; method 2a).

7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepine (56)



MF: $C_{17}H_{18}ClNO_3$ MW: 319.79 g/mol

Standard protocol 2 with 1.5 g (5.8 mmol) **9** and 1.3 g (7.0 mmol) 3-chloro-4-methoxyphenylboronic acid. FCC with EtOAc with 5 % triethylamine (Rf 0.1) gave 0.90 g (2.8 mmol, 48 %) of **56** as a white solid.

mp: 117 – 118 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.56 (d, *J* = 2.3 Hz, 1H, 2‘-H), 7.40 (dd, *J* = 8.5, 2.3 Hz, 1H, 6‘-H), 6.98 (d, *J* = 8.5 Hz, 1H, 5‘-H), 6.95 (d, *J* = 2.1 Hz, 1H, 8-H), 6.89 (d, *J* = 2.1 Hz, 1H, 6-H), 4.14 – 4.10 (m, 2H, 2-H), 4.02 (s, 2H, 5-H), 3.94 (s, 3H, 4’-OCH_3), 3.93 (s, 3H, 9-OCH_3), 3.29 – 3.24 (m, 2H, 3-H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 154.3 (C-4‘), 151.8 (C-9), 148.5 (C-9a), 136.7 (C-5a), 135.0 (C-7), 134.4 (C-1‘), 128.7 (C-2‘), 126.1 (C-6‘), 122.7 (C-7), 119.5 (C-6), 112.2 (C-5‘), 109.8 (C-8), 75.5 (C-2), 56.33 (OCH_3), 56.27 (OCH_3), 53.2 (C-5), 52.3 (C-3).

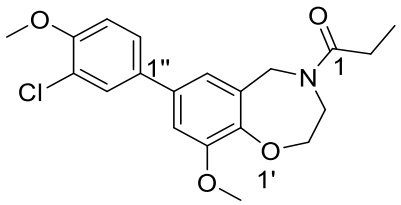
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3431, 3290, 3025, 2922, 2904, 2839, 1587, 1493, 1466, 1281, 1244, 1202, 1182, 1061, 1013, 856, 786, 697, 604.

MS (EI+): m/z (%) = 139 (11), 198 (11), 261 (22), 263 (9), 276 (58), 278 (21), 319 (100) $[M]^{+\bullet}$, 321 (36).

HRMS (EI+): m/z calcd for $C_{17}H_{18}{}^{35}ClNO_3$ 319.0975, found 319.0972.

Purity (HPLC): 97 % (210 nm; method 1c).

1-[7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]propan-1-one (57)



MF: $C_{20}H_{22}ClNO_4$ MW: 375.85 g/mol

Standard protocol 1 with 0.16 g (0.50 mmol) **56** and 0.16 mL (0.65 mmol) propionyl chloride. FCC with EtOAc and hexanes (3:1, Rf 0.4) gave 0.075 g (0.20 mmol, 40 %) of **57** as a white solid.

mp: 66 – 67 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.52 (d, *J* = 2.3 Hz, 1H, 2‘‘-H), 7.37 (dd, *J* = 8.5, 2.3 Hz, 1H, 6‘‘-H), 7.06 – 6.86 (m, 3H, 6‘-H, 8‘-H, 5‘‘-H), 4.59 (s, 2H, 5‘-H), 4.15 – 4.06 (m, 2H, 2‘-H), 3.94 – 3.84 (m, 8H, 3‘-H, OCH_3), 2.47 – 2.24 (m, 2H, 2-H), 1.10 (t, *J* = 7.4 Hz, 3H, 3-H).

^{13}C NMR (ambient temperature, mixture of rotamers, 101 MHz, $C_2D_2Cl_4$): δ = 172.9 (C-1), 172.3 (C-1), 154.3 (C-4‘‘), 154.1 (C-4‘‘), 151.9 (C-9‘), 151.3 (C-9‘), 147.6 (C-9a‘), 147.5 (C-9a‘), 135.1 (C-7‘), 134.7 (C-7‘), 133.7 (C-1‘‘), 133.6 (C-1‘‘), 132.1 (C-5a‘), 131.8 (C-5a‘), 128.5 (C-2‘‘), 128.4 (C-2‘‘), 126.2 (C-6‘‘), 126.1 (C-6‘‘), 122.5 (C-3‘‘), 122.4 (C-3‘‘), 120.0 (C-6‘), 118.8 (C-6‘), 112.3 (C-5‘‘), 112.2 (C-5‘‘), 110.7 (C-8‘), 110.0 (C-8‘), 72.4 (C-2‘), 72.3 (C-2‘), 56.29 (OCH_3), 56.24 (OCH_3), 56.22 (OCH_3), 50.8 (C-3‘, C-5‘), 48.3 (C-3‘), 47.9 (C-5‘), 26.7 (C-2), 26.4 (C-2), 9.19 (C-3), 9.15 (C-3).

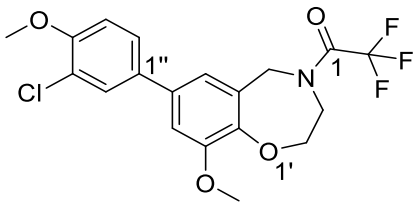
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3434, 2935, 2839, 1648, 1586, 1486, 1463, 1291, 1256, 1202, 1063, 1020, 808, 701, 605.

MS (EI+): m/z (%) = 261 (12), 263 (4), 276 (100), 278 (30), 375 (66) $[M]^{+\bullet}$, 376 (15), 377 (23).

HRMS (EI+): m/z calcd for $C_{20}H_{22}{}^{35}ClNO_4$ 375.1237, found 375.1235.

Purity (HPLC): 99 % (210 nm; method 1c).

1-[7-(3-chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-2,2,2-trifluoroethan-1-one (58)



MF: $C_{19}H_{17}ClF_3NO_4$ MW: 415.79 g/mol

To a solution of 0.040 g (0.13 mmol) **56** and 5 mg (0.04 mmol) DMAP in 1.0 mL DCM was added 0.070 mL (0.50 mmol) trifluoroacetic anhydride at 0 °C. The mixture was stirred and warmed to rt. 0.70 mL (41 mmol) DIPEA was added and the solution was stirred for further 12 h. Then 10 mL saturated $NaHCO_3$ solution was added and this mixture was extracted with DCM (3 x 10 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:2, Rf 0.2) gave 0.050 g (0.12 mmol, 92 %) of **58** as a white solid.

mp: 143 - 144 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.52 (d, *J* = 2.3 Hz, 1H, 2''-H), 7.36 (dd, *J* = 8.6, 2.3 Hz, 1H, 6''-H), 7.09 – 6.90 (m, 3H, 6'-H, 8'-H, 5''-H), 4.69 (s, 2H, 5'-H), 4.18 – 4.13 (m, 2H, 2'-H), 4.03 – 3.93 (m, 2H, 3'-H), 3.90 (s, 3H, 4''-OCH_3), 3.88 (s, 3H, 9'-OCH_3).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 156.0 (C-1), 155.1 (C-4''), 152.2 (C-9'), 148.3 (C-9a'), 136.0 (C-7'), 134.2 (C-1''), 130.6 (C-5a'), 128.8 (C-2''), 126.2 (C-6''), 123.6 (C-3''), 120.4 (C-6'), 116.6 (d, $^1J_{CF}$ = 287.9 Hz, C-2), 113.4 (C-8'/C-5''), 112.9 (C-8'/C-5''), 72.4 (C-2'), 57.0 (9'-OCH_3), 56.7 (4''-OCH_3), 50.9 (C-3'), 50.4 (C-5').

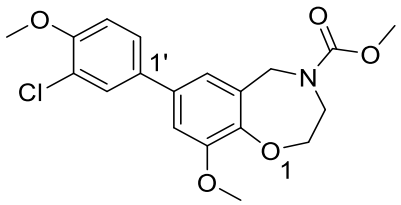
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3433, 3025, 3009, 2970, 2937, 1691, 1488, 1453, 1287, 1237, 1200, 1174, 1141, 1080, 1046, 815, 705, 580.

MS (EI+): m/z (%) = 139 (8), 198 (11), 261 (11), 276 (22), 302 (20), 415 (100) $[M]^{+\bullet}$, 417 (31).

HRMS (EI+): m/z calcd for $C_{19}H_{17}{}^{35}ClF_3NO_4$ 415.0798, found 415.0792.

Purity (HPLC): > 99 % (210 nm; method 3a).

Methyl 7-(3-chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carboxylate (59)



MF: $C_{19}H_{20}ClNO_5$ MW: 377.82 g/mol

Standard protocol 1 with 0.10 g (0.31 mmol) **56** and 0.20 mL (2.6 mmol) methyl chloroformate. FCC with EtOAc and hexanes (1:1, Rf 0.3) gave 0.11 g (0.29 mmol, 94 %) of **59** as a white solid.

mp: 67 – 68 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.53 (d, *J* = 2.3 Hz, 1H, 2‘-H), 7.37 (dd, *J* = 8.5, 2.3 Hz, 1H, 6‘-H), 6.99 – 6.94 (m, 3H, 6-H, 8-H, 5‘-H), 4.53 (s, 2H, 5-H), 4.10 – 4.06 (m, 2H, 2-H), 3.90 (s, 3H, 4‘-OCH_3), 3.87 (s, 3H, 9-OCH_3), 3.84 – 3.81 (m, 2H, 3-H), 3.66 (s, 3H, O=C-OCH_3).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 156.0 (C=O), 154.9 (C-4‘), 152.2 (C-9), 148.6 (C-9a), 135.2 (C-7), 134.6 (C-1‘), 133.0 (C-5a), 128.8 (C-2‘), 126.2 (C-6‘), 123.6 (C-3‘), 120.3 (C-6), 113.4 (C-5‘), 112.3 (C-8), 72.6 (C-2), 57.1 (9-OCH_3), 56.7 (4‘-OCH_3), 52.7 (O=C-OCH_3), 50.2 (C-5), 50.1 (C-3).

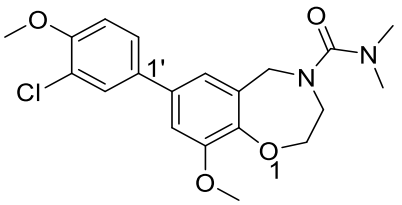
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3433, 2934, 1702, 1637, 1485, 1291, 1235, 1127, 1063, 980, 811, 700.

MS (EI+): m/z (%) = 198 (11), 261 (14), 263 (5), 276 (42), 277 (15), 278 (17), 377 (100) $[M]^{+\bullet}$, 378 (21), 379 (34), 380 (7).

HRMS (EI+): m/z calcd for $C_{19}H_{20}{}^{35}ClNO_5$ 377.1030, found 377.1026.

Purity (HPLC): 97 % (210 nm; method 1c).

7-(3-Chloro-4-methoxyphenyl)-9-methoxy-*N*,*N*-dimethyl-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carboxamide (60)



MF: $C_{20}H_{23}ClN_2O_4$ MW: 390.86 g/mol

Standard protocol 1 with 0.10 g (0.31 mmol) **56** and 0.24 mL (2.6 mmol) N,N-dimethylcarbamoyl chloride. FCC with EtOAc and hexanes (5:1, Rf 0.1) gave 0.090 g (0.23 mmol, 74 %) of **60** as a white solid.

mp: 85 - 86 °C.

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.58 (d, *J* = 2.3 Hz, 1H, 2‘-H), 7.44 (dd, *J* = 8.5, 2.3 Hz, 1H, 6‘-H), 7.05 – 6.94 (m, 3H, 6-H, 8-H, 5‘-H), 4.38 (s, 2H, 5-H), 4.17 – 4.14 (m, 2H, 2-H), 3.92 (s, 3H, 4‘-OCH_3), 3.88 (s, 3H, 9-OCH_3), 3.67 – 3.62 (m, 2H, 3-H), 2.80 (s, 6H, $N(CH_3)_2$).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 164.9 (C=O), 154.7 (C-4‘), 152.0 (C-9), 148.7 (C-9a), 135.0 (C-7), 134.5 (C-1‘), 133.2 (C-5a), 128.9 (C-2‘), 126.6 (C-6‘), 122.8 (C-3‘), 120.2 (C-6), 112.7 (C-5‘), 110.4 (C-8), 73.0 (C-2), 56.60 (OCH_3), 56.57 (OCH_3), 52.8 (C-5), 52.7 (C-3), 39.1 ($N(CH_3)_2$).

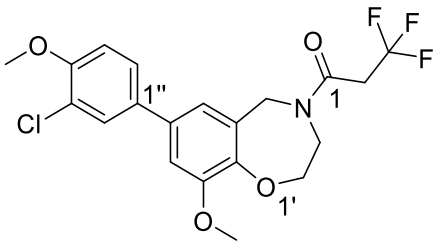
IR (Film): $\tilde{\nu}$ (cm^{-1}) = 3424, 3299, 3002, 2933, 2851, 1644, 1486, 1462, 1391, 1255, 1202, 1063, 1022, 807, 752, 701.

MS (EI+): m/z (%) = 72 (100), 276 (56), 277 (20), 278 (22), 317 (83), 318 (60), 319 (36), 320 (16), 390 (75) $[M]^{+\bullet}$, 391 (21), 392 (23).

HRMS (EI+): m/z calcd for $C_{20}H_{23}{}^{35}ClN_2O_4$ 390.1346, found 390.1348.

Purity (HPLC): 96 % (210 nm; method 1c).

1-[7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-3,3,3-trifluoropropan-1-one (61)



MF: $C_{20}H_{19}ClF_3NO_4$ MW: 429.82 g/mol

To a solution of 0.066 g (0.21 mmol) **56** and 5 mg (0.04 mmol) DMAP in 2.0 mL DCM was added 0.035 mL (0.40 mmol) 3,3,3-trifluoropropionic acid at 0 °C. After five minutes 0.058 g (0.30 mmol) EDC-HCl was added, the mixture warmed to rt and stirred for further 12 h. To this mixture was added 10 mL saturated $NaHCO_3$ solution and this mixture was extracted with DCM (3 x 10 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:1, Rf 0.4) gave 0.082 g (0.19 mmol, 94 %) of **61** as a white solid.

mp: 105 - 106 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$): δ = 7.56 (d, *J* = 2.3 Hz, 0.4H, 2‘‘-H), 7.54 (d, *J* = 2.3 Hz, 0.6H, 2‘‘-H), 7.46 – 7.34 (m, 1H, 6‘‘-H), 7.11 (d, *J* = 2.1 Hz, 0.4H, 6‘-H), 7.07 – 6.92 (m, 2H, 8‘-H, 5‘‘-H), 6.88 (d, *J* = 2.1 Hz, 0.6H, 6‘-H), 4.72 (s, 0.8H, 5‘-H), 4.57 (s, 1.2H, 5-H), 4.24 – 4.13 (m, 2H, 2‘-H), 4.11 – 4.01 (m, 1.2H, 3‘-H), 4.00 – 3.84 (m, 6.8H, 3‘-H, OCH_3), 3.31 (q, $^3J_{HF}$ = 9.9 Hz, 1.2H, 2-H), 3.23 (q, $^3J_{HF}$ = 9.9 Hz, 0.8H, 2-H).

^{13}C NMR (mixture of rotamers, 101 MHz, $CDCl_3$): δ = 162.7 (q, $^3J_{CF}$ = 2.9 Hz, C-1), 161.9 (q, $^3J_{CF}$ = 2.9 Hz, C-1), 154.7 (C-4‘‘), 154.4 (C-4‘‘), 152.3 (C-9‘), 151.4 (C-9‘), 147.6 (C-9a‘), 147.4 (C-9a‘), 136.1 (C-7‘), 135.7 (C-7‘), 133.9 (C-1‘‘), 133.7 (C-1‘‘), 131.2 (C-5a‘), 131.0 (C-5a‘), 128.8 (C-2‘‘), 128.7 (C-2‘‘), 126.2 (C-6‘‘), 125.3 (C-3), 122.9 (C-3’’), 122.7 (C-3’’), 122.6 (C-3), 120.5 (C-6‘), 118.3 (C-6‘), 112.3 (C-5‘‘), 112.2 (C-5‘‘), 111.3 (C-8‘), 110.4 (C-8‘), 72.3 (C-2‘), 72.2 (C-2‘), 56.32 (OCH_3), 56.31 (OCH_3), 56.27 (OCH_3), 56.23 (OCH_3), 51.8 (C-3‘), 51.6 (C-5‘), 48.8 (C-3‘), 48.3 (C-5‘), 38.4 (q, $^2J_{CF}$ = 29.4 Hz, C-2), 38.1 (q, $^2J_{CF}$ = 29.4 Hz, C-2).

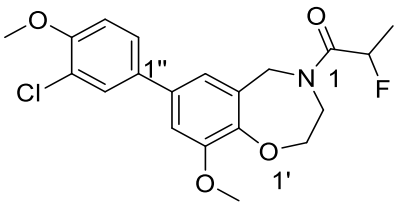
IR (Film): $\tilde{\nu}$ (cm^{-1}) = 3387, 2925, 1659, 1486, 1465, 1371, 1290, 1255, 1114, 854, 800.

MS (EI+): m/z (%) = 111 (18), 198 (15), 247 (13), 261 (17), 276 (72), 278 (29), 318 (17), 429 (100) $[M]^{+\bullet}$, 430 (19), 431 (41), 432 (8).

HRMS (EI+): m/z calcd for $C_{20}H_{19}{}^{35}ClF_3NO_4$ 429.0954, found 429.0950.

Purity (HPLC): 96 % (210 nm; method 1c).

(±)-1-[7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-2-fluoropropan-1-one (62)



MF: $C_{20}H_{21}ClFNO_4$ MW: 393.84 g/mol

To a solution of 0.050 g (0.16 mmol) **56** and 5 mg (0.04 mmol) DMAP in 1.0 mL DCM was added 0.019 mL (0.23 mmol) (±)-2-fluoropropionic acid at 0 °C. After five minutes 0.061 g (0.32 mmol) EDC-HCl was added, then the solution was warmed to rt and stirred for further 12 h. To this mixture was added 10 mL saturated $NaHCO_3$ solution and this mixture was extracted with DCM (3 x 10 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:1, Rf 0.3) gave 0.50 g (0.13 mmol, 81 %) of **62** as a colorless oil.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.53 (d, *J* = 2.4 Hz, 1H, 2‘‘-H), 7.37 (dd, *J* = 8.5, 2.4 Hz, 1H, 6‘‘-H), 7.05 – 6.91 (m, 3H, 6‘-H, 8‘-H, 5‘‘-H), 5.23 (dq, $^2J_{HF}$ = 48.5 Hz, $^3J_{HH}$ 6.7 Hz, 1H, 2-H), 4.75 – 4.59 (m, 2H, 5‘-H), 4.25 – 4.06 (m, 2H, 2‘-H), 4.02 – 3.91 (m, 2H, 3’-H), 3.90 (s, 3H, 4’’-OCH_3), 3.87 (s, 3H, 9’-OCH_3), 1.52 (dd, $^3J_{HF}$ = 24.6, $^3J_{HH}$ = 6.7 Hz, 3H).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 168.1 (d, $^2J_{CF}$ = 18.8 Hz, C-1), 155.0 (C-4‘‘), 152.1 (C-9‘), 148.5 (C-9a‘), 135.5 (C-7‘), 134.4 (C-1‘‘), 131.8 (C-5a‘), 128.8 (C-2‘‘), 126.2 (C-6‘‘), 123.6 (C-3‘‘), 120.3 (C-6‘), 113.4 (C-5‘‘), 112.5 (C-8‘), 87.2 (d, $^1J_{CF}$ = 178.2 Hz, C-2), 72.5 (C-2‘), 57.1 (9’-OCH_3), 56.7 (4’’-OCH_3), 49.9 (C-3‘, C-5‘), 17.8 (d, $^2J_{CF}$ = 22.9 Hz).

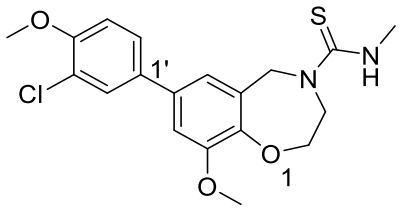
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3442, 2936, 2840, 1659, 1587, 1487, 1440, 1378, 1291, 1255, 1080, 1063, 1033, 854, 807, 701.

MS (EI+): m/z (%) = 139 (11), 198 (15), 261 (20), 263 (8), 276 (100), 278 (33), 393 (100) $[M]^{+\bullet}$, 395 (36).

HRMS (EI+): m/z calcd for $C_{20}H_{21}{}^{35}ClFNO_4$ 393.1143, found 393.1139.

Purity (HPLC): 98 % (210 nm; method 3a).

7-(3-Chloro-4-methoxyphenyl)-9-methoxy-*N*-methyl-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carbothioamide (63)



MF: $C_{19}H_{21}ClN_2O_3S$ MW: 392.90 g/mol

To a solution of 0.15 g (0.47 mmol) **56** in 3.0 mL anhydrous THF was added 0.028 g (0.71 mmol) of a 60 % suspension of NaH. After 15 minutes a solution of 0.068 g (0.94 mmol) methyl isothiocyanate in 1.0 mL anhydrous THF was added and the mixture was stirred for three hours at rt. To this mixture was added 30 mL saturated $NaHCO_3$ solution and this mixture was extracted with DCM (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (3:2, Rf 0.3) gave 0.13 g (0.32 mmol, 69 %) of **63** as a white solid.

mp: 200 - 201 °C.

^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$): δ = 7.54 (d, *J* = 2.3 Hz, 1H, 2‘-H), 7.38 (dd, *J* = 8.5, 2.3 Hz, 1H, 6‘-H), 7.07 – 6.81 (m, 3H, 6-H, 8-H, 5‘-H), 5.80 – 5.63 (m, 1H, NH), 4.85 – 4.66 (m, 2H, 5-H), 4.51 – 4.42 (m, 2H, 3-H), 4.28 – 4.15 (m, 2H, 2-H), 4.01 – 3.79 (m, 6H, OCH_3), 3.15 – 3.05 (m, 3H, NCH_3).

^{13}C NMR (mixture of rotamers, 126 MHz, $CDCl_3$): δ = 182.9 (C=S), 154.5 (C-4‘), 152.2 (C-9), 147.2 (C-9a), 135.6 (C-7), 133.8 (C-1‘), 130.3 (C-5a), 130.1 (C-5a), 128.7 (C-2‘), 126.2 (C-6‘), 124.0, 122.8 (C-3‘), 120.4 (C-6), 119.0 (C-6), 112.6 (C-8), 112.3 (C-5‘), 111.0 (C-8), 72.0 (C-2), 71.9 (C-2), 56.3 (OCH_3), 56.1 (OCH_3), 54.7 (C-3), 54.4 (C-3), 52.0 (C-5), 51.8 (C-5), 33.2 (NCH_3), 33.1 (NCH_3).

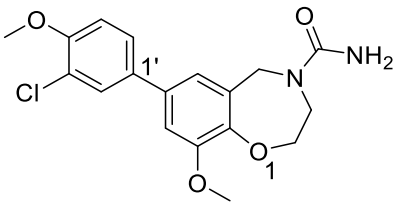
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3388, 2936, 2837, 1530, 1485, 1388, 1342, 1255, 1080, 1063, 1032, 976, 812, 702, 602.

MS (EI+): m/z (%) = 115 (97), 139 (17), 261 (28), 263 (12), 275 (28), 276 (98), 277 (31), 278 (36), 319 (100), 321 (34), 392 (51) $[M]^{+\bullet}$, 393 (12), 394 (20).

HRMS (EI+): m/z calcd for $C_{19}H_{21}{}^{35}ClN_2O_3S$ 392.0961, found 392.0953.

Purity (HPLC): 96 % (210 nm; method 1c).

7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carboxamide (64)



MF: $C_{18}H_{19}ClN_2O_4$ MW: 362.81 g/mol

To a solution of 0.10 g (0.31 mmol) **56** in 2 mL DCM was added 0.42 mL (3.1 mmol) (trimethylsilyl)isocyanate and the mixture was stirred for 2.5 h. The solvent was removed in vacuo. The residue was dissolved in 7 mL DCM and 7 mL of a solution of 4 M HCl in 1,4-dioxane was added, and the mixture stirred for 1 h. After adjustment to pH 9 with 1 M NaOH, the mixture was extracted with DCM (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and 3 % MeOH (Rf 0.2) gave 0.098 g (0.27 mmol, 87 %) of **64** as a white solid.

mp: 153 - 154 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46 (d, *J* = 2.3 Hz, 1H, 2‘-H), 7.30 (dd, *J* = 8.5, 2.3 Hz, 1H, 6‘-H), 6.92 – 6.87 (m, 3H, 6-H, 8-H, 5‘-H), 4.72 (s, 2H, NH_2), 4.41 (s, 2H, 5-H), 4.10 – 4.05 (m, 2H, 2-H), 3.85 (s, 3H, 4’-OCH_3), 3.83 (s, 3H, 9-OCH_3), 3.81 – 3.76 (m, 2H, 3-H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 157.0 (C=O), 153.4 (C-4‘), 151.0 (C-9), 146.5 (C-9a), 134.4 (C-7), 132.9 (C-1‘), 131.1 (C-5a), 127.6 (C-2‘), 125.1 (C-6‘), 121.7 (C-3‘), 118.1 (C-6), 111.2 (C-5‘), 109.6 (C-8), 71.5 (C-2), 55.3 (OCH_3), 49.4 (C-5), 49.0 (C-3).

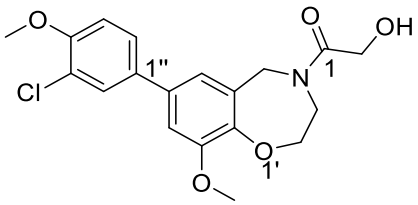
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3370, 3216, 2959, 2936, 2839, 1731, 1653, 1601, 1485, 1440, 1290, 1255, 1237, 1084, 1063, 1020, 809, 702, 607, 580.

MS (EI+): m/z (%) = 139 (13), 198 (15), 261 (23), 263 (8), 276 (100), 278 (35), 319 (56), 321 (20), 362 (80) $[M]^{+\bullet}$, 364 (28).

HRMS (EI+): m/z calcd for $C_{18}H_{19}{}^{35}ClN_2O_4$ 362.1033, found 362.1029.

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

1-[7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzoxazepin-4(5*H*)-yl]-2-hydroxyethan-1-one (65)



MF: $C_{19}H_{20}ClNO$ MW: 377.82 g/mol

A solution of 0.10 g (0.31 mmol) **56** in 0.95 mL (10 mmol) ethyl glycolate was heated to 60 °C. After 24 h and 48 h, EtOH was removed in vacuo. After 84 h purification by FCC with EtOAc and hexanes (5:1, Rf 0.2) gave 0.060 g (0.16 mmol, 51 %) of **65** as a white solid.

mp: 160 – 161 °C.

^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$): δ = 7.57 (d, *J* = 2.3 Hz, 0.4H, 2''-H), 7.54 (d, *J* = 2.3 Hz, 0.6H, 2''-H), 7.42 (dd, *J* = 8.5, 2.3 Hz, 0.4H, 6''-H), 7.38 (dd, *J* = 8.5, 2.3 Hz, 0.6H, 6''-H), 7.12 (d, *J* = 2.2 Hz, 0.4H, 6'-H), 7.02 – 6.96 (m, 2H, 8'-H, 5''-H), 6.87 (d, *J* = 2.2 Hz, 0.6H, 6'-H), 4.76 (s, 0.8H, 5'-H), 4.42 (s, 1.2H, 5'-H), 4.35 – 4.31 (m, 1.2H, 2-H), 4.21 – 4.06 (m, 4H, 2-H, 2'-H, 3'-H), 3.98 – 3.89 (m, 6H, OCH_3), 3.72 – 3.65 (m, 0.8H, 3'-H), 3.52 (t, *J* = 4.4 Hz, 0.4H, OH), 3.48 (t, *J* = 4.4 Hz, 0.6H, OH).

^{13}C NMR (mixture of rotamers, 126 MHz, $CDCl_3$): δ = 171.2 (C-1), 170.6 (C-1), 154.6 (C-4''), 154.5 (C-4''), 152.2 (C-9'), 151.6 (C-9'), 147.6 (C-9a'), 147.4 (C-9a'), 136.0 (C-7'), 135.8 (C-7'), 133.9 (C-1''), 133.7 (C-1''), 131.4 (C-5a'), 131.1 (C-5a'), 128.72 (C-2''), 128.71 (C-2''), 126.24 (C-6''), 126.21 (C-6''), 122.9 (C-3''), 122.8 (C-3''), 120.1 (C-6'), 119.0 (C-6'), 112.3 (C-5''), 112.2 (C-5''), 111.1 (C-8'), 110.4 (C-8'), 72.5 (C-2'), 72.1 (C-2'), 60.0 (C-2), 60.0 (C-2), 56.31 (OCH_3), 56.28 (OCH_3), 56.24 (OCH_3), 49.4 (C-5'), 49.3 (C-3'), 49.2 (C-3'), 48.7 (C-5').

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3399, 3001, 2934, 2842, 1638, 1488, 1389, 1289, 1254, 1076, 1054, 1022, 861, 808, 707, 584, 573.

MS (EI+): m/z (%) = 198 (10), 261 (14), 276 (67), 277 (29), 278 (30), 318 (23), 320 (6), 377 (100) $[M]^{+\bullet}$, 378 (20), 379 (36).

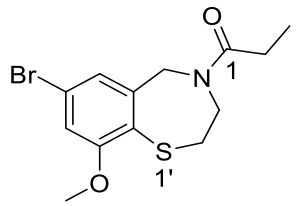
HRMS (EI+): m/z calcd for $C_{19}H_{20}{}^{35}ClNO$ 377.1030, found 377.1021.

Purity (HPLC): 97 % (210 nm; method 1c).

66 and 68

A solution of 1.3 g (4.5 mmol) **14** in 16 mL anhydrous THF and cooled to - 30 °C under N_2. 45 mL 1 M BH_3-THF solution (45 mmol) was added and the mixture refluxed for 40 h. A mixture of 15 mL MeOH, 15 mL conc. HCl and 30 mL water was added at rt and the mixture was refluxed for 1 h. pH was adjusted to 12 with K_2CO_3 and the mixure was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. A short FCC with DCM with 5 % MeOH and 2 % triethylamine gave the secondary amine as crude intermediate. The standard protocol for the *N*-acylation was applied to equal portions of this intermediate with propionyl chloride to obtain **66** or with cyclopropanecarbonyl chloride to obtain **68**.

1-(7-Bromo-9-methoxy-2,3-dihydro-1,4-benzothiazepin-4(5*H*)-yl)propan-1-one (66)



MF: $C_{13}H_{16}BrNO_2S$ MW: 330.24 g/mol

FCC with EtOAc and hexanes (1:3, Rf 0.2) gave 0.36 g (1.1 mmol, 49 %) of **66** as a white solid.

mp: 118 - 119 °C.

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 7.20 (d, J = 2.1 Hz, 1H, 6‘-H), 7.08 (d, J = 2.1 Hz, 1H, 8‘-H), 4.62 (s, 2H, 5‘-H), 3.93 – 3.86 (m, 2H, 3‘-H), 3.82 (s, 3H, OCH_3), 2.98 – 2.91 (m, 2H, 2‘-H), 2.30 (q, J = 7.4 Hz, 2H, 2-H), 0.97 (t, J = 7.4 Hz, 3H, 3-H).

^{13}C NMR (ambient temperature, mixture of rotamers, 126 MHz, DMSO-d_6): δ = 172.8 (C-1), 171.9 (C-1), 158.5 (C-9‘), 158.2 (C-9‘), 144.3 (C-5a‘), 142.6 (C-5a‘), 125.8 (C-6‘), 124.6 (C-6‘), 124.0 (C-9a‘), 123.7 (C-9a‘), 119.9 (C-7‘), 119.6 (C-7‘), 113.4 (C-8‘), 113.0 (C-8‘), 56.5 (OCH_3), 56.4 (OCH_3) 50.9 (C-3‘), 50.6 (C-5‘), 50.3 (C-5‘), 47.9 (C-3‘), 33.6 (C-2‘), 31.7 (C-2‘), 25.8 (C-2), 25.6 (C-2), 9.2 (C-3), 9.1 (C-3).

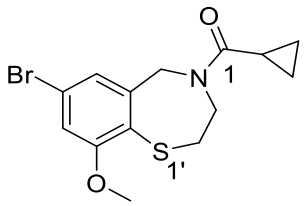
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 2975, 2936, 1647, 1561, 1452, 1436, 1402, 1286, 1266, 1074, 833, 756.

MS (ESI+): m/z (%) = 274 (22), 276 (23), 330 (92) $[M + H]^+$, 332 (100).

HRMS (ESI+): m/z calcd for $[C_{13}H_{17}{}^{79}BrNO_2S]^+$ 330.0163, found 330.0158

Purity (HPLC): > 99 % (210 nm; method 5a).

1-(7-Bromo-9-methoxy-2,3-dihydro-1,4-benzothiazepin-4(5*H*)-yl)-1-(cyclopropyl)methanone (68)



MF: $C_{14}H_{16}BrNO_2S$ MW: 342.25 g/mol

FCC with EtOAc and hexanes (1:3, Rf 0.2) gave 0.34 g (1.0 mmol, 45 %) of **68** as a white solid.

mp: 87 – 88 °C.

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 7.34 – 7.19 (m, 1H, 6‘-H), 7.08 (d, *J* = 2.0 Hz, 1H, 8‘-H), 4.73 (s, 2H, 5‘-H), 4.11 – 3.93 (m, 2H, 3‘-H), 3.83 (s, 3H, OCH_3), 2.96 – 2.90 (m, 2H, 2‘-H), 1.95 – 1.85 (m, 1H, C*H*-CH_2), 0.73 – 0.66 (m, 4H, CH-C*H*$_2$).

^{13}C NMR (ambient temperature, mixture of rotamers, 101 MHz, DMSO-d_6): δ = 173.1 (C-1), 172.0 (C-1), 158.9 (C-9‘), 158.8 (C-9‘), 144.7 (C-5a‘), 144.0 (C-5a‘), 126.2 (C-6‘), 125.1 (C-6‘), 124.4 (C-9a‘), 124.0 (C-9a‘), 120.35 (C-7‘), 120.33 (C-7‘), 113.9 (C-8‘), 113.5 (C-8‘), 56.94 (OCH_3), 56.91 (OCH_3), 51.6 (C-3‘), 51.4 (C-5‘), 51.1 (C-5‘), 49.0 (C-3‘), 34.3 (C-2‘), 32.4 (C-2‘), 11.7 (*C*H-CH_2), 11.1 (*C*H-CH_2), 8.0 (CH-*C*H_2), 7.5 (CH-*C*H_2).

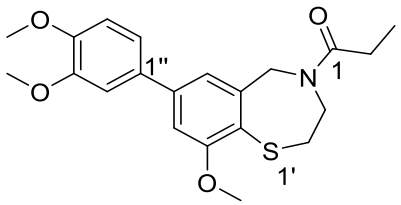
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3005, 2924, 2854, 1639, 1561, 1451, 1436, 1402, 1285, 1266, 1081, 1058, 834.

MS (EI+): m/z (%) = 276 (27), 278 (27), 342 (92) $[M + H]^+$, 344 (100), 434 (87), 436 (84).

HRMS (ESI+): m/z calcd for $[C_{14}H_{17}{}^{79}BrNO_2S]^+$ 342.0163, found 342.0157.

Purity (HPLC): 88 % (210 nm; method 5a).

1-[7-(3,4-Dimethoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzothiazepin-4(5*H*)-yl]propan-1-one (67)



MF: $C_{21}H_{25}NO_4S$ MW: 387.49 g/mol

Standard protocol 2 with 0.20 g (0.61 mmol) **66** and 0.22 g (1.2 mmol) 3,4-dimethoxyphenylboronic acid. FCC with EtOAc and hexanes (1:4, Rf 0.3) gave 0.14 g (0.36 mmol, 59 %) of **67** as a white solid.

mp: 82 - 83 °C.

^{1}H NMR (mixture of rotamers, 500 MHz, CD_2Cl_2): δ = 7.37 (d, *J* = 1.9 Hz, 0.6H, 6'-H), 7.17 (dd, *J* = 8.3, 2.2 Hz, 0.6H, 6''-H), 7.14 (dd, *J* = 8.3, 2.2 Hz, 0.4H, 6''-H), 7.11 (d, *J* = 2.2 Hz, 0.6H, 2''-H), 7.10 (d, *J* = 1.9 Hz, 0.4H, 6'-H), 7.08 (d, *J* = 2.2 Hz, 0.4H, 2''-H), 7.01 (d, *J* = 1.9 Hz, 0.4H, 8'-H), 6.98 (d, *J* = 1.9 Hz, 0.6H, 8'-H), 6.98 – 6.93 (m, 1H, 5''-H), 4.82 – 4.64 (m, 2H, 5'-H), 4.08 – 3.89 (m, 8H, 3'-H, OCH_3), 3.89 – 3.86 (m, 3H, OCH_3), 2.93 – 2.76 (m, 2H, 2'-H), 2.44 (q, *J* = 7.4 Hz, 0.8H, 2-H), 2.27 (q, *J* = 7.4 Hz, 1.2H, 2-H), 1.2 (t, *J* = 7.4 Hz, 3H, 3-H).

^{13}C NMR (mixture of rotamers, 126 MHz, CD_2Cl_2): δ = 173.6 (C-1), 172.6 (C-1), 159.4 (C-9'), 159.2 (C-9'), 149.9 (C-3''), 149.8 (C-3''), 149.7 (C-4''), 149.6 (C-4''), 144.6 (C-5a'), 143.4 (C-5a'), 141.4 (C-7'), 141.2 (C-7'), 133.6 (C-1''), 133.5 (C-1''), 123.8 (C-9a'), 123.2 (C-9a'), 122.7 (C-6'), 121.2 (C-6'), 119.84 (C-6''), 119.79 (C-6''), 112.2 (C-5''), 112.1 (C-5''), 111.1 (C-2''), 111.0 (C-2''), 109.4 (C-8'), 108.8 (C-8'), 56.74 (OCH_3), 56.67 (OCH_3), 56.4 (OCH_3), 56.30 (OCH_3), 56.26 (C-5'), 52.33 (C-3'), 52.25 (C-5'), 49.7 (C-3'), 35.3 (C-2'), 34.0 (C-2'), 27.11 (*C*H-CH_2), 27.06 (*C*H-CH_2), 9.42 (CH-*C*H_2), 9.40 (CH-*C*H_2).

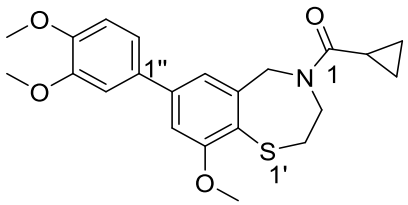
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3441, 2934, 2835, 1646, 1557, 1517, 1458, 1256, 1170, 1025, 952, 846, 806, 763.

MS (ESI+): m/z (%) = 388 (100) $[M + H]^+$, 410 (18).

HRMS (ESI+): m/z calcd for $[C_{21}H_{26}NO_4S]^+$ 388.1583, found 388.1575.

Purity (HPLC): > 99 % (210 nm; method 2b).

1-Cyclopropyl-1-[7-(3,4-dimethoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzothiazepin-4(5*H*)-yl]methanone (69)



MF: $C_{22}H_{25}NO_4S$ MW: 399.51 g/mol

Standard protocol 2 with 0.18 g (0.53 mmol) **68** and 0.25 g (1.4 mmol) 3,4-dimethoxyphenylboronic acid. FCC with EtOAc and hexanes (1:1, Rf 0.1) gave 0.18 g (0.45 mmol, 85 %) of **69** as a white solid.

mp: 92 - 93 °C.

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 7.34 – 7.25 (m, 1H, 6'-H), 7.24 – 7.18 (m, 2H, 2''-H, 6''-H), 7.12 (d, J = 1.9 Hz, 1H, 8'-H), 7.07 – 7.01 (m, 1H, 5''-H), 4.84 (s, 2H, 5'-H), 4.08 – 3.98 (m, 2H, 3'-H), 3.90 (s, 3H, 9'-OCH_3), 3.87 (s, 3H, 3''-OCH_3), 3.83 (s, 3H, 4''-OCH_3), 2.97 – 2.90 (m, 2H, 2'-H), 2.06 – 1.92 (m, 1H, C*H*-CH_2), 0.73 – 0.64 (m, 4H, CH-CH_2).

^{13}C NMR (100 °C, 101 MHz, DMSO-d_6): δ = 171.5 (C-1), 157.8 (C-9'), 149.1 (C-3''), 148.9 (C-4''), 142.1 (C-5a'), 139.1 (C-7'), 132.4 (C-1''), 122.7 (C-9a'), 120.5 (C-6'), 118.9 (C-2''/C-6''), 112.9 (C-5''), 111.6 (C-2''/C-6''), 108.7 (C-8'), 56.0 (OCH_3), 55.8 (OCH_3), 55.6 (OCH_3), 51.2 (C-5'), 49.2 (C-3'), 32.5 (C-2'), 10.7 (*C*H-CH_2), 6.3 (CH-*C*H_2).

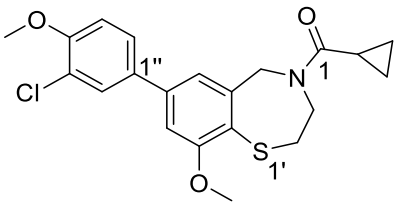
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3432, 3075, 3001, 2933, 2835, 1639, 1517, 1455, 1439, 1256, 1170, 1025, 897, 806, 764.

MS (ESI+): m/z (%) = 400 (100) $[M + H]^+$.

HRMS (ESI+): m/z calcd for $[C_{22}H_{26}NO_4S]^+$ 400.1583, found 400.1577.

Purity (HPLC): > 99 % (210 nm; method 2b).

1-[7-(3-Chloro-4-methoxyphenyl)-9-methoxy-2,3-dihydro-1,4-benzothiazepin-4(5*H*)-yl]-1-(cyclopropyl)methanone (70)



MF: $C_{21}H_{22}ClNO_3S$ MW: 403.92 g/mol

Standard protocol 2 with 0.18 g (0.53 mmol) **68** and 0.26 g (1.4 mmol) 3-chloro-4-methoxyphenylboronic acid. FCC with EtOAc and hexanes (1:1, Rf 0.2) gave 0.15 g (0.37 mmol, 70 %) of **70** as a white solid.

mp: 95 - 96 °C.

^{1}H NMR (110 °C, 400 MHz, $C_2D_2Cl_4$): δ = 7.55 (d, *J* = 2.4 Hz, 1H, 2‘‘-H), 7.40 (dd, *J* = 8.5, 2.4 Hz, 1H, 6‘‘-H), 7.22 – 7.09 (m, 1H, 6‘-H), 7.05 – 6.87 (m, 2H, 8‘-H, 5‘‘-H), 4.81 (s, 2H, 5‘-H), 4.12 – 3.99 (m, 2H, 3‘-H), 3.98 – 3.84 (m, 6H, OCH_3), 2.97 – 2.81 (m, 2H, 2‘-H), 1.81 – 1.64 (m, 1H, C*H*-CH_2), 0.95 – 0.84 (m, 2H, CH-C*H*$_2$), 0.77 – 0.63 (m, 2H, CH-C*H*$_2$).

^{13}C NMR (110 °C, 101 MHz, $C_2D_2Cl_4$): δ = 172.4 (C-1), 159.2 (C-9‘), 155.2 (C-4‘‘), 143.4 (C-5a‘), 139.4 (C-7‘), 134.2 (C-1‘‘), 128.9 (C-2‘‘), 126.3 (C-6‘‘), 124.7 (C-9a‘), 123.7 (C-3‘‘), 121.7 (C-6‘), 113.4 (C-8‘/C-5‘‘), 109.8 (C-8‘/C-5‘‘), 56.9 (OCH_3), 56.7 (OCH_3), 52.9 (C-5‘), 50.6, (C-3‘) 34.3 (C-2‘), 12.0 (*C*H-CH_2), 7.3 (CH-*C*H$_2$).

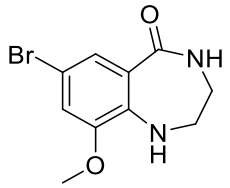
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3426, 3003, 2932, 2838, 1638, 1552, 1507, 1452, 1439, 1291, 1258, 1212, 1063, 1020, 944, 853, 810, 706.

MS (EI+): m/z (%) = 292 (22), 294 (11), 302 (18), 304 (9), 334 (81), 336 (33), 403 (100) $[M]^{+\bullet}$, 405 (40).

HRMS (EI+): m/z calcd for $C_{21}H_{22}{}^{35}ClNO_3S$ 403.1009, found 403.0998.

Purity (HPLC): 95 % (210 nm; method 2b).

7-bromo-9-methoxy-1,2,3,4-tetrahydro-1,4-benzodiazepin-5*H*-5-one (71)



MF: $C_{10}H_{11}BrN_2O_2$ MW: 271,11 g/mol

2.0 g (7.1 mmol) **17** was dissolved under nitrogen atmosphere in 75 mL 1 M (75 mmol) BH_3-THF. The mixture was refluxed for 24 h. Then the mixture was cooled to 0 °C, diluted with 20 mL MeOH, and concentrated in vacuo. 30 mL MeOH and 30 mL conc. HCl were added and the mixture was refluxed for one hour. The mixture was then cooled, and saturated K_2CO_3 was added until neutral pH. The mixture was extracted with ethyl acetate (3 x 50 mL) three times, the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with DCM with 3 % MeOH (Rf 0.1) gave 1.4 g (5.2 mmol, 73 %) of **71** as a colorless oil.

^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.67 (d, *J* = 2.3 Hz, 1H, 6-H), 6.90 (d, *J* = 2.3 Hz, 1H, 8-H), 6.77 (br s, 1H, NH), 3.85 (s, 3H, OCH_3), 3.64 – 3.55 (m, 2H, 2-H), 3.51 – 3.40 (m, 2H, 3-H).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 170.1 (C-5), 148.6 (C-9), 136.7 (C-9a), 127.0 (C-6), 117.3 (C-5a), 114.9 (C-8), 107.1 (C-7), 56.8 (OCH_3), 48.4 (C-2), 42.7 (C-3).

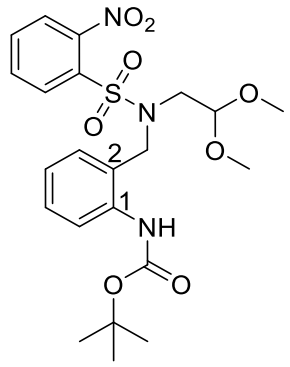
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3355, 2936, 2793, 1580, 1486, 1257, 1237, 828.

MS (EI+): m/z (%) = 104 (14), 106 (17), 226 (20), 228 (19), 241 (23), 242 (12), 270 (100) $[M]^{+\bullet}$, 272 (80).

HRMS (EI+): m/z calcd for $C_{10}H_{11}Br^{79}N_2O_2$ 270.0004, found 270.0004.

Purity (HPLC): 76 % (210 nm; method 5).

tert-Butyl [2-({[_N_-(2,2-dimethoxyethyl)-2-nitrophenyl]sulfonylamino}methyl)phenyl]carbamate[a] (79)



MF: $C_{22}H_{29}N_3O_8S$ MW: 495.55 g/mol

To a vigorously stirred solution of 0.68 g (2.6 mmol) triphenylphosphine in 2.0 mL anhydrous THF under N_2 atmosphere, 0.48 mL (2.5 mmol) DIAD was added. When a homogenous white precipitate formed, 0.72 g (2.5 mmol) *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide[67] was added and the reaction mixture was treated in an ultrasonic bath. After 10 min a solution of 0.50 g (2.2 mmol) *tert*-butyl [2-(hydroxymethyl)phenyl]carbamate[97] in 0.5 mL anhydrous THF was added and the suspension was sonicated until a clear solution was obtained. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:3, Rf 0.3) followed by a second FCC with pure CH_2Cl_2 gave 0.65 g (1.3 mmol, 50 %) of **79** as a colorless oil.

^{1}H NMR (500 MHz, CD_2Cl_2) δ = 7.93 (dd, *J* = 7.9, 1.4 Hz, 1H, Ar-H_{Nosyl}), 7.80 – 7.64 (m, 4H, 6-H, Ar-H_{Nosyl}), 7.57 (br s, 1H, NH), 7.28 (ddd, *J* = 8.5, 7.3, 1.7 Hz, 1H, 5-H), 7.10 (dd, *J* = 7.7, 1.7 Hz, 1H, 3-H), 7.03 – 6.98 (m, 1H, 4-H), 4.57 (s, 2H, 2-CH_2-N), 4.30 (t, *J* = 5.2 Hz, 1H, (N-CH_2-C*H*$(OR)_2$)), 3.31 (s, 6H, OCH_3), 3.27 (d, *J* = 5.2 Hz, 2H, (N-C*H*$_2$-CH$(OR)_2$)), 1.49 (s, 9H, $OC(CH_3)_3$).

^{13}C NMR (126 MHz, CD_2Cl_2) δ = 153.6 (C=O), 148.4 (quart. C_{Nosyl}), 138.3 (C-1), 134.3 (CH_{Nosyl}), 133.7 (CH_{Nosyl}), 132.3 (C-3, CH_{Nosyl}), 131.3 (quart. C_{Nosyl}), 129.5 (C-4/C-5), 124.9 (C-2), 124.7 (CH_{Nosyl}), 124.1 (C-4/C-5), 122.8 (C-6), 104.3 (N-CH_2-*C*H$(OR)_2$), 80.5 (O*C*$(CH_3)_3$), 55.4 (OCH_3), 49.4 (2-CH_2-N), 48.4 (N-C*H*$_2$-CH$(OR)_2$), 28.5 (OC(*C*$H_3)_3$).

IR (film): $\tilde{\nu}$ (cm^{-1}) = 3355, 3095, 2978, 2936, 1726, 1545, 1368, 1236, 1067, 1160.

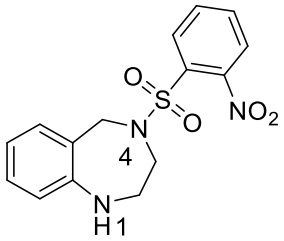
MS (EI+): m/z (%) = 305 (30), 332 (50), 363 (15), 395 (100), 495 (20) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{22}H_{29}N_3O_8S$ 495.1675, found 495.1669.

Purity (HPLC): 98 % (210 nm; method 1b).

[a]prepared and characterized by Edgar Uhl for his master thesis.

4-[(2-Nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine[a] (80)



MF: $C_{15}H_{15}N_3O_4S$ MW: 333.36 g/mol

To a solution of 0.50 g (1.0 mmol) **79** in 2.0 mL CH_2Cl_2 under N_2 atmosphere, 1.0 mL trifluoroacetic acid and 0.40 mL (2.5 mmol) triethylsilane were added in rapid succession. After 24 h of stirring 2 M NaOH was added and the mixture extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:2, Rf 0.3) gave 0.31 g (0.93 mmol, 93 %) of **80** as a yellow solid.

mp: 125 - 127 °C.

^{1}H NMR (500 MHz, CD_2Cl_2) δ = 7.91 – 7.85 (m, 1H, Ar-H_{Nosyl}), 7.70 – 7.62 (m, 1H, Ar-H_{Nosyl}), 7.65 – 7.55 (m, 2H, Ar-H_{Nosyl}), 7.20 (dd, *J* = 7.4, 1.5 Hz, 1H, 6-H), 7.12 (td, *J* = 7.6, 1.5 Hz, 1H, 8-H), 6.87 (td, *J* = 7.4, 1.2 Hz, 1H, 7-H), 6.73 (dd, *J* = 7.9, 1.2 Hz, 1H, 9-H), 4.44 (s, 2H, 5-H), 3.99 (br s, 1H, NH), 3.64 – 3.58 (m, 2H, 3-H), 3.25 – 3.19 (m, 2H, 2-H).

^{13}C NMR (126 MHz, CD_2Cl_2) δ = 150.0 (C-9a), 148.5 (quart. C_{Nosyl}), 133.9 (CH_{Nosyl}), 133.5 (quart. C_{Nosyl}), 132.0 (CH_{Nosyl}), 130.8 (CH_{Nosyl}), 130.4 (C-6), 129.1 (C-8), 128.0 (C-5a), 124.3 (CH_{Nosyl}), 121.3 (C-7), 119.5 (C-9), 52.8 (C-5), 51.9 (C-3), 48.8 (C-2).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3378, 3367, 3088, 3020, 2929, 1604, 1548, 1372, 1334, 1163, 766.

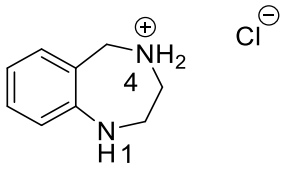
MS (EI+): m/z (%) = 147 (100), 333 (20) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{15}H_{15}N_3O_4S$ 333.0783, found 333.0782.

Purity (HPLC): 98 % (210 nm; method 1b).

[a]prepared and characterized by Edgar Uhl for his master thesis.

4-[(2-Nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine[a] (81)



MF: $C_9H_{13}{}^{35}ClN_2$ MW: 184.67 g/mol

To a solution of 0.20 g (0.60 mmol) **80** in 1.5 mL acetonitrile were added 0.33 g (2.4 mmol) K_2CO_3 and 0.18 mL (1.8 mmol) thiophenol. The mixture was stirred at 50 °C for 24 h. Then 2 M NaOH was added. The mixture was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solvent was evaporated, and the residue dissolved in methanol. A 4 M solution of HCl in 1,4-dioxane was added and the mixture cooled to -18 °C overnight. The obtained precipitate was washed with diethyl ether to give 67 mg (0.36 mmol, 61 %) of **81** as a white solid.

mp: 232 – 234 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.58 (s, 2H, NH), 7.37 (dd, *J* = 7.7, 1.5 Hz, 1H, 6-H), 7.27 (td, *J* = 7.7, 1.5 Hz, 1H, 8-H), 7.18 (d, *J* = 7.7 Hz, 1H, 9-H), 7.03 – 6.97 (m, 1H, 7-H), 4.28 – 4.20 (m, 2H, 5-H), 3.35 – 3.26 (m, 4H, 2-H, 3-H).

^{13}C NMR (126 MHz, DMSO-d_6) δ = 147.6 (C-9a), 131.8 (C-6), 129.6 (C-8), 123.6 (C-5a), 122.3 (C-7), 120.1 (C-9), 49.1 (C-5), 47.5 (C-3), 44.0 (C-2).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3433, 3057, 2926, 2617, 2074, 1977, 1416, 778.

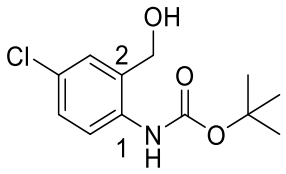
MS (EI+): m/z (%) = 148 (100) $[M]^{+\bullet}$.

HRMS (ESI+): m/z calcd for $[(C_9H_{13}N_2)^+]$ 149.1073, found 149.1073.

Purity (HPLC): > 99 % (210 nm; method 7b).

[a]prepared and characterized by Edgar Uhl for his master thesis.

tert-Butyl [4-chloro-2-(hydroxymethyl)phenyl]carbamate (84)



MF: $C_{12}H_{16}ClNO_3$ MW: 257.71 g/mol

To a solution of 1.5 g (6.9 mmol) di-*tert*-butyl dicarbonate in 5 mL anhydrous THF under N_2 atmosphere, 1.0 g (6.3 mmol) commercial (2-amino-5-chlorophenyl)methanol was added and the resulting solution was stirred at 40 °C for 20 h. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:8, Rf 0.1) gave 0.84 g (3.3 mmol, 52 %) of **84** as a white solid.

mp: 88 - 89 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (d, *J* = 8.8 Hz, 1H, 6-H), 7.62 (br s, 1H, NH), 7.25 (dd, *J* = 8.8, 2.5 Hz, 1H, 5-H), 7.14 (d, *J* = 2.5 Hz, 1H, 3-H), 4.63 (d, *J* = 5.7 Hz, 2H, CH_2), 2.31 (t, *J* = 5.7 Hz, 1H, OH), 1.51 (s, 9H, $OC(CH_3)_3$).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 153.4 (C=O), 136.7 (C-1), 130.6 (C-2), 129.0 (C-5), 128.8 (C-3), 128.1 (C-4), 122.5 (C-6), 81.0 ($OC(CH_3)_3$), 63.9 (CH_2), 28.5 ($OC(CH_3)_3$).

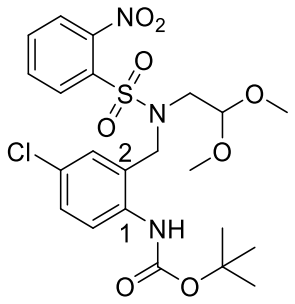
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3423, 3354, 3006, 2984, 1694, 1515, 1163.

MS (EI+): m/z (%) = 57 (100), 139 (36), 141 (11), 157 (21), 159 (7), 201 (20), 257 (6) $[M]^{+\bullet}$, 259 (2).

HRMS (EI+): m/z calcd for $C_{12}H_{16}{}^{35}ClNO_3$ 257.0819, found 257.0812.

Purity (HPLC): 95 % (210 nm; method 7a).

tert-Butyl [4-chloro-2-({[_N_-(2,2-dimethoxyethyl)-2-nitrophenyl]sulfonylamino}methyl)phenyl]carbamate (85)



MF: $C_{22}H_{28}ClN_3O_8S$ MW: 529.99 g/mol

To a vigorously stirred solution of 0.66 g (2.5 mmol) triphenylphosphine in 2.0 mL anhydrous THF under N_2 atmosphere, 0.51 mL (2.5 mmol) DIAD was added. When a homogenous white precipitate formed, 0.73 g (2.5 mmol) *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide[67] was added and the reaction mixture was treated in an ultrasonic bath. After 10 min a solution of 0.55 g (2.1 mmol) **84** in 1.0 mL anhydrous THF was added and the suspension was sonicated until a clear solution was obtained. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:2, Rf 0.4) gave 0.46 g (0.86 mmol, 41 %) of **85** as a colorless oil.

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.96 (dd, *J* = 7.9, 1.4 Hz, 1H, Ar-H_{Nosyl}), 7.77 – 7.63 (m, 4H, 6-H, Ar-H_{Nosyl}), 7.61 (br s, 1H, NH), 7.21 (dd, *J* = 8.8, 2.5 Hz, 1H, 5-H), 6.97 (d, *J* = 2.5 Hz, 1H, 3-H), 4.55 (s, 2H, 2-CH_2-N), 4.40 (t, *J* = 5.1 Hz, 1H, N-CH_2-C*H*$(OR)_2$), 3.38 (s, 6H, (OCH_3)), 3.32 (d, *J* = 5.1 Hz, 2H, N-C*H*$_2$-CH$(OR)_2$), 1.50 (s, 9H, ($OC(CH_3)_3$)).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 153.2 (C=O), 147.8 (quart. C_{Nosyl}), 136.3 (C-1), 133.9 (CH_{Nosyl}), 133.4 (quart. C_{Nosyl}), 131.8 (CH_{Nosyl}), 131.1 (CH_{Nosyl}), 130.5 (C-3), 129.2 (C-5), 128.7 (C-2/C-4), 126.1 (C-2/C-4), 124.4 (C-6/CH_{Nosyl}), 123.8 (C-6/Ns-CH_{Ar}), 104.3 (N-*C*H_2-CH$(OR)_{2)}$, 80.7 (O*C*$(CH_3)_3$), 55.3 (OCH_3), 48.6 (2-CH_2-N), 48.1 (N-*C*H_2-CH$(OR)_2$), 28.3 (OC(*C*$H_3)_3$).

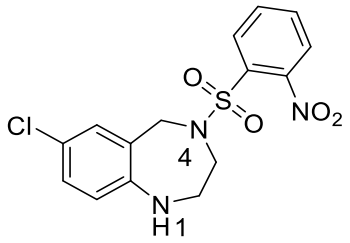
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3333, 2978, 1725, 1544, 1368, 1159.

MS (ESI-): m/z (%) = 289 (28), 528 (100) $[M - H]^-$, 530 (35).

HRMS (ESI-): m/z calcd for $C_{22}H_{27}{}^{35}ClN_3O_8S$ 528.1213, found 528.1218.

Purity (HPLC): 96 % (210 nm; method 7a).

7-Chloro-4-[(2-nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (86)



MF: $C_{15}H_{14}ClN_3O_4S$ MW: 367.80 g/mol

To a solution of 0.15 g (0.28 mmol) **85** in 0.50 mL CH_2Cl_2 under N_2 atmosphere, 0.25 mL trifluoroacetic acid and 0.11 mL (0.71 mmol) triethylsilane were added in rapid succession. After 24 h 30 mL of 2 M NaOH was added and the mixture was extracted with CH_2Cl_2 (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:1, Rf 0.3) followed by a second FCC with pure CH_2Cl_2 gave 0.099 g (0.27 mmol, 96 %) of **86** as a yellow solid.

mp: 140 - 141 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.96 (dd, *J* = 7.8, 1.5 Hz, 1H, Ar-H_{Nosyl}), 7.72 – 7.56 (m, 3H, Ar-H_{Nosyl}), 7.21 (d, *J* = 2.4 Hz, 1H, H-6), 7.06 (dd, *J* = 8.4, 2.4 Hz, 1H, H-8), 6.65 (d, *J* = 8.4 Hz, 1H, H-9), 4.39 (s, 2H, H-5), 3.91 (br s, 1H, NH), 3.66 – 3.58 (m, 2H, H-3), 3.26 – 3.20 (m, 2H, H-2).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 148.0 (C-9a, quart. C_{Nosyl}), 133.5 (CH_{Nosyl}), 133.2 (quart. C_{Nosyl}), 131.5 (CH_{Nosyl}), 130.8 (CH_{Nosyl}), 129.9 (C-6), 129.2 (C-7), 128.5 (C-8), 125.9 (C-5a), 124.0 (CH_{Nosyl}), 120.5 (C-9), 52.0 (C-5), 51.3 (C-3), 48.5 (C-2).

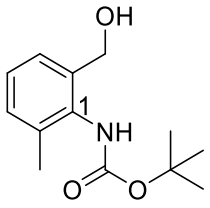
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3408, 3097, 2930, 1531, 1495, 1352, 1126.

MS (ESI+): m/z (%) = 265 (100), 368 (65) $[M + H]^+$, 370 (20).

HRMS (ESI+): m/z calcd for $[C_{15}H_{15}{}^{35}ClN_3O_4S]^+$ 368.0466, found 368.0475.

Purity (HPLC): 97 % (210 nm; method 7b).

tert-Butyl [2-(hydroxymethyl)-6-methylphenyl]carbamate (88)



MF: $C_{13}H_{19}NO_3$ MW: 237.30 g/mol

To a solution of 1.7 g (7.7 mmol) di-*tert*-butyl dicarbonate in 16 mL anhydrous THF under N_2 atmosphere, 0.96 g (7.0 mmol) commercial (2-amino-3-methylphenyl)methanol was added and the resulting solution was stirred at 40 °C for 2 d. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:5, Rf 0.2) gave 1.0 g (4.2 mmol, 55 %) of **88** as a white solid.

mp: 122 - 123 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.29 (br s, 1H, NH), 7.32 – 7.27 (m, 1H, 3-H), 7.15 (t, J = 7.5 Hz, 1H, 4-H), 7.13 – 7.06 (m, 1H, 5-H), 5.04 (t, J = 5.6 Hz, 1H, OH), 4.45 (d, J = 5.6 Hz, 2H, 2-CH_2), 2.16 (s, 3H, 6-CH_3), 1.45 (s, 9H, $OC(CH_3)_3$).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 153.6 (C=O), 139.7 (C-2), 135.1 (C-6), 133.0 (C-1), 128.2 (C-5), 126.1 (C-4), 124.0 (C-3), 78.3 ($OC(CH_3)_3$), 59.4 (CH_2), 28.1 ($OC(CH_3)_3$), 17.7 (6-CH_3).

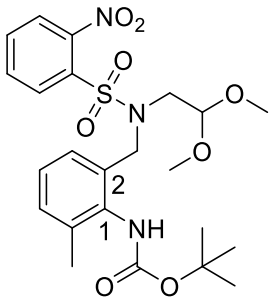
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3406, 3265, 2980, 1688, 1516, 1279, 1176, 1056, 773.

MS (EI+): m/z (%) = 59 (100), 119 (73), 137 (35), 181 (40), 237 (5) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{13}H_{19}NO_3$ 237.1365, found 237.1356.

Purity (HPLC): 91 % (210 nm; method 7a).

tert-Butyl [2-({[_N_-(2,2-dimethoxyethyl)-2-nitrophenyl]sulfonylamino}methyl)-6-methylphenyl]carbamate (89)



MF: $C_{23}H_{31}N_3O_8S$ MW: 509.57 g/mol

To a vigorously stirred solution of 0.55 g (2.1 mmol) triphenylphosphine in 2.0 mL anhydrous THF under N_2 atmosphere, 0.41 mL (2.1 mmol) DIAD was added. When a homogenous white precipitate formed, 0.60 g (2.1 mmol) *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide[67] was added and the reaction mixture was treated in an ultrasonic bath. After 10 min 0.44 g (1.9 mmol) **88** was added and the suspension was sonicated until a clear solution was obtained. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:1, Rf 0.5) followed by a second FCC with pure CH_2Cl_2 gave 0.61 g (1.2 mmol, 63 %) of **89** as a colorless oil.

^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.92 (dd, *J* = 7.9, 1.4 Hz, 1H, Ar-H_{Nosyl}), 7.73 – 7.56 (m, 3H, Ar-H_{Nosyl}), 7.18 – 7.10 (m, 1H, 5-H), 7.12 – 6.99 (m, 2H, 4-H, 3-H), 6.69 (br s, 1H, NH), 4.61 (s, 2H, Ar-CH_2-N), 4.31 (t, *J* = 5.2 Hz, 1H, $CH(OR)_2$), 3.33 – 3.24 (m, 8H, (N-C*H*$_2$-CH(OC*H*$_3$)$_2$)), 2.21 (s, 3H, (6-CH_3)), 1.49 (s, 9H, $OC(CH_3)_3$).

^{13}C NMR (101 MHz, CD_2Cl_2): δ = 154.0 (C=O), 148.2 (quart. C_{Nosyl}), 137.0 (C-1), 135.2 (quart. C_{Nosyl}), 134.1 (CH_{Nosyl}), 133.8 (C-2/C-6), 132.7 (C-2/C-6), 132.1 (CH_{Nosyl}), 131.2 (CH_{Nosyl}), 130.7 (C-5), 127.3 (C-4/C-3), 127.2 (C-4/C-3), 124.5 (CH_{Nosyl}), 104.3 ($CH(OR)_2$), 80.2 (O*C*$(CH_3)_3$), 55.3 (OCH_3), 49.44 (N-*C*H_2-CH$(OR)_2$), 49.37 (2-CH_2-N), 28.4 (OC(*C*H_3)$_3$), 18.4 (6-CH_3).

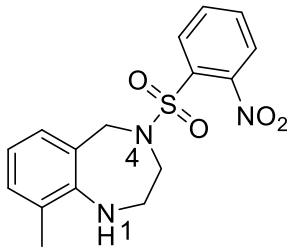
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3330, 3019, 2936, 1710, 1543, 1158.

MS (ESI-): m/z (%) = 289 (20), 508 (100) $[M - H]^-$, 554 (20).

HRMS (ESI-): m/z calcd for $[C_{23}H_{30}N_3O_8S]^-$ 508.1759, found 508.1764.

Purity (HPLC): > 99 % (210 nm; method 7b).

9-Methyl-4-[(2-nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (90)



MF: $C_{16}H_{17}N_3O_4S$ MW: 347.39 g/mol

To a solution of 0.42 g (0.82 mmol) **89** in 1.6 mL CH_2Cl_2 under N_2 atmosphere, 0.80 mL trifluoroacetic acid and 0.33 mL (2.1 mmol) triethylsilane were added in rapid succession. After 48 h 50 mL of 2 M NaOH was added and the mixture was extracted with CH_2Cl_2 (3 x 50 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:3, Rf 0.4) gave 0.17 g (0.43 mmol, 53 %) of **90** as a yellow solid.

mp: 148 - 149 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.77 (dd, *J* = 7.9, 1.4 Hz, 1H, Ar-H_{Nosyl}), 7.58 – 7.50 (m, 1H, Ar-H_{Nosyl}), 7.50 – 7.39 (m, 2H, Ar-H_{Nosyl}), 7.02 (dd, *J* = 7.4, 1.6 Hz, 1H, 6-H), 6.94 (dd, *J* = 7.7, 1.6 Hz, 1H, 8-H), 6.70 (dd, *J* = 7.7, 7.4 Hz, 1H, 7-H), 4.43 (s, 2H, 5-H), 3.81 (br s, 1H, NH), 3.61 – 3.56 (m, 2H, 3-H), 3.25 – 3.18 (m, 2H, 2-H), 2.05 (s, 3H, (9-CH_3)).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 148.2 (quart. C_{Nosyl}), 147.6 (C-9a), 133.4 (quart. C_{Nosyl}), 133.3 (CH_{Nosyl}), 131.4 (CH_{Nosyl}), 130.8 (CH_{Nosyl}), 130.3 (C-8), 128.6 (C-6), 126.8 (C-5/C-9a), 125.5 (C-5/C-9a), 123.8 (CH_{Nosyl}), 120.6 (C-7), 52.4 (C-5), 51.4 (C-3), 47.7 (C-2), 17.7 (9-CH_3).

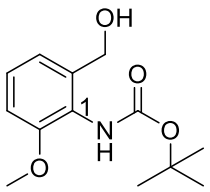
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3406, 3087, 2905, 1732, 1535, 1371, 1160, 1026, 938.

MS (ESI+): m/z (%) = 219 (13), 348 (100) $[M + H]^+$.

HRMS (ESI+): m/z calcd for $[C_{16}H_{18}N_3O_4S]^+$ 348.1013, found 348.1015.

Purity (HPLC): > 99 % (210 nm; method 7a).

tert-Butyl [2-(hydroxymethyl)-6-methoxyphenyl]carbamate (92)



MF: $C_{13}H_{19}NO_4$ MW: 253.30 g/mol

To a solution of 1.3 g (5.9 mmol) di-*tert*-butyl dicarbonate in 12 mL anhydrous THF under N_2 atmosphere, 0.83 g (5.4 mmol) (2-amino-3-methoxyphenyl)methanol[102] was added and the resulting solution was stirred at 40 °C for 20 h. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:3, Rf 0.6) gave 0.79 g (3.1 mmol, 58 %) of **92** as a white solid.

mp: 111 - 112 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.18 – 7.12 (m, 1H, 4-H), 7.04 (dd, *J* = 7.8, 1.3 Hz, 1H, 3-H), 6.79 (dd, *J* = 8.3, 1.3 Hz, 1H, 5-H), 6.52 (br s, 1H, NH), 4.50 (d, *J* = 5.8 Hz, 2H, CH_2), 4.14 (br s, 1H), 3.77 (s, 3H, OCH_3), 1.49 (s, 9H, $OC(CH_3)_3$).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 155.8 (C=O), 153.1 (C-6), 137.9 (C-2), 126.8 (C-4), 124.0 (C-1), 122.3 (C-3), 110.0 (C-5), 80.7 ($O\underline{C}(CH_3)_3$), 61.8 (CH_2), 55.6 (OCH_3), 28.1 ($OC(\mathit{C}H_3)_3$).

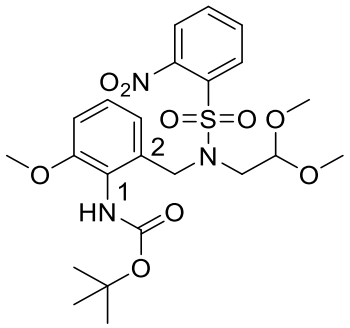
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3463, 3361, 3274, 3016, 2924, 1686, 1531, 1158.

MS (EI+): m/z (%) = 57 (100), 107 (54), 135 (44), 153 (78), 197 (32), 253 (5) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{13}H_{19}NO_4$ 253.1314, found 253.1327.

Purity (HPLC): 99 % (210 nm; method 7b).

tert-Butyl [2-({[_N_-(2,2-dimethoxyethyl)-2-nitrophenyl]sulfonylamino}methyl)-6-methoxyphenyl]carbamate (93)



MF: $C_{23}H_{31}ClN_3O_9S$ MW: 525.57 g/mol

To a vigorously stirred solution of 0.66 g (2.5 mmol) triphenylphosphine in 2.0 mL anhydrous THF under N_2 atmosphere, 0.51 mL (2.5 mmol) DIAD was added. When a homogenous white precipitate formed, 0.73 g (2.5 mmol) *N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide[67] was added and the reaction mixture was treated in an ultrasonic bath. After 10 min a solution of 0.50 g (2.0 mmol) **92** in 1.0 mL anhydrous THF was added and the suspension was sonicated until a clear solution was obtained. The solvent was evaporated under reduced pressure and FCC with EtOAc and hexanes (1:2, Rf 0.2) followed by a second FCC with pure CH_2Cl_2 gave 0.39 g (0.74 mmol, 37 %) of **93** as a colorless oil.

^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.97 – 7.91 (m, 1H, Ar-H_{Nosyl}), 7.71 – 7.57 (m, 3H, Ar-H_{Nosyl}), 7.11 (t, *J* = 8.1 Hz, 1H, 4-H), 6.88 (dd, *J* = 8.1, 1.2 Hz, 1H, 3-H), 6.81 (dd, *J* = 8.1, 1.2 Hz, 1H, 5-H), 6.31 (br s, 1H, NH), 4.64 (s, 2H, 2-CH_2-N), 4.32 (t, *J* = 5.3 Hz, 1H, (N-CH_2-C*H*$(OR)_2$), 3.81 (s, 3H, 6-OCH_3), 3.32 (d, *J* = 5.3 Hz, 2H, (N-C*H*$_2$-$CH(OR)_2$), 3.22 (s, 6H, $CH(OCH_3)_2$), 1.48 (s, 9H, $OC(CH_3)_3$).

^{13}C NMR (101 MHz, CD_2Cl_2): δ = 154.4 (C=O), 154.3 (C-6), 148.2 (quart. C_{Nosyl}), 134.1 (quart. C_{Nosyl}), 133.9 (CH_{Nosyl}), 133.8 (C-2), 132.0 (CH_{Nosyl}), 131.2 (CH_{Nosyl}), 127.2 (C-4), 125.3 (C-1), 124.4 (CH_{Nosyl}), 120.5 (C-3), 110.4 (C-5), 103.7 (N-CH_2-*C*H$(OR)_2$), 80.6 (O*C*$(CH_3)_3$), 56.2 (6-OCH_3), 55.0 ($(OCH_3)_2$), 49.6 (N-*C*H_2-$CH(OR)_2$, 49.1 (2-CH_2-N), 28.4 (OC(*C*$H_3)_3$).

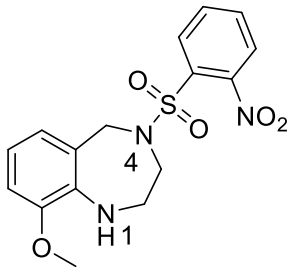
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3011, 2936, 1718, 1543, 1366, 1160, 1068.

MS (ESI-): m/z (%) = 289 (17), 524 (100) $[M - H]^-$, 570 (14).

HRMS (ESI-): m/z calcd for $[C_{23}H_{30}{}^{35}ClN_3O_9S]^-$ 524.1708, found 524.1707.

Purity (HPLC): > 99 % (210 nm; method 7b).

9-Methoxy-4-[(2-nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (94)



MF: $C_{16}H_{17}N_3O_5S$ MW: 363.39 g/mol

To a solution of 0.11 g (0.21 mmol) **93** in 0.5 mL CH_2Cl_2 under N_2 atmosphere, 0.25 mL trifluoroacetic acid and 0.084 mL (0.53 mmol) triethylsilane were added in rapid succession. After 24 h 30 mL of 2 M NaOH was added and the mixture was extracted with CH_2Cl_2 (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:3, Rf 0.3) gave 0.065 g (0.18 mmol, 86 %) of **94** as a yellow solid.

mp: 132 – 133 °C.

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.85 (dd, *J* = 8.2, 1.5 Hz, 1H, Ar-H_{Nosyl}), 7.69 – 7.54 (m, 3H, Ar-H_{Nosyl}), 6.91 – 6.68 (m, 3H, 6-H, 7-H, 8-H), 4.74 (br s, 1H, NH), 4.45 (s, 2H, 5-H), 3.80 (s, 3H, OCH_3), 3.65 – 3.59 (m, 2H, 3-H), 3.24 – 3.19 (m, 2H, 2-H).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 149.6 (C-9), 148.5 (quart. C_{Nosyl}), 139.6 (C-9a), 133.8 (CH_{Nosyl}), 133.5 (quart. C_{Nosyl}), 131.9 (CH_{Nosyl}), 130.8 (CH_{Nosyl}), 128.0 (C-5a), 124.2 (CH_{Nosyl}), 122.2 (C-6), 120.4 (C-7/C-8), 110.3 (C-7/C-8), 56.2 (OCH_3), 52.6 (C-5), 52.0 (C-3), 48.3 (C-2).

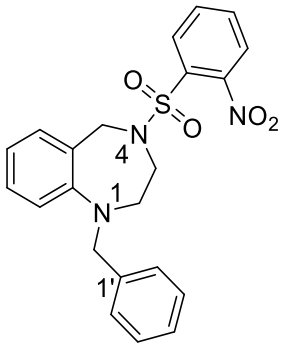
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3361, 3013, 2920, 1532, 1158, 1074.

MS (ESI+): m/z (%) = 364 (100) $[M + H]^+$, 365 (13).

HRMS (ESI+): m/z calcd for $[C_{16}H_{18}N_3O_5S]^+$ 364.0962, found 364.0959.

Purity (HPLC): > 99 % (210 nm; method 7a).

1-Benzyl-4-[(2-nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine[a] (95)



MF: $C_{22}H_{21}N_3O_4S$ MW: 423.49 g/mol

To a solution of 0.15 g (0.30 mmol) **79** in 0.6 mL CH_2Cl_2, 0.30 mL trifluoroacetic acid and 0.17 mL (1.1 mmol) triethylsilane were added in rapid succession and the resulting solution was stirred for 20 h at rt. Then 0.061 mL (0.61 mmol) benzaldehyde were added. After 1 d another equivalent of each benzaldehyde, TFA and TES were added. The reaction mixture was stirred for 2 d, then diluted with CH_2Cl_2 and washed with $NaHCO_3$. The phases were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were washed with brine (1x 30 mL), dried over Na_2SO_4 and concentrated in vacuo. FCC with EtOAc and hexanes (1:4, Rf 0.2) gave 0.068 g (0.21 mmol, 68 %) of **80** and 14 mg (33 µmol, 11 %) of **95** as a yellow oil.

^{1}H NMR (400 MHz, CD_2Cl_2) δ = 7.75 (dd, *J* = 8.1, 1.5 Hz, 1H, Ar-H_{Nosyl}), 7.62 – 7.47 (m, 3H, Ar-H_{Nosyl}), 7.30 – 7.11 (m, 7H, 6-H, 8-H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H), 6.90 – 6.81 (m, 2H, 7-H, 9-H), 4.49 (s, 2H, 5-H), 4.23 (s, 2H, 1'-CH_2), 3.41 – 3.31 (m, 2H, 3-H), 3.05 – 2.97 (m, 2H, 2-H).

^{13}C NMR (101 MHz, CD_2Cl_2) δ = 150.9 (C-9a), 147.3 (quart. C_{Nosyl}), 138.0 (C-1'), 132.7 (CH_{Nosyl}), 132.2 (quart. C_{Nosyl}), 130.8 (CH_{Nosyl}), 129.7 (CH_{Nosyl}), 129.3 (C-6/C-8), 128.3 (C-5a), 128.1 (C-6/C-8), 127.7 (C-3', C-5'), 127.4 (C-2', C-6'), 126.4 (C-4'), 123.1 (CH_{Nosyl}), 120.7 (C-7/C-9), 117.0 (C-7/C-9), 56.9 (1'-CH_2), 52.2 (C-2), 51.2 (C-5), 48.9 (C-3).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3028, 2920, 1599, 1543, 1495, 1371, 1357, 1163, 762.

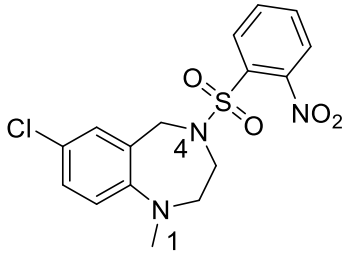
MS (EI+): m/z (%) = 194 (100), 237 (50), 423 (20) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{22}H_{21}N_3O_4S$ 423.1253, found 423.1252.

Purity (HPLC): 98 % (210 nm; method 1b).

[a]prepared and characterized by Edgar Uhl for his master thesis.

7-Chloro-1-methyl-4-[(2-nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (96)



MF: $C_{16}H_{16}ClN_3O_4S$ MW: 381.83 g/mol

To a solution of 0.15 g (0.28 mmol) **85** in 0.50 mL CH_2Cl_2 under N_2 atmosphere, 0.25 mL trifluoroacetic acid and 0.11 mL (0.71 mmol) triethylsilane were added in rapid succession. After 24 h further 0.33 mL (2.1 mmol) triethylsilane and 0.076 g trioxane (0.84 mmol) were added and stirred for further 24 h. 30 mL of 2 M NaOH was added and the mixture was extracted with CH_2Cl_2 (3 x 30 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:1, Rf 0.6) gave 0.081 g (0.21 mmol, 79 %) of **96** as a yellow oil.

^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.80 – 7.74 (m, 1H, Ar-H_{Nosyl}), 7.62 – 7.56 (m, 1H, Ar-H_{Nosyl}), 7.54 – 7.48 (m, 2H, Ar-H_{Nosyl}), 7.14 – 7.06 (m, 2H, 6-H, 8-H), 6.69 (d, *J* = 8.4 Hz, 1H, 9-H), 4.34 (s, 2H, 5-H), 3.54 – 3.47 (m, 2H, 3-H), 3.07 – 2.99 (m, 2H, 2-H), 2.74 (s, 3H, NCH_3).

^{13}C NMR (101 MHz, CD_2Cl_2): δ = 151.2 (C-9a), 148.6 (quart. C_{Nosyl}), 134.2 (CH_{Nosyl}), 133.3 (quart. C_{Nosyl}), 132.1 (CH_{Nosyl}), 131.0 (CH_{Nosyl}), 130.24 (C-5a/C-7), 130.21 (C-6), 128.9 (C-8) , 125.9 (C-5a/C-7), 124.4 (CH_{Nosyl}), 118.1 (C-9), 56.7 (C-2), 52.0 (C-5), 50.0 (C-3), 42.8 (NCH_3).

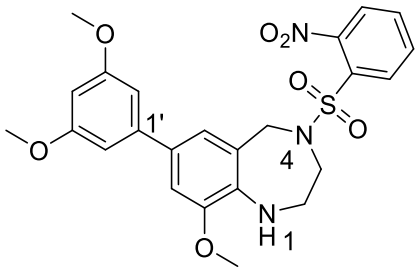
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3009, 2860, 1737, 1546, 1494, 1355, 1165, 1084.

MS (ESI+): m/z (%) = 265 (100), 266 (15), 382 (90) $[M + H]^+$, 384 (26).

HRMS (ESI+): m/z calcd for $[C_{16}H_{17}{}^{35}ClN_3O_4S]^+$ 382.0623, found 382.0630.

Purity (HPLC): 99 % (210 nm; method 7b).

7-(3,5-Dimethoxyphenyl)-9-methoxy-4-[(2-nitrophenyl)sulfonyl]-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (97)



MF: $C_{24}H_{26}N_3O_7S$ MW: 499.54 g/mol

To a solution of 0.30 g (0.68 mmol) **22**, 0.18 g (1.0 mmol) 3,5-dimethoxyphenylboronic acid, and 0.051 g (0.070 mmol) [1.1′-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) in a mixture of 1.0 mL H_2O and 4.0 mL 1,4-dioxane, were added 0.47 mL (2.8 mmol) DIPEA. The mixture was heated to 95 °C for 3.5 h. After cooling 50 mL water was added and the mixture was extracted with CH_2Cl_2 (3 x 50 mL) three times. The combined organic layers were dried over $MgSO_4$, and concentrated in vacuo. FCC with with EtOAc and hexanes (1:1, Rf 0.3) gave 0.22 g (0.44 mmol, 65 %) of **97** as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.93 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-H_{Nosyl}), 7.67 – 7.50 (m, 3H, Ar-H_{Nosyl}), 7.07 (d, *J* = 1.9 Hz, 1H, 6-H), 6.94 (d, *J* = 1.9 Hz, 1H, 8-H), 6.68 (d, *J* = 2.2 Hz, 2H, 2‘-H, 6‘-H), 6.44 (t, J = 2.2 Hz, 1H, 4‘-H), 4.75 (br s, 1H, NH), 4.54 (s, 2H, 5-H), 3.89 – 3.83 (m, 9H, OCH_3), 3.73 – 3.65 (m, 2H, 3-H), 3.32 – 3.24 (m, 2H, 2-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 161.0 (C-3‘, C-5‘), 149.1 (C-9), 148.1 (quart. C_{Nosyl}), 143.2 (C-1‘), 138.7 (C-9a), 133.3 (C-7, CH_{Nosyl}), 133.2 (quart. C_{Nosyl}), 131.3 (CH_{Nosyl}), 130.8 (CH_{Nosyl}), 127.3 (C-5a), 123.9 (CH_{Nosyl}), 120.8 (C-6), 108.7 (C-8), 105.2 (C-2‘, C-6‘), 98.6 (C-4‘), 55.9 (OCH_3), 55.5 (OCH_3), 52.4 (C-5), 51.5 (C-3), 47.9 (C-2).

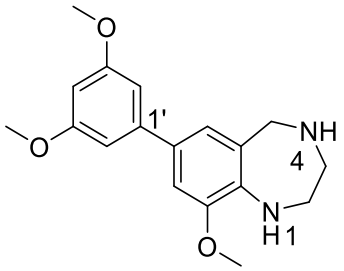
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3380, 3004, 2934, 1589, 1544, 1463, 1342, 1158.

MS (ESI+): m/z (%) = 223 (14), 500 (100) $[M + H]^+$, 501 (16), 522 (14).

HRMS (ESI+): m/z calcd for $[C_{24}H_{26}N_3O_7S]^+$ 500.1486, found 500.1489.

Purity (HPLC): 98 % (210 nm; method 7b).

7-(3,5-Dimethoxyphenyl)-9-methoxy-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (98)



MF: $C_{18}H_{22}N_2O_3$ MW: 314.39 g/mol

To a solution of 0.32 g (0.64 mmol) **97** in 1.6 mL MeCN, 0.36 g (2.9 mmol) K_2CO_3 and 0.20 mL (1.9 mmol) thiophenol were added and the mixture was warmed under N_2 atmosphere to 50 °C for 12 h. The solvent was evaporated, and the residue dissolved in a mixture of EtOAc and 2 M NaOH (70 mL each). This mixture was extracted with EtOAc (5 x 35 mL) five times and the combined organic layers were concentrated in vacuo. FCC on a short column with CH_2Cl_2 with 1→10 % MeOH (Rf 0.1) gave 0.15 g (0.48 mmol, 75 %) of **98** as a colorless oil.

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 6.99 – 6.95 (m, 2H, 6-H, 8-H), 6.68 (d, *J* = 2.2 Hz, 2H, 2‘-H, 6‘-H), 6.40 (t, *J* = 2.2 Hz, 1H, 4‘-H), 4.84 (br s, 1H, NH), 3.93 (s, 2H, 5-H), 3.89 (s, 3H, 9-OCH_3), 3.82 (s, 6H, 3‘-OCH_3, 5‘-OCH_3), 3.15 – 3.09 (m, 2H, 2-H), 3.09 – 3.02 (m, 2H, 3-H).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 161.5 (C-3‘, C-5‘), 149.7 (C-9), 143.7 (C-1‘), 139.9 (C-9a), 132.8 (C-7), 131.5 (C-5a), 121.0 (C-6), 108.4 (C-8), 105.2 (C-2‘, C-6‘), 98.8 (C-4‘), 56.4 (9-OCH_3), 55.8 (3‘-OCH_3, 5‘-OCH_3), 54.2 (C-5), 51.8 (C-3), 50.0 (C-2).

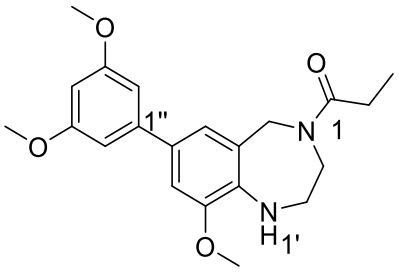
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3377, 2936, 2837, 1586, 1461, 1153.

MS (ESI+): m/z (%) = 315 (100) $[M + H]^+$, 316 (12).

HRMS (ESI+): m/z calcd for $[C_{18}H_{23}N_2O_3]^+$ 315.1703, found 315.1705.

Purity (HPLC): 90 % (210 nm; method 1a).

1-[7-(3,5-Dimethoxyphenyl)-9-methoxy-1,2,3,5-tetrahydro-4*H*-1,4-benzodiazepin-4-yl]propan-1-one (99)



MF: $C_{21}H_{26}N_2O_4$ MW: 370.45 g/mol

To a solution of 0.050 g (0.16 mmol) **98** in 1.0 mL CH_2Cl_2 under N_2 atmosphere was added 0.080 mL (0.47 mmol) DIPEA and the mixture was cooled to - 78 °C. Then 0.014 mL (0.16 mmol) propionyl chloride were added. After warming to rt, 20 mL of 2 M NaOH was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:1, Rf 0.1) gave 0.030 g (0.09 mmol, 56 %) of **99** as a yellow oil.

^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$): δ = 7.20 (d, *J* = 1.9 Hz, 0.3H, 6‘-H), 6.99 – 6.93 (m, 1.7H, 6‘-H, 8‘-H), 6.70 (d, *J* = 2.2 Hz, 0.7H, H-2‘‘, H-6‘‘), 6.68 (d, *J* = 2.2 Hz, 1.3H, H-2‘‘, H-6‘‘), 6.44 (t, *J* = 2.2 Hz, 0.7H, H-4‘‘), 6.41 (t, *J* = 2.2 Hz, 0.3H, H-4‘‘), 4.90 – 4.77 (m, 1H, NH), 4.64 (s, 0.7H, 5‘-H), 4.51 (s, 1.3H, 5‘-H), 3.92 – 3.82 (m, 10.3H, 3‘-H, OCH_3), 3.76 – 3.69 (m, 0.7H, 3‘-H), 3.29 – 3.19 (m, 2H), 2.46 (q, *J* = 7.4 Hz, 1.3H, 2-H), 2.33 (q, *J* = 7.4 Hz, 0.7H, 2-H), 1.16 – 1.07 (m, 3H, 3-H).

^{13}C NMR (mixture of rotamers, 126 MHz, $CDCl_3$): δ = 173.2 (C-1), 172.4 (C-1), 161.1 (C-3‘‘, C-5‘‘), 160.9 (C-3‘‘, C-5‘‘), 149.4 (C-9‘), 149.0 (C-9‘), 143.4 (C-1‘‘), 143.3 (C-1‘‘), 138.8 (C-9a‘), 138.2 (C-9a‘), 133.1 (C-7‘), 132.7 (C-7‘), 128.8 (C-5a‘), 127.7 (C-5a‘), 121.5 (C-6‘), 120.2 (C-6‘), 108.6 (C-8‘), 108.2 (C-8‘), 105.3 (C-2‘‘, C-6‘‘), 105.1 (C-2‘‘, C-6‘‘), 98.8 (C-4‘‘), 98.3 (C-4‘‘), 56.0 (OCH_3), 55.9 (OCH_3), 55.47 (OCH_3), 55.45 (OCH_3), 52.3 (C-5‘), 51.1 (C-3‘), 49.3 (C-5‘), 48.5 (C-3‘), 48.1 (C-2‘), 47.2 (C-2‘), 27.0 (C-2), 26.7 (C-2), 9.3 (C-3).

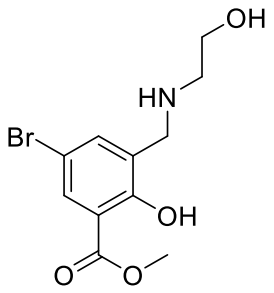
IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3384, 3000, 2937, 1639, 1585, 1461, 1154, 751.

MS (ESI+): m/z (%) = 371 (100) $[M + H]^+$, 372 (11), 741 $[2xM + H]^+$.

HRMS (ESI+): m/z calcd for $[C_{21}H_{27}N_2O_4]^+$ 371.1965, found 371.1969.

Purity (HPLC): 97 % (210 nm; method 7a).

Methyl 5-bromo-2-hydroxy-3-{[(2-hydroxyethyl)amino]methyl}benzoate (100)



MF: $C_{11}H_{14}BrNO_4$ MW: 304.14 g/mol

To a solution of 1.7 g (6.5 mmol) methyl 5-bromo-3-formyl-2-hydroxybenzoate[7] in a mixture of 4 mL MeOH and 36 mL anhydrous THF, 0.48 mL (8.1 mmol) 2-aminoethanol was added. The solution was stirred for 0.5 h and then 0.22 g (5.8 mmol) $NaBH_4$ was added in portions over 15 minutes. After 1 hour of stirring, the solution was concentrated and water and EtOAc were added. The product partially precipitated as white solid, the remaining product was extracted from the water phase at alkaline pH with EtOAc. After drying over $MgSO_4$ and evaporation of the solvent 1.8 g (6.0 mmol, 93 %) of **100** were obtained.

mp: 150 – 151 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.69 (d, J = 2.7 Hz, 1H, 6-H), 7.57 (d, J = 2.7 Hz, 1H, 4-H), 3.86 – 3.80 (m, 5H, OCH_3, 3-CH_2), 3.49 (t, J = 5.7 Hz, 2H, CH_2OH), 2.61 (t, J = 5.7 Hz, 2H, HNC$H_2$$CH_2$OH).

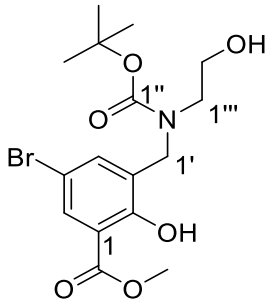
^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.3 (C=O), 159.1 (C-2), 135.7 (C-4), 130.6 (C-6), 129.8 (C-3), 116.1 (C-1/C-5), 108.3 (C-1/C-5), 59.7 (CH_2OH), 52.4 (OCH_3), 50.4 (HNC$H_2$$CH_2$OH), 48.3 (3-$CH_2$).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3418, 3261, 3000, 2953, 2924, 2844, 2629, 1680, 1445, 1429, 1288, 1230, 1148, 1016, 978, 882, 804, 684.

MS (EI+): m/z (%) = 131 (100), 169 (96), 181 (92), 219 (49), 281 (41), 303 (4) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{11}H_{14}{}^{79}BrNO_4$ 303.0106, found 303.0095.

Methyl 5-bromo-3-{[(*tert*-butoxycarbonyl)(2-hydroxyethyl)amino]methyl}-2-hydroxybenzoate (101)



MF: $C_{16}H_{22}BrNO_6$ MW: 404.26 g/mol

To a dispersion of 2.8 g (9.2 mmol) **100** in a mixture of 100 mL EtOAc and 60 mL saturated $NaHCO_3$ solution, 2.8 g (13 mmol) di-tert-butyl dicarbonate was added and the mixture was stirred for 12 h. The organic layer was separated and the aqueous phase extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:2, Rf 0.5) gave 2.1 g (5.2 mmol, 56 %) of **101** as a colorless oil.

^{1}H NMR (70 °C, 400 MHz, DMSO-d_6): δ = 7.81 (d, *J* = 2.6 Hz, 1H, 6-H), 7.45 (d, *J* = 2.6 Hz, 1H, 4-H), 4.44 (s, 2H, 1‘-H), 3.94 (s, 3H, OCH_3), 3.51 (t, *J* = 6.1 Hz, 2H, 2‘‘‘-H), 3.31 (t, *J* = 6.1 Hz, 2H, 1‘‘‘-H), 3.10 (br s, 1H, OH), 1.38 (s, 9H, 4‘‘-H).

^{13}C NMR (70 °C, 101 MHz, DMSO-d_6): δ = 168.2 (O=CO), 156.8 (C-2), 154.7 (C-1‘‘), 135.7 (C-4), 129.9 (C-6), 129.7 (C-1/C-3/C-5), 114.0 (C-1/C-3/C-5), 109.6 (C-1/C-3/C-5), 78.7 (C-3‘‘), 59.0 (C-2‘‘‘), 52.5 (OCH_3), 49.5 (C-1‘‘‘), 45.3 (C-1‘), 27.7 (C-4‘‘).

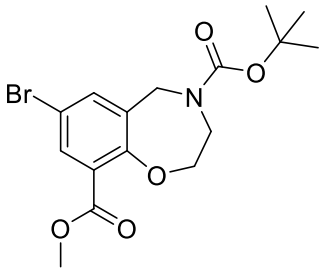
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3441, 3160, 2975, 1677, 1609, 1442, 1366, 1326, 1236, 1162, 996, 880, 794, 699.

MS (EI+): m/z (%) = 131 (100), 169 (87), 252 (71), 368 (87), 403 (3) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{16}H_{22}{}^{79}BrNO_6$ 403.0630, found 403.0637.

Purity (HPLC): 96 % (210 nm; method 3a).

4-(*tert*-Butyl) 9-methyl 7-bromo-2,3-dihydro-1,4-benzoxazepine-4,9(5*H*)-dicarboxylate (102)



MF: $C_{16}H_{20}BrNO_5$ MW: 386.24 g/mol

To a solution of 2.0 g (7.6 mmol) triphenylphosphine in 50 mL anhydrous THF was added 1.5 mL (7.6 mmol) DIAD at 0 °C under N_2. After 20 minutes a solution of 2.0 g (5.0 mmol) **101** in 80 mL anhydrous THF was added and the mixture was stirred for 16 h. After concentration in vacuo FCC with EtOAc and hexanes (1:4, Rf 0.3) gave 1.7 g (4.3 mmol, 85 %) of **102** as a white solid.

mp: 89 - 90 °C.

^{1}H NMR (70 °C, 400 MHz, DMSO-d_6): δ = 7.65 (d, J = 2.6 Hz, 1H, 8-H), 7.60 (d, J = 2.6 Hz, 1H, 6-H), 4.44 (s, 2H, 5-H), 4.09 – 4.03 (m, 2H, 2-H), 3.82 (s, 3H, OCH_3), 3.78 – 3.71 (m, 2H, 3-H), 1.35 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (70 °C, 101 MHz, DMSO-d_6): δ = 164.5 (O=CO), 156.5 (C-9a), 153.8 (NC=O), 135.5 (C-5a/C-7/C-9), 134.9 (C-6), 130.6 (C-8), 126.6 (C-5a/C-7/C-9), 113.8 (C-5a/C-7/C-9), 79.2 ($C(CH_3)_3$), 71.9 (C-2), 51.9 (OCH_3), 48.8 (C-3), 48.4 (C-5), 27.6 ($C(CH_3)_3$).

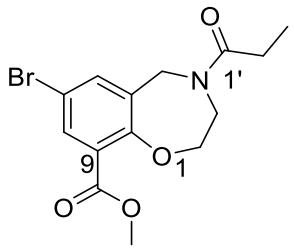
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3420, 3075, 2981, 2962, 2924, 1716, 1691, 1438, 1395, 1292, 1214, 1170, 1014, 794, 657.

MS (EI+): m/z (%) = 131 (100), 169 (97), 252 (66), 331 (37), 385 (3) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{16}H_{20}{}^{79}BrNO_5$ 385.0525, found 385.0503.

Purity (HPLC): > 99 % (210 nm; method 3a).

Methyl 7-bromo-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxylate (103)



MF: $C_{14}H_{16}BrNO_4$ MW: 342.19 g/mol

To a solution of 0.89 g (2.3 mmol) **102** in 5 mL 1,4-dioxane, 10 mL of a 4 M solution of HCl in 1,4-dioxane was added. After 5 h the supernatant was removed and the white precipitate washed with 5 mL diethyl ether. The solid was dissolved in 10 mL DCM and cooled to 0 °C. 1.6 mL (9.2 mmol) DIPEA and 0.40 mL (4.6 mmol) propionyl chloride were added. The mixture was warmed to rt and stirred for 12 h. Then 20 mL $NaHCO_3$ solution was added and the mixture extracted with DCM (3 x 20 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc and hexanes (1:1, Rf 0.3) gave 0.58 g (1.7 mmol, 74 %) of **103** as a white solid.

mp: 112 - 113 °C.

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 7.80 – 7.57 (m, 2H, 6-H, 8-H), 4.62 (s, 2H, 5-H), 4.17 – 4.11 (m, 2H, 2-H), 3.90 – 3.86 (m, 2H, 3-H), 3.83 (s, 3H, OCH_3), 2.34 (q, *J* = 7.4 Hz, 2H, 2‘-H), 0.99 (t, *J* = 7.4 Hz, 3H, 3‘-H).

^{13}C NMR (100 °C, 126 MHz, DMSO-d_6): δ = 171.8 (C-1’), 164.3 (O=CO), 156.2 (C-9a), 134.6 (C-6), 134.5 (C-5a), 130.5 (C-8), 126.3 (C-7/C-9), 113.6 (C-7/C-9), 71.7 (C-2), 51.6 (OCH_3), 47.9 (C-3), 47.2 (C-5), 25.1 (C-2’), 8.5 (C-3’).

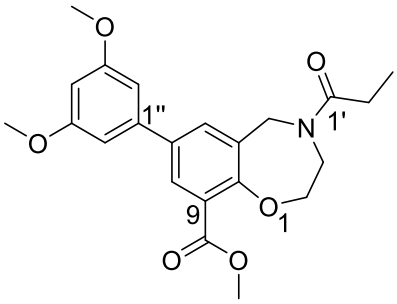
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3084, 3023, 2993, 2948, 1733, 1647, 1462, 1443, 1286, 1221, 1187, 1155, 1024, 889, 798, 681, 640.

MS (EI+): m/z (%) = 206 (89), 252 (97), 286 (76), 310 (26), 312 (26), 341 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{14}H_{16}{}^{79}BrNO_4$ 341.0263, found 341.0260.

Purity (HPLC): 98 % (210 nm; method 4a).

Methyl 7-(3,5-dimethoxyphenyl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxylate (104)



MF: $C_{22}H_{25}NO_6$ MW: 399.44 g/mol

Standard protocol 2 with 1.0 g (2.9 mmol) **103** and 1.1 g (6.0 mmol) 3,5-dimethoxyphenylboronic acid. FCC with EtOAc and hexanes (2:1, Rf 0.3) gave 0.84 g (2.1 mmol, 72 %) of **104** as a colorless oil.

^{1}H NMR (100 °C, 400 MHz, DMSO-d_6): δ = 7.84 – 7.66 (m, 2H, 6-H, 8-H), 6.76 (d, *J* = 2.2 Hz, 2H, 3‘‘-H, 5‘‘-H), 6.53 (t, *J* = 2.2 Hz, 1H, 4‘‘-H), 4.71 (s, 2H, 5-H), 4.20 – 4.13 (m, 2H, 2-H), 3.95 – 3.86 (m, 2H, 3-H), 3.85 (s, 3H, O=COCH_3), 3.83 (s, 6H, 3’’-OCH_3, 5’’-OCH_3), 2.38 (q, *J* = 7.3 Hz, 2H, 2‘-H), 1.00 (t, *J* = 7.3 Hz, 3H, 3‘-H).

^{13}C NMR (100 °C, 101 MHz, DMSO-d_6): δ = 171.8 (C-1’), 165.7 (O=CO), 160.7 (C-3’’, C-5’’), 156.3 (C-9a), 140.4 (C-1’’), 134.5 (C-7), 132.2 (C-5a/C-9), 130.5 (C-6/C-8), 126.3 (C-6/C-8), 124.9 (C-5a/C-9), 104.8 (C-2’’, C-6’’), 99.5 (C-4’’), 71.6 (C-2), 55.0 (3’’-OCH_3, 5’’-OCH_3), 51.3 (O=CO*C*H_3), 48.3 (C-5/C-3), 47.5 (C-5/C-3), 25.2 (C-2’), 8.5 (C-3’).

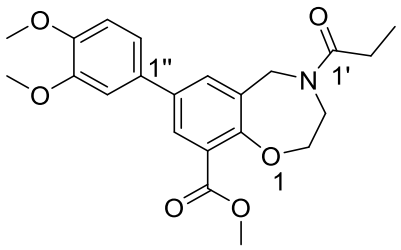
IR (film): $\tilde{\nu}$ (cm^{-1}) = 2938, 1728, 1648, 1596, 1462, 1295, 1209, 1155, 1041, 816.

MS (EI+): m/z (%) = 154 (100), 399 (5) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{22}H_{25}NO_6$ 399.1682, found 399.1662.

Purity (HPLC): 99 % (210 nm; method 3a).

Methyl 7-(3,4-dimethoxyphenyl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxylate (105)



MF: $C_{22}H_{25}NO_6$ MW: 399.44 g/mol

Standard protocol 2 with 0.30 g (0.88 mmol) **103** and 0.32 g (1.8 mmol) 3,4-dimethoxyphenylboronic acid. FCC with EtOAc and hexanes (3:1, Rf 0.3) gave 0.28 g (0.70 mmol, 80 %) of **105** as a white solid.

mp: 107 - 108 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$): δ = 7.83 (d, J = 2.5 Hz, 0.5H, 8-H), 7.81 (d, J = 2.5 Hz, 0.5H, 8-H), 7.72 (d, *J* = 2.5 Hz, 0.5H, 6-H), 7.49 (d, *J* = 2.5 Hz, 0.5H, 6-H), 7.19 – 7.00 (m, 2H, 2‘‘-H, 6‘‘-H), 7.00 – 6.86 (m, 1H, 5‘‘-H), 4.71 (s, 1H, 5-H), 4.61 (s, 1H, 5-H), 4.23 – 4.14 (m, 2H, 2-H), 4.09 – 4.03 (m, 1H, 3-H), 3.98 – 3.87 (m, 10H, 3-H, OCH_3), 2.46 (q, *J* = 7.4 Hz, 1H, 2‘-H), 2.32 (q, *J* = 7.4 Hz, 1H, 2‘-H), 1.18 – 1.08 (m, 3H, 3‘-H).

^{13}C NMR (mixture of rotamers, 101 MHz, $CDCl_3$): δ = 172.9 (C-1‘), 172.4 (C-1‘), 166.72 (O=CO), 166.70 (O=CO), 157.5 (C-9a), 157.4 (C-9a), 149.3 (C-3‘‘/C-4‘‘), 149.2 (C-3‘‘/C-4‘‘), 149.0 (C-3‘‘/C-4‘‘), 148.8 (C-3‘‘/C-4‘‘), 136.64 (C-7), 136.57 (C-7), 133.6 (C-5a), 133.20 (C-5a), 132.28 (C-1‘‘), 132.25 (C-1‘‘), 132.18 (C-6), 130.5 (C-6), 128.5 (C-8), 127.9 (C-8), 125.8 (C-9), 124.7 (C-9), 119.5 (C-6‘‘), 119.4 (C-6‘‘), 111.6 (C-5‘‘), 111.4 (C-5‘‘), 110.3 (C-2‘‘), 110.2 (C-2‘‘), 73.12 (C-2), 73.06 (C-2), 56.1 (OCH_3), 56.0 (OCH_3), 52.4 (OCH_3), 52.3 (OCH_3), 51.1 (C-3/C-5), 50.9 (C-3/C-5), 48.3 (C-5), 48.3 (C-3), 26.8 (C-2‘), 26.5 (C-2‘), 9.2 (C-3‘).

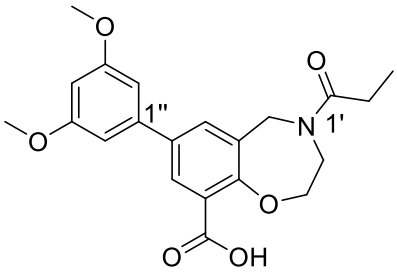
IR (film): $\tilde{\nu}$ (cm^{-1}) = 2939, 1730, 1651, 1519, 1476, 1256, 1222, 1142, 1025.

MS (EI+): m/z (%) = 300 (37), 399 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{22}H_{25}NO_6$ 399.1682, found 399.1682.

Purity (HPLC): > 99 % (210 nm; method 1c).

7-(3,5-Dimethoxyphenyl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxylic acid (106)



MF: $C_{21}H_{23}NO_6$ MW: 385.42 g/mol

A solution of 0.30 g (0.75 mmol) **105** in a mixture of 5 mL MeOH, 5 mL THF and 10 mL 1 M NaOH was heated to 70 °C for 45 min. Then the mixture was acidified with 20 mL 2 M HCl and extracted with EtOAc (3 x 20 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc with 2 % AcOH (Rf 0.5) gave 0.27 g (0.70 mmol, 93 %) of **106** as a colorless oil.

^{1}H NMR (90 °C, 400 MHz, DMSO-d_6): δ = 7.80 – 7.68 (m, 2H, 6-H, 8-H), 6.76 (d, *J* = 2.2 Hz, 2H, 2‘‘-H, 6‘‘-H), 6.52 (t, *J* = 2.2 Hz, 1H, 4‘‘-H), 4.70 (s, 2H, 5-H), 4.24 – 4.12 (m, 2H, 2-H), 3.96 – 3.87 (m, 2H, 3-H), 3.83 (s, 6H, OCH_3), 2.44 – 2.32 (m, 2H, 2‘-H), 0.99 (t, *J* = 7.4 Hz, 3H, 3‘-H).

^{13}C NMR (90 °C, 101 MHz, DMSO-d_6): δ = 171.8 (C-1’), 166.6 (O=COH), 160.7 (C-3’’, C-5’’), 156.2 (C-9a), 140.6 (C-1’’), 134.4 (C-7), 132.1 (C-5a), 130.1 (C-6), 130.0 (C-9), 126.3 (C-8), 104.7 (C-2’’, C-6’’), 99.4 (C-4’’), 71.6 (C-2), 55.0 (OCH_3), 49.1 (C-5), 47.1 (C-3), 25.3 (C-2’), 8.6 (C-3’).

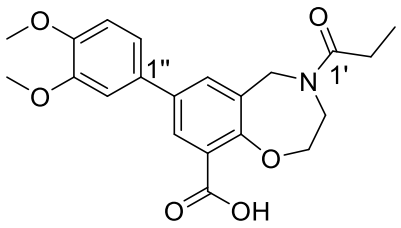
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3431, 2938, 2839, 1719, 1647, 1597, 1466, 1205, 1155, 839, 729.

MS (EI+): m/z (%) = 162 (100), 186 (95), 319 (84), 385 (21) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{21}H_{23}NO_6$ 385.1525, found 385.1519.

Purity (HPLC): 96 % (210 nm; method 4a).

7-(3,4-Dimethoxyphenyl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxylic acid (107)



MF: $C_{21}H_{23}NO_6$ MW: 385.42 g/mol

A solution of 0.30 g (0.75 mmol) **105** in a mixture of 5 mL MeOH, 5 mL THF and 10 mL 1 M NaOH was heated to 70 °C for 45 min. Then the mixture was acidified with 20 mL 2 M HCl and extracted with EtOAc (3 x 20 mL) three times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC with EtOAc with 2 % AcOH (Rf 0.5) gave 0.25 g (0.65 mmol, 87 %) of **107** as a white solid.

mp: 97 - 98 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, $C_2D_2Cl_4$): δ = 8.28 – 8.16 (m, 1H, 8-H), 7.79 (d, *J* = 2.5 Hz, 0.5H, 6-H), 7.59 (d, *J* = 2.5 Hz, 0.5H, 6-H), 7.16 (dd, *J* = 8.4, 2.1 Hz, 0.5H, 6''-H), 7.13 (dd, *J* = 8.4, 2.1 Hz, 0.5H, 6''-H), 7.08 (d, *J* = 2.1 Hz, 0.5H, 2''-H), 7.05 (d, *J* = 2.1 Hz, 0.5H, 2''-H), 6.99 – 6.90 (m, 1H, 5''-H), 4.73 (s, 1H, 5-H), 4.65 (s, 1H, 5-H), 4.42 – 4.31 (m, 2H, 2-H), 4.14 – 4.06 (m, 1H, 3-H), 4.00 – 3.92 (m, 4H, 3-H, OCH_3), 3.92 – 3.87 (m, 3H, OCH_3), 2.45 (q, *J* = 7.4 Hz, 1H, 2'-H), 2.31 (q, *J* = 7.4 Hz, 1H, 2'-H), 1.15 – 1.05 (m, 3H, 3'-H).

^{13}C NMR (mixture of rotamers, 101 MHz, $C_2D_2Cl_4$): δ = 173.0 (C-1'), 172.7 (C-1'), 165.2 (O=COH), 165.1 (O=COH), 156.8 (C-9a), 149.4 (C-3''/C-4''), 149.2 (C-3''/C-4''), 137.9 (C-7), 137.7 (C-7), 134.1 (C-6), 132.7 (C-6), 132.0 (C-5a), 131.6 (C-5a), 131.4 (C-1''), 131.3 (C-1''), 130.5 (C-8), 129.8 (C-8), 121.8 (C-9), 121.0 (C-9), 119.7 (C-6''), 111.9 (C-5''), 111.8 (C-5''), 110.5 (C-2''), 110.3 (C-2''), 74.6 (C-2), 56.3 (OCH_3), 56.1 (OCH_3), 50.7 (C-5), 50.0 (C-3), 47.9 (C-5), 47.4 (C-3), 26.8 (C-2'), 26.6 (C-2'), 9.32 (C-3'), 9.28 (C-3').

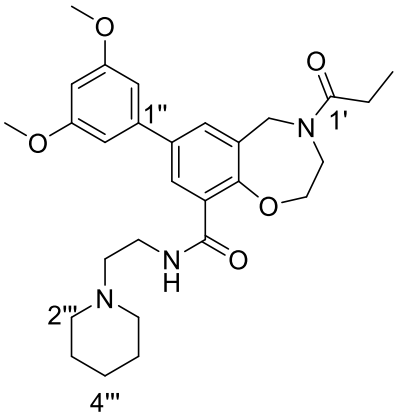
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3429, 2975, 2937, 2836, 1724, 1646, 1520, 1475, 1255, 1219, 1145, 1203, 814, 763, 673.

MS (EI+): m/z (%) = 307 (13), 330 (13), 368 (11), 385 (100) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{21}H_{23}NO_6$ 385.1525, found 385.1518.

Purity (HPLC): > 99 % (210 nm; method 4a).

7-(3,5-Dimethoxyphenyl)-*N*-[2-(piperidin-1-yl)ethyl]-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxamide (108)



MF: $C_{28}H_{37}N_3O_5$ MW: 495.62 g/mol

Standard protocol 3 with 0.11 g (0.29 mmol) **106** and 0.050 mL (0.35 mmol) 1-(2-aminoethyl)piperidine. FCC with DCM with 0.6 % MeOH and 3 % triethylamine (Rf 0.3) gave 0.055 g (0.11 mmol, 31 %) of **108** as a white solid.

mp: 88 – 89 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$): δ = 8.55 – 8.48 (m, 0.7H, NH), 8.45 – 8.39 (m, 0.3H, NH), 8.38 (d, *J* = 2.5 Hz, 0.7H, 8-H), 8.29 (d, *J* = 2.5 Hz, 0.3H, 8-H), 7.71 (d, *J* = 2.5 Hz, 0.3H, 6-H), 7.49 (d, *J* = 2.5 Hz, 0.7H, 6-H), 6.74 (d, *J* = 2.2 Hz, 0.7H, 2''-H, 6''-H), 6.72 (d, *J* = 2.2 Hz, 1.3H, 2''-H, 6''-H), 6.48 (t, *J* = 2.2 Hz, 0.7H, 4''-H), 6.45 (t, *J* = 2.2 Hz, 0.3H, 4''-H), 4.73 (s, 0.7H, 5-H), 4.63 (s, 1.3H, 5-H), 4.30 – 4.23 (m, 2H, 2-H), 4.13 – 4.07 (m, 1.3H, 3-H), 3.96 – 3.92 (m, 0.7H, 3-H), 3.87 – 3.82 (m, 6H, OCH_3), 3.62 – 3.55 (m, 2H, OCHN-C*H*$_2$), 2.62 – 2.38 (m, 7.3H, 2'-H, $N(CH_2)_3$), 2.33 (q, *J* = 7.4 Hz, 0.7H, 2'-H), 1.65 – 1.55 (m, 4H, 3'''-H, 5'''-H), 1.55 – 1.44 (m, 2H, 4'''-H), 1.16 – 1.10 (m, 3H, 3'-H).

^{13}C NMR (mixture of rotamers, 126 MHz, $CDCl_3$): δ = 173.0 (C-1'), 172.3 (C-1'), 164.8 (O=CNH), 164.4 (O=CNH), 161.2 (C-3'', C-5''), 161.0 (C-3'', C-5''), 157.1 (C-9a), 156.9 (C-9a), 141.7 (C-1''), 141.6 (C-1''), 137.1 (C-7), 136.9 (C-7), 132.2 (C-6), 132.1 (C-5a/C-9), 131.7 (C-5a/C-9), 130.6 (C-6), 130.0 (C-8), 129.2 (C-8), 126.0 (C-5a/C-9), 125.4 (C-5a/C-9), 105.3 (C-2'', C-6''), 105.1 (C-2'', C-6''), 100.0 (C-4''), 99.5 (C-4''), 73.3 (C-2), 73.2 (C-2), 57.3 ($N(CH_2)_3$), 57.2 ($N(CH_2)_3$), 55.5 (OCH_3), 54.3 ($N(CH_2)_3$),

51.0 (C-5), 50.4 (C-3), 48.1 (C-5), 47.7 (C-3), 36.62 (OCHN-*C*H_2), 36.56 (OCHN-*C*H_2), 26.8 (C-2‘), 26.5 (C-2‘), 26.1 (C-3‘‘‘, C-5‘‘‘), 24.4 (C-4‘‘‘), 9.21 (C-3‘), 9.18 (C-3‘).

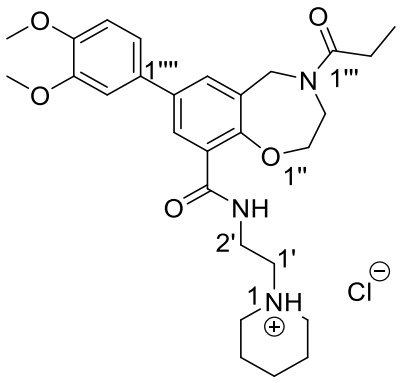
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3381, 2936, 2850, 2807, 1649, 1597, 1518, 1464, 1205, 1155, 1041, 843, 753.

MS (EI+): m/z (%) = 162 (24), 269 (100), 353 (26), 410 (34), 495 (14) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{28}H_{37}N_3O_5$ 495.2733, found 495.2734.

Purity (HPLC): 97 % (210 nm; method 1c).

1-{2-[7-(3,4-Dimethoxyphenyl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxamido]ethyl}piperidin-1-ium chloride (109)



MF: $C_{28}H_{38}ClN_3O_5$ MW: 532.08 g/mol

Standard protocol 3 with 0.090 g (0.23 mmol) **107** and 0.033 mL (0.27 mmol) 1-(2-aminoethyl)piperidine. FCC with DCM with 10 % MeOH (Rf 0.46) gave a colourless oil. **109** was then precipitated from a solution in 1,4-dioxane as hydrochloride by addition of 4 N solution of HCl in 1,4-dioxane. The precipitate was washed with diethyl ether to obtain 0.043 g (0.10 mmol, 43 %) of **109** as a white solid.

mp: 98 – 99 °C.

^{1}H NMR (mixture of rotamers, 400 MHz, $C_2D_2Cl_4$): δ = 11.93 (br s, 1H, N^+H), 8.78 (br s, 1H, OCNH), 8.29 – 8.10 (m, 1H, 8''-H), 7.75 – 7.61 (m, 0.5H, 6''-H), 7.55 – 7.44 (m, 0.5H, 6''-H), 7.23 – 7.03 (m, 2H, 2''''-H, 6''''-H), 7.04 – 6.92 (m, 1H, 5''''-H), 4.84 – 4.31 (m, 4H, 2''-H, 5''-H), 4.18 – 3.78 (m, 10H, 1'-H/2'-H, 3''-H, OCH_3), 3.66 – 3.44 (m, 2H, 2-H/6-H), 3.38 – 3.03 (m, 2H, 1'-H/2'-H), 2.90 – 2.55 (m, 2H, 2-H, 6-H), 2.55 – 2.14 (m, 3H, 3-H/5-H, 2'''-H), 2.05 – 1.72 (m, 4H, 3-H, 5-H, 4-H), 1.53 – 1.31 (m, 1H, 4-H), 1.19 – 1.01 (m, 3H, 3'''-H).

^{13}C NMR (mixture of rotamers, 101 MHz, $C_2D_2Cl_4$): δ = 172.9 (C-1'''), 172.6 (C-1'''), 165.8 (OCNH), 165.7 (OCNH), 156.5 (C-9a''), 156.5 (C-9a''), 149.04 (C-3''''), 148.95 (C-3''''), 148.8 (C-4''''), 148.6 (C-4''''), 136.4 (C-7''), 136.1 (C-7''), 132.2 (C-6''), 131.9 (C-1''''), 131.8 (C-1''''), 131.4 (C-5a''), 131.3 (C-5a''), 130.8 (C-6''), 129.0 (C-8''), 128.4 (C-8''), 124.9 (C-9''), 123.9 (C-9''), 119.3 (C-2''''/C-6''''), 111.54 (C-5''''), 111.50 (C-5''''), 110.3 (C-2''''/C-6''''), 110.1 (C-2''''/C-6''''), 73.0 (C-2''), 57.5 (C-1'/C-2'), 57.3 (C-1'/C-2'), 56.1 (OCH_3), 55.9 (OCH_3), 54.9 (C-2, C-6), 50.6 (C-5''), 50.0 (C-3''), 47.6

(C-5''), 47.5 (C-3''), 35.6 (C-1'/C-2'), 35.5 (C-1'/C-2'), 26.7 (C-2'''), 26.4 (C-2'''), 22.5 (C-4, C-3, C-5), 21.7 (C-4), 9.2 (C-3'''), 9.1 (C-3''').

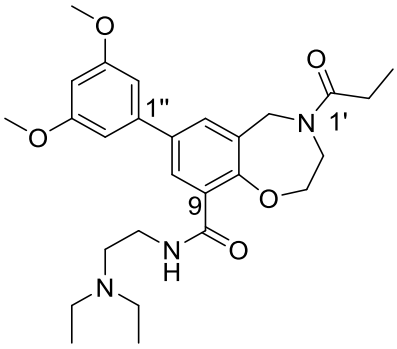
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3432, 2939, 2639, 2535, 1642, 1519, 1470, 1253, 1218, 1022, 872, 764.

MS (ESI+): m/z (%) = 496 (100) $[M + H]^+$, 497 (16).

HRMS (ESI+): m/z calcd for $[C_{28}H_{38}N_3O_5]^+$ 496.2811, found 496.2804.

Purity (HPLC): > 99 % (210 nm; method 1c).

_N_-[2-(Diethylamino)ethyl]-7-(3,5-dimethoxyphenyl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxamide (110)



MF: $C_{27}H_{37}N_3O_5$ MW: 483.61 g/mol

Standard protocol 3 with 0.050 g (0.13 mmol) **106** and 0.028 mL (0.20 mmol) *N*,*N*-diethylethylenediamine. FCC with DCM with 0.6 % MeOH and 3 % triethylamine (Rf 0.3) gave 0.029 g (0.060 mmol, 46 %) of **110** as a colourless oil.

^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$): δ = 8.50 (s, 0.6H, NH), 8.40 (s, 0.4H, NH), 8.37 (d, *J* = 2.5 Hz, 0.6H, 8-H), 8.29 (d, *J* = 2.5 Hz, 0.4H, 8-H), 7.70 (d, *J* = 2.5 Hz, 0.4H, 6-H), 7.49 (d, *J* = 2.5 Hz, 0.6H, 6-H), 6.74 (d, *J* = 2.3 Hz, 0.8H, 2''-H, 6''-H), 6.72 (d, *J* = 2.3 Hz, 1.2H, 2''-H, 6''-H), 6.48 (t, *J* = 2.3 Hz, 0.6H, 4''-H), 6.45 (t, *J* = 2.3 Hz, 0.4H, 4''-H), 4.73 (s, 0.8H, 5-H), 4.62 (s, 1.2H, 5-H), 4.29 – 4.19 (m, 2H, 2-H), 4.11 – 4.05 (m, 1.4H, 3-H), 3.95 – 3.90 (m, 0.6H, 3-H), 3.87 – 3.81 (m, 6H, OCH_3), 3.61 – 3.52 (m, 2H, HNC*H*$_2$), 2.75 – 2.56 (m, 6H, $N(CH_2)_3$), 2.47 (q, *J* = 7.4 Hz, 1.2H, 2'-H), 2.33 (q, *J* = 7.4 Hz, 0.8H, 2'-H), 1.18 – 1.02 (m, 9H, CH_2CH_3).

^{13}C NMR (mixture of rotamers, 101 MHz, $CDCl_3$): δ = 173.0 (C-1'), 172.3 (C-1'), 164.9 (O=CNH), 164.5 (O=CNH), 161.2 (C-3'', C-5''), 161.1 (C-3'', C-5''), 157.1 (C-9a), 156.9 (C-9a), 141.7 (C-1''), 141.6 (C-1''), 137.0 (C-7), 136.9 (C-7), 132.2 (C-6), 132.1 (C-5a), 131.7 (C-5a), 130.6 (C-6), 130.0 (C-8), 129.2 (C-8), 126.1 (C-9), 125.4 (C-9), 105.3 (C-2'', C-6''), 105.1 (C-2'', C-6''), 100.0 (C-4''), 99.6 (C-4''), 73.3 (C-2), 73.1 (C-2), 55.5 (OCH_3), 51.5 ($N(CH_2)_3$), 51.4 ($N(CH_2)_3$), 51.0 (C-5), 50.4 (C-3), 48.1 (C-5), 47.7 (C-3), 46.8 ($N(CH_2)_3$), 46.6 ($N(CH_2)_3$), 37.5 ($HNCH_2$), 37.4 ($HNCH_2$), 26.8 (C-2'), 26.5 (C-2'), 11.7 (NCH_2CH_3), 9.2 (C-3').

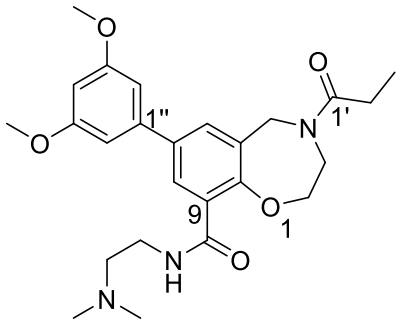
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3375, 2969, 2936, 2838, 1649, 1597, 1517, 1462, 1205, 1153, 1066, 1042, 985.

MS (EI+): m/z (%) = 269 (100), 483 (17) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{27}H_{37}N_3O_5$ 483.2733, found 483.2720.

Purity (HPLC): 98 % (210 nm; method 1c).

7-(3,5-Dimethoxyphenyl)-*N*-[2-(dimethylamino)ethyl]-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxamide (111)



MF: $C_{25}H_{33}N_3O_5$ MW: 455.56 g/mol

Standard protocol 3 with 0.11 g (0.29 mmol) **106** and 0.039 mL (0.35 mmol) *N*,*N*-dimethylethylenediamine. FCC with DCM with 1 % MeOH and 1 % triethylamine (Rf 0.3) gave 0.09 g (0.20 mmol, 69 %) of **111** as a colourless oil.

^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$): δ = 8.53 – 8.46 (m, 0.7H, NH), 8.40 – 8.34 (m, 1H, NH, 8-H), 8.28 (d, *J* = 2.5 Hz, 0.3H, 8-H), 7.71 (d, *J* = 2.5 Hz, 0.3H, 6-H), 7.49 (d, *J* = 2.5 Hz, 0.7H, 6-H), 6.74 (d, *J* = 2.3 Hz, 0.6H, 2''-H, 6''-H), 6.72 (d, *J* = 2.3 Hz, 1.4H, 2''-H, 6''-H), 6.48 (t, *J* = 2.3 Hz, 0.7H, 4''-H), 6.45 (t, *J* = 2.3 Hz, 0.3H, 4''-H), 4.72 (s, 0.7H, 5-H), 4.62 (s, 1.3H, 5-H), 4.23 – 4.16 (m, 2H, 2-H), 4.13 – 4.07 (m, 1.3H, 3-H), 3.96 – 3.91 (m, 0.7H, 3-H), 3.87 – 3.82 (m, 6H, OCH_3), 3.61 – 3.52 (m, 2H, HNCH_2), 2.57 – 2.50 (m, 2H, (CH_2N$(CH_3)_2$)), 2.46 (q, *J* = 7.4 Hz, 1.4H, 2'-H), 2.36 – 2.25 (m, 6.6H, 2'-H, (N$(CH_3)_2$)), 1.18 – 1.09 (m, 3H).

^{13}C NMR (mixture of rotamers, 101 MHz, $CDCl_3$): δ = 173.0 (C-1'), 172.2 (C-1'), 164.8 (O=CNH), 164.5 (O=CNH), 161.2 (C-3'', C-5''), 161.0 (C-3'', C-5''), 157.0 (C-9a), 156.8 (C-9a), 141.7 (C-1''), 141.6 (C-1''), 137.2 (C-7), 137.0 (C-7), 132.4 (C-5a), 132.2 (C-6), 131.9 (C-5a), 130.6 (C-6), 129.9 (C-8), 129.2 (C-8), 126.2 (C-9), 125.5 (C-9), 105.3 (C-2'', C-6''), 105.2 (C-2'', C-6''), 100.0 (C-4''), 99.6 (C-4''), 73.3 (C-2), 73.2 (C-2), 57.8 (CH_2N$(CH_3)_2$), 57.7 (CH_2N$(CH_3)_2$), 55.5 (OCH_3), 51.1 (C-5), 50.5 (C-3), 48.2 (C-5), 47.8 (C-3), 45.23 (N$(CH_3)_2$), 45.19 (N$(CH_3)_2$), 37.3 (HNCH_2), 26.8 (C-2'), 26.5 (C-2'), 9.2 (C-3').

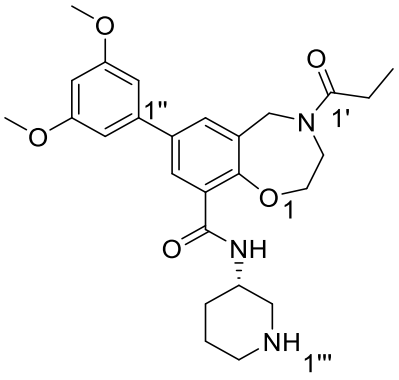
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3376, 2939, 2821, 2771, 1650, 1596, 1521, 1461, 1254, 1205, 1155, 1065, 1041, 1019, 943, 844.

MS (EI+): m/z (%) = 269 (74), 385 (100), 455 (10) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{25}H_{33}N_3O_5$ 455.2420, found 455.2426.

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

(*S*)-7-(3,5-Dimethoxyphenyl)-*N*-(piperidin-3-yl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxamide (112)



MF: $C_{26}H_{33}N_3O_5$ MW: 467.57 g/mol

Standard protocol 3 with 0.75 g (2.0 mmol) **106** and 0.50 g (2.5 mmol) (S)-(+)-3-amino-1-boc-piperidine. FCC with EtOAc and hexanes (5:1, Rf 0.2) gave a white solid, which was dissolved in 3 mL 1,4-dioxane. Then 3 mL of a 4 N solution of HCl in 1,4-dioxane was added and the mixture stirred for 16 h. Then 100 mL 1 M NaOH was added and the mixture was extracted with DCM (3 x 100mL) three times. The combined organic layers were dried over $MgSO_4$. After concentration in vacuo purification by FCC with DCM with 5 % MeOH and 2 % triethylamine (Rf 0.2) gave 0.29 g (0.62 mmol, 31 %) of **112** as a white solid.

mp: 91 - 92 °C.

$[\alpha]^{20}_D$ = -4.5 (c 0.16, MeOH).

^{1}H NMR (mixture of rotamers, 500 MHz, $C_2D_2Cl_4$): δ = 8.40 (br s, 0.6H, O=CNH), 8.35 – 8.27 (m, 1H, O=CNH, 8-H), 8.27 – 8.22 (m, 0.4H, 8-H), 7.69 (d, *J* = 2.6 Hz, 0.4H, 6-H), 7.51 (d, *J* = 2.6 Hz, 0.6H, 6-H), 6.76 – 6.70 (m, 2H, 2‘‘-H, 6‘‘-H), 6.50 – 6.43 (m, 1H, 4‘‘-H), 4.77 – 4.56 (m, 2H, 5-H), 4.31 – 3.87 (m, 5H, 2-H, 3-H, 3‘‘‘-H), 3.87 – 3.82 (m, 6H, OCH_3), 3.21 – 3.05 (m, 1H, 2‘‘‘-H), 2.92 – 2.69 (m, 3H, 2‘‘‘-H, 6‘‘‘-H), 2.46 – 2.39 (m, 1.2H, 2‘-H), 2.32 – 2.26 (m, 0.8H, 2‘-H), 1.91 – 1.55 (m, 4H, 4‘‘‘-H, 5‘‘‘-H), 1.13 – 1.05 (m, 3H, 3‘-H).

^{13}C NMR (mixture of rotamers, 126 MHz, $C_2D_2Cl_4$): δ = 172.8 (C-1‘), 172.2 (C-1‘), 163.7 (O=CNH), 163.5 (O=CNH), 160.9 (C-3‘‘, C-5‘‘), 156.8 (C-9a), 156.7 (C-9a), 141.2 (C-1‘‘), 136.5 (C-7), 132.2 (C-5a), 131.9 (C-5a), 131.8 (C-6), 130.5 (C-6), 129.5 (C-8),

128.8 (C-8), 125.9 (C-9), 125.3 (C-9), 105.2 (C-2‘‘), 104.9 (C-6‘‘), 99.8 (C-4‘‘), 99.4 (C-4‘‘), 73.1 (C-2), 55.5 (OCH_3), 50.9 (C-5, C-2‘‘‘), 50.7 (C-3‘‘‘), 50.1 (C-3‘‘‘), 47.9 (C-5), 47.5 (C-3), 46.1 (C-6‘‘‘), 46.0 (C-6‘‘‘), 45.5 (C-3), 29.8 (C-4‘‘‘), 26.6 (C-2‘), 26.4 (C-2‘), 23.2 (C-5‘‘‘), 23.1 (C-5‘‘‘), 9.1 (C-3‘).

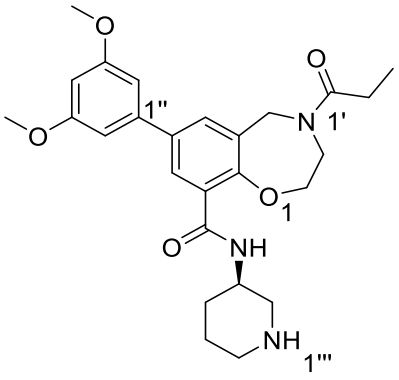
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3381, 2937, 2839, 2734, 1648, 1597, 1523, 1463, 1205, 1154, 1040, 831, 811, 756.

MS (EI+): m/z (%) = 252 (100), 269 (58), 368 (32), 404 (13), 467 (9) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{26}H_{33}N_3O_5$ 467.2420, found 467.2419.

Purity (HPLC): 98 % (210 nm; method 1c).

(*R*)-7-(3,5-Dimethoxyphenyl)-*N*-(piperidin-3-yl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxamide (113)



MF: $C_{26}H_{33}N_3O_5$ MW: 467.57 g/mol

Standard protocol 3 with 0.75 g (2.0 mmol) **106** and 0.50 g (2.5 mmol) (R)-(-)-3-amino-1-boc-piperidine. FCC with EtOAc and hexanes (5:1, Rf 0.2) gave a white solid, which was dissolved in 3 mL 1,4-dioxane. 3 mL of a 4 N solution of HCl in 1,4-dioxane was added and the mixture stirred for 16 h. Then 100 mL of 1 M NaOH was added and the mixture was extracted with DCM (3 x 100 mL) three times. The combined organic layers were dried over $MgSO_4$. It was concentrated in vacuo and purification by FCC with DCM with 5 % MeOH and 2 % triethylamine (Rf 0.2) gave 0.50 g (1.1 mmol, 55 %) of **113** as a white solid.

mp: 91 – 92 °C.

$[\alpha]^{20}_D$ = +4.4 (c 0.16, MeOH).

^{1}H NMR (mixture of rotamers, 500 MHz, $C_2D_2Cl_4$): δ = 8.44 (br s, 0.6H, O=CNH), 8.35 (br s, 0.4H, O=CNH), 8.32 (d, *J* = 2.5 Hz, 0.6H, 8-H), 8.27 (d, *J* = 2.5 Hz, 0.4H, 8-H), 7.69 (d, J = 2.5 Hz, 0.4H, 6-H), 7.51 (d, J = 2.5 Hz, 0.6H, 6-H), 6.75 (d, *J* = 2.3 Hz, 0.8H, 2''-H, 6''-H), 6.73 (d, *J* = 2.3 Hz, 1.2H, 2''-H, 6''-H), 6.48 (t, *J* = 2.3 Hz, 0.6H, 4''-H), 6.46 (t, *J* = 2.3 Hz, 0.4H, 4''-H), 4.79 – 4.57 (m, 2H, 5-H), 4.30 – 3.89 (m, 5H, 2-H, 3-H, 3'''-H), 3.87 – 3.82 (m, 6H, OCH_3), 3.10 – 3.03 (m, 1H, 2'''-H), 2.83 – 2.67 (m, 3H, 2'''-H, 6'''-H), 2.47 – 2.38 (m, 1.2H, 2'-H), 2.35 – 2.24 (m, 0.8H, 2'-H), 1.91 – 1.55 (m, 4H, 4'''-H, 5'''-H), 1.12 – 1.07 (m, 3H, 1'-H).

^{13}C NMR (mixture of rotamers, 126 MHz, $C_2D_2Cl_4$): δ = 172.8 (C-1'), 172.2 (C-1'), 163.7 (O=CNH), 163.4 (O=CNH), 160.88 (C-3'', C-5''), 160.85 (C-3'', C-5''), 156.8 (C-9a),

156.7 (C-9a), 141.2 (C-1''), 136.5 (C-7), 132.3 (C-5a), 131.9 (C-5a), 131.8 (C-6), 130.5 (C-6), 129.5 (C-8), 128.8 (C-8), 125.9 (C-9), 125.3 (C-9), 105.2 (C-2'', C-6''), 104.9 (C-2'', C-6''), 99.8 (C-4''), 99.4 (C-4''), 73.1 (C-2), 55.5 (OCH_3), 50.7 (C-5, C-2'''), 50.1 (C-3'''), 47.9 (C-5), 47.5 (C-3), 46.1 (C-6'''), 45.7 (C-6'''), 45.5 (C-3), 29.8 (C-4'''), 26.6 (C-2'), 26.4 (C-2'), 23.2 (C-5'''), 9.2 (C-3'), 9.1 (C-3').

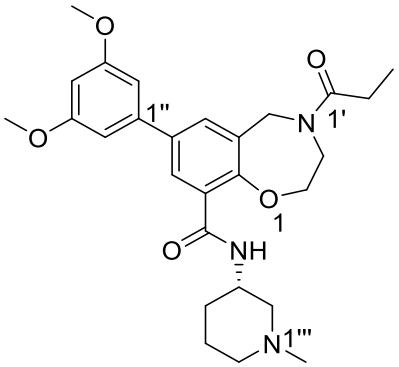
IR (film): $\tilde{\nu}$ (cm^{-1}) = 3374, 2938, 1651, 1597, 1525, 1463, 1205, 1155.

MS (EI+): m/z (%) = 83 (100), 269 (10), 368 (2), 467 (0.3) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{26}H_{33}N_3O_5$ 467.2420, found 467.2420.

Purity (HPLC): 97 % (210 nm; method 1c).

(*S*)-7-(3,5-Dimethoxyphenyl)-*N*-(1-methylpiperidin-3-yl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxamide (114)



MF: $C_{27}H_{35}N_3O_5$ MW: 481.59 g/mol

To a solution of 0.16 g (0.34 mmol) **112** in 1.0 mL acetonitrile 0.14 mL (1.7 mmol) of a 35 % solution of formaldehyde in water and 34 mg (0.54 mmol) $NaCNBH_3$ were added. The mixture was stirred for 1 h, then 20 mL 2 M NaOH was added and the mixture was extracted with DCM (3 x 20 mL) three times. The combined organic layers were dried over $MgSO_4$. FCC with DCM with 5 % MeOH (Rf 0.2) gave 0.060 g (0.12 mmol, 35 %) of **114** as a white solid.

mp: 75 – 76 °C.

$[\alpha]^{20}_D$ = -1.7 (c 0.41, MeOH).

^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$): δ = 8.56 (br s, 0.7H, NH), 8.43 (br s, 0.3H, NH), 8.36 (d, *J* = 2.5 Hz, 0.7H, 8-H), 8.28 (d, *J* = 2.5 Hz, 0.3H, 8-H), 7.71 (d, *J* = 2.5 Hz, 0.3H, 6-H), 7.49 (d, *J* = 2.5 Hz, 0.7H, 6-H), 6.74 (d, *J* = 2.3 Hz, 0.6H, 2''-H, 6''-H), 6.72 (d, *J* = 2.3 Hz, 1.4H, 2''-H, 6''-H), 6.48 (t, *J* = 2.2 Hz, 0.7H, 4''-H), 6.45 (t, *J* = 2.2 Hz, 0.3H, 4''-H), 4.72 (s, 0.6H, 5-H), 4.62 (s, 1.4H, 5-H), 4.34 – 4.28 (m, 1H, 3'''-H), 4.24 – 4.19 (m, 2H, 2-H), 4.14 – 4.08 (m, 1.4H, 3-H), 3.97 – 3.91 (m, 0.6H, 3-H), 3.87 – 3.83 (m, 6H, OCH_3), 2.65 – 2.30 (m, 5H, 2'-H, 2'''-H, 6'''-H), 2.29 – 2.26 (s, 3H, NCH_3), 2.23 – 2.13 (m, 1H, 6'''-H), 1.78 – 1.71 (m, 2H, 4'''-H, 5'''-H), 1.67 – 1.58 (m, 2H, 4'''-H, 5'''-H), 1.15 – 1.11 (m, 3H, 3'-H).

^{13}C NMR (mixture of rotamers, 101 MHz, $CDCl_3$): δ = 173.0 (C-1'), 172.2 (C-1'), 163.8 (O=CNH), 163.4 (O=CNH), 161.2 (C-3'', C-5''), 161.0 (C-3'', C-5''), 157.0 (C-9a), 156.8 (C-9a), 141.7 (C-1''), 141.6 (C-1''), 137.2 (C-7), 137.1 (C-7), 132.5 (C-5a), 132.1 (C-

6), 132.0 (C-5a), 130.5 (C-6), 129.9 (C-8), 129.2 (C-8), 126.4 (C-9), 125.8 (C-9), 105.3 (C-2‘‘, C-6‘‘), 105.2 (C-2‘‘, C-6‘‘), 100.0 (C-4‘‘), 99.6 (C-4‘‘), 73.3 (C-2), 60.5 (C-2‘‘‘), 60.4 (C-2‘‘‘), 56.0 (C-6‘‘‘), 55.5 (OCH_3), 51.1 (C-5), 50.5 (C-3), 48.3 (C-5), 47.9 (C-3), 46.7 (NCH_3), 45.5 (C-3‘‘‘), 45.4 (C-3‘‘‘), 28.5 (C-4‘‘‘), 28.4 (C-4‘‘‘), 26.8 (C-2‘), 26.5 (C-2‘), 22.1 (C-5‘‘‘), 22.0 (C-5‘‘‘), 9.20 (C-3‘), 9.18 (C-3‘).

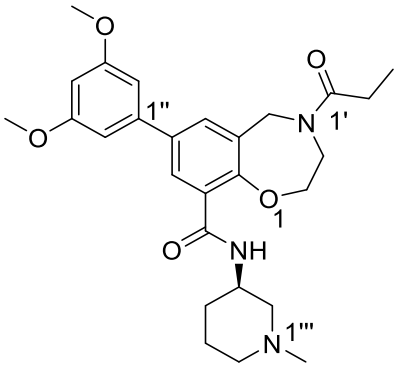
IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3429, 2937, 2842, 2786, 1654, 1599, 1521, 1463, 1205, 1154, 1041, 1019, 843, 816.

MS (EI+): m/z (%) = 269 (100), 385 (47), 481 (5) $[M]^{+\bullet}$.

HRMS (EI+): m/z calcd for $C_{27}H_{35}N_3O_5$ 481.2577, found 481.2591.

Purity (HPLC): 96 % (210 nm; method 1c).

(*R*)-7-(3,5-Dimethoxyphenyl)-*N*-(1-methylpiperidin-3-yl)-4-propionyl-2,3,4,5-tetrahydro-1,4-benzoxazepine-9-carboxamide (115)



MF: $C_{27}H_{35}N_3O_5$ MW: 481.59 g/mol

To a solution of 0.27 g (0.58 mmol) **113** in 1.7 mL acetonitrile 0.23 mL (2.9 mmol) of a 35 % solution of formaldehyde in water and 58 mg (0.92 mmol) $NaCNBH_3$ was added. The mixture was stirred for 1 h, then 50 mL 2 M NaOH was added and the mixture was extracted with DCM (3 x 50 mL) three times. The combined organic layers were dried over $MgSO_4$. FCC with DCM with 5 % MeOH (Rf 0.2) gave 0.10 g (0.21 mmol, 36 %) of **115** as a white solid.

mp: 75 – 76 °C.

$[\alpha]^{20}_D$ = +1.6 (c 0.40, MeOH)

^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$): δ = 8.56 (br s, 0.7H, NH), 8.43 (br s, 0.3H, NH), 8.35 (d, *J* = 2.5 Hz, 0.7H, 8-H), 8.27 (d, *J* = 2.5 Hz, 0.3H, 8-H), 7.71 (d, *J* = 2.5 Hz, 0.3H, 6-H), 7.49 (d, *J* = 2.5 Hz, 0.7H, 6-H), 6.74 (d, *J* = 2.3 Hz, 0.6H, 2''-H, 6''-H), 6.71 (d, *J* = 2.3 Hz, 1.4H, 2''-H, 6''-H), 6.48 (t, *J* = 2.2 Hz, 0.7H, 4''-H), 6.45 (t, *J* = 2.3 Hz, 0.3H, 4''-H), 4.72 (s, 0.6H, 5-H), 4.62 (s, 1.4H, 5-H), 4.34 – 4.28 (m, 1H, 3'''-H), 4.25 – 4.18 (m, 2H, 2-H), 4.13 – 4.08 (m, 1.4H, 3-H), 3.98 – 3.90 (m, 0.6H, 3-H), 3.86 – 3.82 (m, 6H, OCH_3), 2.62 – 2.30 (m, 5H, 2'-H, 2'''-H, 6'''-H), 2.28 (s, 3H, NCH_3), 2.23 – 2.13 (m, 1H, 6'''-H), 1.78 – 1.70 (m, 2H, 4'''-H, 5'''-H), 1.67 – 1.57 (m, 2H, 4'''-H, 5'''-H), 1.16 – 1.09 (m, 3H, 3'-H).

^{13}C NMR (mixture of rotamers, 101 MHz, $CDCl_3$): δ = 173.1 (C-1'), 172.2 (C-1'), 163.8 (O=CNH), 163.4 (O=CNH), 161.2 (C-3'', C-5''), 161.1 (C-3'', C-5''), 157.0 (C-9a), 156.8 (C-9a), 141.7 (C-1''), 141.6 (C-1''), 137.2 (C-7), 137.1 (C-7), 132.5 (C-5a), 132.1 (C-

6), 132.0 (C-5a), 130.5 (C-6), 129.9 (C-8), 129.2 (C-8), 126.4 (C-9), 125.8 (C-9), 105.3 (C-2‘‘, C-6‘‘), 105.2 (C-2‘‘, C-6‘‘), 100.0 (C-4‘‘), 99.6 (C-4‘‘), 73.3 (C-2), 60.4 (C-2‘‘‘), 56.0 (C-6‘‘‘), 55.5 (OCH_3), 51.1 (C-5), 50.5 (C-3), 48.3 (C-5), 47.9 (C-3), 46.7 (NCH_3), 45.5 (C-3‘‘‘), 45.4 (C-3‘‘‘), 28.4 (C-4‘‘‘), 26.8 (C-2‘), 26.5 (C-2‘), 22.1 (C-5‘‘‘), 9.2 (C-3‘).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3376, 2938, 2842, 2789, 1652, 1600, 1523, 1465, 1206, 1156.

MS (ESI+): m/z (%) = 482 (100) $[M + H]^+$, 483 (12).

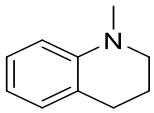
HRMS (ESI+): m/z calcd for $[C_{27}H_{36}N_3O_5]^+$ 482.2649, found 482.2650.

Purity (HPLC): > 99 % (210 nm; method 1a).

Standard protocol for the *N*-methylation of aromatic amines and N-heterocycles

Under nitrogen atmosphere 1.0 mmol of N-containing substance and 3.0 mmol of trioxane were dissolved in 1.5 mL CH_2Cl_2. To this solution 0.75 mL TFA and 1.45 mL (10 mmol) triethylsilane were added. Reaction was monitored by tlc. After 24 or 48 hours (in case of incomplete conversion after 24 hours), 20 mL of 2 N NaOH solution were carefully added and the mixture was extracted three times with 20 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. FCC was used for purification.

1-Methyl-1,2,3,4-tetrahydroquinoline* (118)



MF: $C_{10}H_{13}N$ MW: 147.22 g/mol

Standard TTT protocol with 0.45 g (3.4 mmol) 1,2,3,4-tetrahydroquinoline. Standard protocol workup after 48 h and purification by FCC with hexanes and EtOAc (20:1, Rf 0.5) gave 0.32 g (2.2 mmol, 64 %) of **118** as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.15 – 7.11 (m, 1H, 7-H), 7.02 – 6.99 (m, 1H, 5-H), 6.68 – 6.63 (m, 2H, 8-H, 6-H), 3.28 – 3.25 (m, 2H, 2-H), 2.93 (s, 3H, CH_3), 2.84 – 2.80 (m, 2H, 4-H), 2.07 – 2.00 (m, 2H, 3-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 146.9 (8a), 128.9 (C-5), 127.1 (C-7), 123.0 (C-4a), 116.3 (C-6), 111.1 (C-8), 51.4 (C-2), 39.2 (CH_3), 27.9 (C-4), 22.6 (C-3).

IR (film): $\tilde{\nu}$ (cm^{-1}) = 3065, 2927, 2862, 1602, 1507, 1321, 1208.

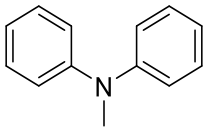
MS (EI+): *m*/*z* (%) = 91 (21), 131 (23), 146 (100) $[M - H]^+$, 147 (87) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{10}H_{13}N$: 147.1048; found: 147.1031.

Purity (HPLC): > 99 % (210 nm; method 6a).

***Known compound, novel synthesis.

_N_-Methyl-_N_-phenylaniline* (120)



MF: $C_{13}H_{13}N$ MW: 183.25 g/mol

Standard TTT protocol with 0.36 g (2.2 mmol) diphenylamine. Standard protocol workup after 24 h and purification by FCC with hexanes and EtOAc (10:1, Rf 0.5) gave 0.35 g (1.9 mmol, 89 %) of **120** as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.28 – 7.22 (m, 4H, 3-H, 5-H), 7.03 – 6.98 (m, 4H, 2-H, 6-H), 6.93 (tt, *J* = 7.3, 1.1 Hz, 2H, 4-H), 3.29 (s, 3H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 149.1 (C-1), 129.3 (C-3, C-5), 121.4 (C-4), 120.5 (C-2, C-6), 40.3 (CH_3).

IR (film): $\tilde{\nu}$ (cm^{-1}) = 3060, 3035, 2939, 2878, 1591, 1496, 1342, 1252, 1131.

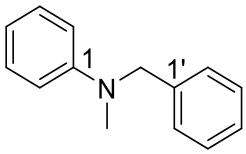
MS (EI+): *m*/*z* (%) = 77 (32), 104 (17), 168 (11), 183 (100) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{13}H_{13}N$: 183.1048; found: 183.1036.

Purity (HPLC): > 99 % (210 nm; method 6a).

*****Known compound, novel synthesis.

_N_-Benzyl-_N_-methylaniline* (121)



MF: $C_{14}H_{15}N$ MW: 197.28 g/mol

Standard TTT protocol with 0.35 g (1.9 mmol) *N*-benzylaniline. Standard protocol workup after 48 h and purification by FCC with hexanes and EtOAc (10:1, Rf 0.6) gave 0.19 g (0.96 mmol, 51 %) of **121** as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.33 – 7.27 (m, 2H, 3‘-H, 5‘-H), 7.25 – 7.18 (m, 5H, 3-H, 5-H, 2‘-H, 4‘-H, 6‘-H), 6.77 – 6.72 (m, 2H, 2-H, 6-H), 6.72 – 6.68 (m, 1H, 4-H), 4.51 (s, 2H, CH_2), 2.99 (s, 3H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 149.9 (C-1), 139.1 (C-1‘), 129.3 (C-3, C-5), 128.7 (C-3‘, C-5‘), 127.0 (C-4‘), 126.8 (C-2‘, C-6‘), 116.6 (C-4), 112.5 (C-2, C-6), 56.7 (CH_2), 38.6 (CH_3).

IR (film): $\tilde{\nu}$ (cm^{-1}) = 3061, 3026, 2894, 1599, 1506, 1451, 1354.

MS (EI+): *m*/*z* (%) = 91 (100), 120 (59), 197 (71) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{14}H_{15}N$: 197.1204; found: 197.1198.

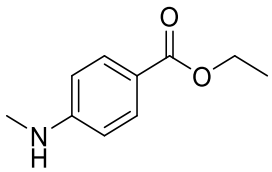
Purity (HPLC): 96 % (210 nm; method 6a).

*****Known compound, novel synthesis.

Ethyl 4-(*N*-methylamino)benzoate (123) and ethyl 4-(*N*,*N*-dimethylamino)benzoate (124)

Standard TTT protocol with 0.31 g (1.9 mmol) ethyl 4-aminobenzoate (benzocaine). Standard protocol workup after 48 h and purification by FCC with hexanes and EtOAc (5:1, Rf 0.5 and 0.3) gave 0.13 g (0.73 mmol, 38 %) of ethyl 4-(methylamino)benzoate (**123**) and 0.21 g (1.1 mmol, 57 %) of ethyl 4-(dimethylamino)benzoate (**124**) as white solids.

Ethyl 4-(*N*-methylamino)benzoate* (123)



MF: $C_{10}H_{13}NO_2$ MW: 179.22 g/mol

mp: 62 - 63 °C [Lit[128].: 59 – 62 °C].

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.90 – 7.86 (m, 2H, 2-H, 6-H), 6.57 – 6.53 (m, 2H, 3-H, 5-H), 4.31 (q, *J* = 7.1 Hz, 2H, C*H*$_2$CH$_3$), 4.19 (br s, 1H, NH), 2.88 (s, 3H, NCH$_3$), 1.36 (t, *J* = 7.1 Hz, 3H, CH$_2$C*H*$_3$).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.0 (C=O), 152.9 (C-4), 131.6 (C-2, C-6), 118.7 (C-1), 111.2 (C-3, C-5), 60.3 (*C*H$_2$CH$_3$), 30.3 (NCH$_3$), 14.6 (CH$_2$*C*H$_3$).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3383, 2962, 2936, 2903, 1680, 1602, 1538, 1276, 1174, 835.

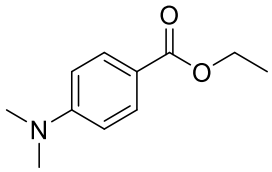
MS (EI+): *m*/*z* (%) = 106 (10), 134 (100), 151 (19), 179 (68) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{10}H_{13}NO_2$: 179.0946; found: 179.0947.

Purity (HPLC): > 99 % (210 nm; method 6a).

*****Known compound, novel synthesis.

Ethyl 4-(*N*,*N*-dimethylamino)benzoate* (124)



MF: $C_{11}H_{15}NO_2$ MW: 193.25 g/mol

mp: 61 - 62 °C [Lit[129].: 65 – 66 °C].

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.93 – 7.89 (m, 2H, 2-H, 6-H), 6.64 – 6.60 (m, 2H, 3-H, 5-H), 4.31 (q, *J* = 7.1 Hz, 2H, OCH_2), 3.00 (s, 6H, $N(CH_3)_2$), 1.36 (t, *J* = 7.1 Hz, 3H, $CH_2C\mathit{H}_3$).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.0 (C=O), 153.2 (C-4), 131.2 (C-2, C-6), 117.3 (C-1), 110.7 (C-3, C-5), 60.1 (OCH_2), 40.0 ($N(CH_3)_2$), 14.5 ($CH_2\mathit{C}H_3$).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 2982, 2903, 2820, 1695, 1611, 1365, 1283, 1186, 1106.

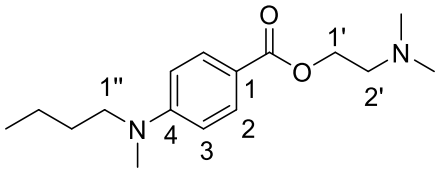
MS (EI+): *m*/*z* (%) = 148 (100), 164 (41), 193 (68) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{11}H_{15}NO_2$: 193.1103; found: 193.1088.

Purity (HPLC): > 99 % (210 nm; method 6a).

***Known compound, novel synthesis.

2-(Dimethylamino)ethyl 4-(*N*-butyl-*N*-methylamino)benzoate (122)



MF: $C_{16}H_{26}N_2O_2$ MW: 278.40 g/mol

Standard TTT protocol with 0.57 g (1.9 mmol) 2-(dimethylamino)ethyl 4-(*N*-butylamino)benzoate (tetracaine) hydrochloride. Standard protocol workup after 48 h and purification by FCC with CH_2Cl_2 with 10 % MeOH (Rf 0.2) gave 0.27 g (0.97 mmol, 51 %) of **122** as a colorless oil.

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.76 – 7.73 (m, 2H, 2-H, 6-H), 6.54 – 6.51 (m, 2H, 3-H, 5-H), 4.21 (t, *J* = 5.9 Hz, 2H, 1‘-H), 3.28 – 3.24 (m, 2H, 1‘‘-H), 2.88 (s, 3 H, 4-N-CH_3), 2.54 (t, *J* = 5.9 Hz, 2H, 2‘-H), 2.19 (s, 6H, $N(CH_3)_2$), 1.50 – 1.43 (m, 2H, 2‘‘-H), 1.29 – 1.20 (m, 2H, 3‘‘-H), 0.85 (t, *J* = 7.3 Hz, 3H, 4‘‘-H).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 167.1 (C=O), 153.0 (C-4), 131.7 (C-2, C-6), 117.0 (C-1), 110.9 (C-3, C-5), 62.7 (C-1‘), 58.6 (C-2‘), 52.6 (C-1‘‘), 46.1 ($N(CH_3)_2$), 38.7 (4-N-CH_3), 29.5 (C-2‘‘), 20.8 (C-3‘‘), 14.3 (C-4‘‘).

IR (film): $\tilde{\nu}$ (cm^{-1}) = 2956, 2873, 2770, 1703, 1607, 1525, 1278, 1184, 1111.

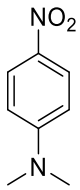
MS (EI+): *m*/*z* (%) = 58 (100), 164 (53), 207 (38), 278 (0.2) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{16}H_{26}N_2O_2$: 278.1994; found: 278.1997.

Purity (HPLC): > 99 % (210 nm; method 6b).

*****Known compound, novel synthesis.

N,*N*-Dimethyl-4-nitroaniline* (125)



MF: $C_8H_{10}N_2O_2$ MW: 166.18 g/mol

Standard TTT protocol with 0.26 g (1.9 mmol) 4-nitroaniline. Standard protocol workup after 48 h and purification by FCC with hexanes and EtOAc (5:1, Rf 0.3) gave 0.31 g (1.9 mmol, 98 %) of **125** as a yellow solid.

mp: 162 - 163 °C [Lit[130].: 162 – 165 °C].

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 8.12 – 8.05 (m, 2H, 3-H, 5-H), 6.65 – 6.60 (m, 2H, 2-H, 6-H), 3.09 (s, 6H, $N(CH_3)_2$).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 154.7 (C-1), 137.1 (C-4), 126.3 (C-3, C-5), 110.6 (C-2, C-6), 40.5 ($N(CH_3)_2$).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3424, 2924, 1735, 1601, 1582, 1485, 1457, 1310, 1116.

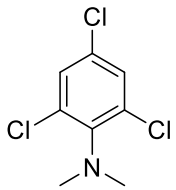
MS (EI+): *m*/*z* (%) = 105 (18), 119 (26), 136 (28), 166 (100) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_8H_{10}N_2O_2$: 166.0742; found: 166.0738.

Purity (HPLC): 96 % (210 nm; method 6a).

*****Known compound, novel synthesis.

_N_,_N_-Dimethyl-2,4,6-trichloroaniline* (126)



MF: $C_8H_8Cl_3N$ MW: 224.51 g/mol

Standard TTT protocol with 0.37 g (1.9 mmol) 2,4,6-trichloroaniline. Standard protocol workup after 48 h and purification by FCC with hexanes (Rf 0.7) gave 0.40 g (1.8 mmol, 94 %) of **126** as a colorless oil.

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.29 (s, 2H, 3-H, 5-H), 2.85 (s, 6H, $N(CH_3)_2$).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 145.8 (C-1), 136.3 (C-2, C-6), 130.4 (C-4), 129.2 (C-3, C-5), 42.2 ($N(CH_3)_2$).

IR (film): $\tilde{\nu}$ (cm^{-1}) = 3054, 2986, 1421, 1265, 739, 705.

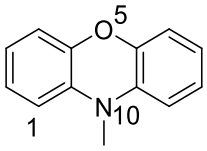
MS (EI+): *m*/*z* (%) = 222 (100) $[M - H]^+$, 223 (49) $[M]^{+\bullet}$, 224 (94), 225 (53), 226 (30), 227 (15).

HRMS (EI+): *m*/*z* calcd for ($C_8H_8Cl_3N$): 222.9722; found: 222.9722.

Purity (HPLC): > 99 % (210 nm; method 6a).

*Known compound, novel synthesis.

10-Methyl-10*H*-phenoxazine* (127)



MF: $C_{13}H_{11}NO$ MW: 197.24 g/mol

Standard TTT protocol with 0.35 g (1.9 mmol) 10*H*-phenoxazine. Standard protocol workup after 24 h and purification by FCC with hexanes and EtOAc (20:1, Rf 0.6) gave 0.37 g (1.9 mmol, 98 %) of **127** as a white to pale violet solid.

mp: 27 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.90 – 6.84 (m, 2H, 4-H, 6-H), 6.75 – 6.70 (m, 4H, 2-H, 3-H, 7-H, 8-H), 6.54 (d, *J* = 7.9 Hz, 2H, 1-H, 9-H), 3.05 (s, 3H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 145.7 (C-4a, C-5a), 135.1 (C-9a, C-10a), 123.9 (C-4, C-6), 121.0 (C-3, C-7), 115.4 (C-2, C-10), 111.5 (C-1, C-9), 31.0 (CH_3).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3063, 2882, 1592, 1486, 1362, 1268, 1217.

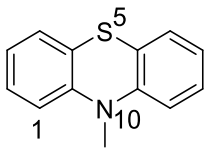
MS (EI+): *m*/*z* (%) = 127 (6), 182 (100), 197 (63) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{13}H_{11}NO$: 197.0841; found: 197.0831.

Purity (HPLC): > 99 % (210 nm; method 6a).

***Known compound, novel synthesis.

10-Methyl-10*H*-phenothiazine* (128)



MF: $C_{13}H_{11}NS$ MW: 213.30 g/mol

Standard TTT protocol with 0.40 g (2.0 mmol) 10*H*-phenothiazine. Standard protocol workup after 24 h and purification by FCC with hexanes and EtOAc (20:1, Rf 0.6) gave 0.41 g (1.9 mmol, 96 %) of **128** as a white solid.

mp: 101 - 102 °C [Lit[131].: 99 – 100 °C].

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.21 – 7.14 (m, 4H, 2-H, 4-H, 6-H, 8-H), 6.97 – 6.92 (m, 2H, 3-H, 7-H), 6.82 (dd, *J* = 8.1, 1.1 Hz, 2H, 1-H, 9-H), 3.38 (s, 3H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 145.9 (C-9a, C-10a), 127.5 (C-2/C-4, C-6/C-8), 127.3 (C-2/C-4, C-6/C-8), 123.5 (C-4a, C-5a), 122.6 (C-3, C-7), 114.2 (C-1, C-9), 35.4 (CH_3).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3058, 2968, 2888, 1592, 1568, 1457, 1331, 1258, 1137.

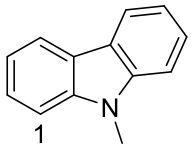
MS (EI+): *m*/*z* (%) = 198 (73), 213 (100) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{13}H_{11}NS$: 213.0612; found: 213.0601.

Purity (HPLC): 99 % (210 nm; method 6a).

*****Known compound, novel synthesis.

9-Methyl-9*H*-carbazole* (129)



MF: $C_{13}H_{11}N$ MW: 181.24 g/mol

Standard TTT protocol with 0.33 g (1.9 mmol) 9*H*-carbazole. Standard protocol workup after 24 h and purification by FCC with hexanes and EtOAc (20:1, Rf 0.3) gave 0.21 g (1.2 mmol, 61 %) of **129** as a white solid. Starting with 0.25 g (0.95 mmol) *N*-Boc-carbazole (**135**), the same product was obtained with slightly lower yield (0.084 g, 0.46 mmol, 49 %).

mp: 84 - 85 °C [Lit[132].: 88 – 90 °C].

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 8.0 Hz, 2H, 4-H, 5-H), 7.45 – 7.40 (m, 2H, 2-H, 7-H), 7.30 (d, *J* = 8.2 Hz, 2H, 1-H, 8-H), 7.22 – 7.18 (m, 2H, 3-H, 6-H), 3.70 (s, 3H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 141.1 (C-8a, C-9a), 125.7 (C-2, C-7), 122.8 (C-4a, C-4b), 120.4 (C-4, C-5), 118.9 (C-3, C-6), 108.5 (C-1, C-8), 29.0 (CH_3).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3433, 3049, 2926, 1598, 1467, 1323, 1246.

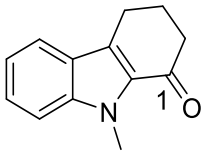
MS (EI+): *m*/*z* (%) = 152 (20), 166 (9), 181 (100) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{13}H_{11}N$: 181.0891; found: 181.0884.

Purity (HPLC): 97 % (210 nm; method 6a).

*****Known compound, novel synthesis.

9-Methyl-2,3,4,9-tetrahydro-1*H*-carbazol-1-one* (130)



MF: $C_{13}H_{13}NO$ MW: 199.25 g/mol

Standard TTT protocol with 0.35 g (1.9 mmol) 2,3,4,9-tetrahydro-1*H*-carbazol-1-one. Standard protocol workup after 48 h and purification by FCC with hexanes and EtOAc (10:1, Rf 0.3) gave 0.11 g (0.55 mmol, 29 %) of **130** as a yellow solid.

mp: 95 - 97 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.62 (d, *J* = 8.1 Hz, 1H, 7-H), 7.39 – 7.35 (m, 1H, 5-H), 7.30 (d, *J* = 8.5 Hz, 1H, 8-H), 7.14 – 7.10 (m, 1H, 6-H), 4.03 (s, 3H, CH_3), 2.97 (t, *J* = 6.1 Hz, 2H, 4-H), 2.63 – 2.59 (m, 2H, 2-H), 2.21 – 2.15 (m, 2H, 3-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 192.3 (C-1), 139.7 (C-8a), 130.4 (C-9a), 129.2 (C-4a), 126.7 (C-5), 124.7 (C-4b), 121.3 (C-7), 120.0 (C-6), 110.3 (C-8), 40.1 (C-2), 31.6 (CH_3), 24.8 (C-3), 21.9 (C-4).

IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3428, 2927, 2838, 1643, 1408, 1230, 935, 760.

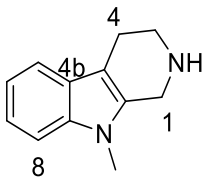
MS (EI+): *m*/*z* (%) = 128 (20), 143 (63), 170 (40), 199 (100) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{13}H_{13}NO$: 199.0997; found: 199.0988.

Purity (HPLC): 98 % (210 nm; method 6a).

*****Known compound, novel synthesis.

9-Methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole* (131)



MF: $C_{12}H_{14}N_2$ MW: 186.26 g/mol

Standard TTT protocol with 0.33 g (1.9 mmol) 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole. Standard protocol workup after 48 h and purification by FCC with CH_2Cl_2 with 10 % MeOH (Rf 0.3) gave 0.24 g (1.3 mmol, 68 %) of **131** as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (ddd, *J* = 7.9, 1.1, 0.8 Hz, 1H, 5-H), 7.27 – 7.24 (m, 1H, 8-H), 7.17 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H, 7-H), 7.08 (ddd, *J* = 7.9, 7.0, 1.1 Hz, 1H, 6-H), 4.01 (t, *J* = 1.7 Hz, 2H, 1-H), 3.56 (s, 3H, CH_3), 3.15 (t, *J* = 5.7 Hz, 2H, 3-H), 2.75 (tt, *J* = 5.7, 1.7 Hz, 2H, 4-H), 1.80 (br s, 1H, NH).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.8 (C-8a), 134.4 (C-9a), 127.2 (C-4b), 121.0 (C-7), 118.9 (C-6), 117.9 (C-5), 108.7 (C-8), 107.7 (C-4a), 44.0 (C-3), 42.5 (C-1), 29.3 (CH_3), 22.7 (C-4).

IR (film): $\tilde{\nu}$ (cm^{-1}) = 3306, 3049, 2918, 2838, 1615, 1471, 1380, 1183, 739.

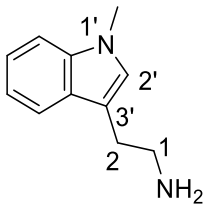
MS (EI+): *m*/*z* (%) = 142 (11), 157 (100), 186 (36) $[M]^{+\bullet}$.

HRMS (EI+): *m*/*z* calcd for $C_{12}H_{14}N_2$: 186.1157; found: 186.1152.

Purity (HPLC): 96 % (210 nm; method 6b).

*****Known compound, novel synthesis.

2-(1-Methyl-1*H*-indol-3-yl)ethan-1-amine* (132)



MF: $C_{11}H_{15}N_2$ MW: 174.25 g/mol

Standard TTT protocol with 0.30 g (1.9 mmol) 2-(1*H*-indol-3-yl)ethan-1-amine (tryptamine). Standard protocol workup after 48 h and purification by FCC with CH_2Cl_2 with 10 % MeOH (Rf 0.1) gave 0.10 g (0.57 mmol, 30 %) of **132** as a colorless oil.

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.55 (d, *J* = 7.9 Hz, 1H, 4‘-H), 7.26 (d, *J* = 8.2 Hz, 1H, 7‘-H), 7.17 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H, 6‘-H), 7.04 (ddd, *J* = 7.9, 7.0, 1.1 Hz, 1H, 5‘-H), 6.89 (s, 1H, 2‘-H), 3.69 (s, 3H, CH_3), 3.13 (br s, 2H, NH_2), 2.97 (t, *J* = 6.8 Hz, 2H, 1-H), 2.89 (t, *J* = 6.8 Hz, 2H, 2-H).

^{13}C NMR (126 MHz, CD_2Cl_2): δ = 137.6 (C-7a‘), 128.2 (C-3a‘), 127.5 (C-2‘), 121.8 (C-6‘), 119.1 (C-4‘), 119.0 (C-5‘), 111.9 (C-3‘), 109.6 (C-7‘), 42.4 (C-1), 32.8 (CH_3), 28.5 (C-2).

IR (film): $\tilde{\nu}$ (cm^{-1}) = 3347, 3050, 2926, 1578, 1473, 1328, 739.

MS (ESI+): *m*/*z* (%) = 158 (100), 175 (78, $[M + H]^+$).

HRMS (ESI+): *m*/*z* calcd for $C_{11}H_{15}N_2$: 175.1230; found: 175.1231.

Purity (HPLC): 96 % (210 nm; method 6a).

***Known compound, novel synthesis.

Abbreviations

Ac	acetyl
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
BET	Bromodomain and extraterminal
CBP, CREBBP	CREB (cAMP responsive element binding protein) binding protein
CI	chemical ionization
CMMP	(cyanomethylene)trimethylphosphorane
CpG	cytosine-phosphate-guanine
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DIPEA	*N*,*N*-diisopropylethylamine
DTBAD	di-*tert*-butylazodicarboxylate
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DSF	differential scanning fluorimetry
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EI	electronic ionization
EMA	European Medicines Agency
ESI	electron spray ionization
EtOAc	ethyl acetate
EtOH	ethanol
FDA	Food and Drug Administration
FRAP	fluorescence recovery after photobleach
GFP	green fluorescent protein
H [number]	histone [number]
HAT	histone acetyltransferase

HDAC	histone deacetylase
HPLC	high-performance liquid chromatography
HR	high resolution
IC	inhibitory concentration
ITC	isothermal titration calorimetry
K	lysine
K_{ac}	acetylated lysine
K_d	dissociation constant
MeCN	acetonitrile
MF	molecular formula
MeOH	methanol
MLL	mixed lineage leukemia
MOZ	monocytic leukaemia zinc finger protein
mp	melting point
MS	mass spectrometry
mTOR	mechanistic Target of Rapamycin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
N	asparagine
NAD(P)	Nicotinamide adenine dinucleotide (phosphate)
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMR	nuclear magnetic resonance
Ns-	nosyl-, 4-nitrophenylsulfonyl-
PCR	polymerase chain reaction
PDB	protein data bank
PPh_3	triphenylphosphine
ppm	part per million
R	arginine
RNA	ribonucleic acid
rt	room temperature
SAR	structure-activity relationship
SGC	Structural Genomics Consortium
TBDMS-	*tert*-butyl-dimethylsilyl-
THF	tetrahydrofuran

TFA	trifluoroacetic acid
TES	triethylsilane
tlc	thin layer chromatography
TTT	1,3,5-trioxane-triethylsilane-trifluoroacetic acid
Y	tyrosine

References

[1] O. T. Avery, C. M. MacLeod, M. McCarty, *The Journal of Experimental Medicine* **1944**, *79*, 137-158.

[2] A. D. Hershey, M. Chase, *The Journal of General Physiology* **1952**, *36*, 39-56.

[3] O. T. Avery, C. M. MacLeod, M. McCarty, *The Journal of Experimental Medicine* **1979**, *149*, 297-326.

[4] A. Riggs, R. Martienssen, V. Russo, *Epigenetic Mechanisms of Gene Regulation* **1996**, *32*, 1-692.

[5] A. Portela, M. Esteller, *Nature Biotechnology* **2010**, *28*, 1057-1068.

[6] R. Jaenisch, A. Bird, *Nature Genetics* **2003**, *33*, 245 - 254.

[7] S. Kacem, R. Feil, *Mamm Genome* **2009**, *20*, 544-556.

[8] A. Annunziato, *Nature Education* **2008**, *1*.

[9] R. K. Chodavarapu, S. Feng, Y. V. Bernatavichute, P.-Y. Chen, H. Stroud, Y. Yu, J. A. Hetzel, F. Kuo, J. Kim, S. J. Cokus, D. Casero, M. Bernal, P. Huijser, A. T. Clark, U. Kramer, S. S. Merchant, X. Zhang, S. E. Jacobsen, M. Pellegrini, *Nature* **2010**, *466*, 388-392.

[10] V. G. Allfrey, R. Faulkner, A. E. Mirsky, *Proceedings of the National Academy of Sciences* **1964**, *51*, 786-794.

[11] A. J. Bannister, T. Kouzarides, *Cell Research* **2011**, *21*, 381-395.

[12] a) Y. Wang, J. Wysocka, J. Sayegh, Y.-H. Lee, J. R. Perlin, L. Leonelli, L. S. Sonbuchner, C. H. McDonald, R. G. Cook, Y. Dou, R. G. Roeder, S. Clarke, M. R. Stallcup, C. D. Allis, S. A. Coonrod, *Science* **2004**, *306*, 279-283; b) G. L. Cuthbert, S. Daujat, A. W. Snowden, H. Erdjument-Bromage, T. Hagiwara, M. Yamada, R. Schneider, P. D. Gregory, P. Tempst, A. J. Bannister, T. Kouzarides, *Cell* **2004**, *118*, 545-553.

[13] J. R. Whetstine, A. Nottke, F. Lan, M. Huarte, S. Smolikov, Z. Chen, E. Spooner, E. Li, G. Zhang, M. Colaiacovo, Y. Shi, *Cell* **2006**, *125*, 467-481.

[14] M. Oki, H. Aihara, T. Ito, *Sub-cellular biochemistry* **2007**, *41*, 319-336.

[15] a) K. Marushige, *Proceedings of the National Academy of Sciences* **1976**, *73*, 3937-3941; b) P. Filippakopoulos, S. Knapp, *Nature Reviews Drug Discovery* **2014**, *13*, 337-356.

[16] B. Barneda-Zahonero, M. Parra, *Molecular Oncology* **2012**, *6*, 579-589.

[17] a) K. Garber, *Nature Biotechnology* **2007**, *25*, 17-19; b) K. T. Thurn, S. Thomas, A. Moore, P. N. Munster, *Future oncology* **2011**, *7*, 263-283.

[18] J. E. Bolden, M. J. Peart, R. W. Johnstone, *Nature Reviews Drug Discovery* **2006**, *5*, 769-784.

[19] S. C. Hodawadekar, R. Marmorstein, *Oncogene* **2007**, *26*, 5528-5540.

[20] B. M. Dancy, P. A. Cole, *Chemical Reviews* **2015**, *115*, 2419-2452.

[21] Z. Arany, W. R. Sellers, D. M. Livingston, R. Eckner, *Cell* **1994**, *77*, 799-800.

[22] a) T. Kouzarides, *Cell* **2007**, *128*, 693-705; b) P. Filippakopoulos, S. Knapp, *FEBS Letters* **2012**, *586*, 2692-2704.

[23] a) J. Boyes, P. Byfield, Y. Nakatani, V. Ogryzko, *Nature* **1998**, *396*, 594-598; b) A. Imhof, X.-J. Yang, V. V. Ogryzko, Y. Nakatani, A. P. Wolffe, H. Ge, *Current Biology* **1997**, *7*, 689-692.

[24] D. C. Bedford, L. H. Kasper, T. Fukuyama, P. K. Brindle, *Epigenetics* **2010**, *5*, 9-15.

[25] a) G. A. Josling, S. A. Selvarajah, M. Petter, M. F. Duffy, *Genes* **2012**, *3*, 320; b) A. S. Neish, S. F. Anderson, B. P. Schlegel, W. Wei, J. D. Parvin, *Nucleic Acids Research* **1998**, *26*, 847-853.

[26] S. Mujtaba, Y. He, L. Zeng, S. Yan, O. Plotnikova, Sachchidanand, R. Sanchez, N. J. Zeleznik-Le, Z. e. Ronai, M.-M. Zhou, *Molecular Cell* **2004**, *13*, 251-263.

[27] a) L. Zeng, M.-M. Zhou, *FEBS Letters* **2002**, *513*, 124-128; b) P. Filippakopoulos, S. Picaud, M. Mangos, T. Keates, J.-P. Lambert, D. Barsyte-Lovejoy, I. Felletar, R. Volkmer, S. Müller, T. Pawson, A.-C. Gingras, Cheryl H. Arrowsmith, S. Knapp, *Cell* **2012**, *149*, 214-231.

[28] D. J. Owen, P. Ornaghi, J. C. Yang, N. Lowe, P. R. Evans, P. Ballario, D. Neuhaus, P. Filetici, A. A. Travers, *The EMBO Journal* **2000**, *19*, 6141-6149.

[29] a) M. Brand, A. M. Measures, B. G. Wilson, W. A. Cortopassi, R. Alexander, M. Höss, D. S. Hewings, T. P. C. Rooney, R. S. Paton, S. J. Conway, *ACS Chemical Biology* **2015**, *10*, 22-39; b) D. S. Hewings, T. P. C. Rooney, L. E. Jennings, D. A. Hay, C. J. Schofield, P. E. Brennan, S. Knapp, S. J. Conway, *Journal of Medicinal Chemistry* **2012**, *55*, 9393-9413.

[30] M. Delvecchio, J. Gaucher, C. Aguilar-Gurrieri, E. Ortega, D. Panne, *Nature Structural Molecular Biology* **2013**, *20*, 1040-1046.

[31] W.-I. Chan, R. L. Hannah, M. A. Dawson, C. Pridans, D. Foster, A. Joshi, B. Göttgens, J. M. Van Deursen, B. J. P. Huntly, *Molecular and Cellular Biology* **2011**, *31*, 5046-5060.

[32] a) T. Katsumoto, N. Yoshida, I. Kitabayashi, *Cancer Science* **2008**, *99*, 1523-1527; b) Y. Zhang, N. Zeleznik-Le, N. Emmanuel, N. Jayathilaka, J. Chen, P. Strissel, R. Strick, L. Li, M. B. Neilly, T. Taki, Y. Hayashi, Y. Kaneko, B. Schlegelberger, J. D. Rowley, *Genes, Chromosomes and Cancer* **2004**, *41*, 257-265.

[33] a) R. H. Giles, D. J. M. Peters, M. H. Breuning, *Trends in Genetics* **1998**, *14*, 178-183; b) M. Chaffanet, L. Gressin, C. Preudhomme, V. Soenen-Cornu, D. Birnbaum, M.-J. Pébusque, *Genes, Chromosomes and Cancer* **2000**, *28*, 138-144.

[34] M. Muraoka, M. Konishi, R. Kikuchi-Yanoshita, K. Tanaka, N. Shitara, J. M. Chong, T. Iwama, M. Miyaki, *Oncogene* **1996**, *12*, 1565-1569.

[35] a) S. Timmermann, H. Lehrmann, A. Polesskaya, A. Harel-Bellan, *Cellular and Molecular Life Sciences* **2001**, *58*, 728-736; b) N. G. Iyer, H. Ozdag, C. Caldas, *Oncogene* **2004**, *23*, 4225-4231.

[36] a) C. H. Arrowsmith, J. E. Audia, C. Austin, J. Baell, J. Bennett, J. Blagg, C. Bountra, P. E. Brennan, P. J. Brown, M. E. Bunnage, C. Buser-Doepner, R. M. Campbell, A. J. Carter, P. Cohen, R. A. Copeland, B. Cravatt, J. L. Dahlin, D. Dhanak, A. M. Edwards, M. Frederiksen, S. V. Frye, N. Gray, C. E. Grimshaw, D. Hepworth, T. Howe, K. V. M. Huber, J. Jin, S. Knapp, J. D. Kotz, R. G. Kruger, D. Lowe, M. M. Mader, B. Marsden, A. Mueller-Fahrnow, S. Muller, R. C. O'Hagan, J. P. Overington, D. R. Owen, S. H. Rosenberg, R. Ross, B. Roth, M. Schapira, S. L. Schreiber, B. Shoichet, M. Sundstrom, G. Superti-Furga, J. Taunton, L. Toledo-Sherman, C. Walpole, M. A. Walters, T. M. Willson, P. Workman, R. N. Young, W. J. Zuercher, *Nature Chemical Biology* **2015**, *11*, 536-541; b) S. Knapp, P. Arruda, J. Blagg, S. Burley, D. H. Drewry, A. Edwards, D. Fabbro, P. Gillespie, N. S. Gray, B. Kuster, K. E. Lackey, P. Mazzafera, N. C. O. Tomkinson, T. M. Willson, P. Workman, W. J. Zuercher, *Nature Chemical Biology* **2013**, *9*, 3-6.

[37] S. Knapp, *DPhG Annual Meeting 2015 Conference Book* **2015**, 59.

[38] a) M. V. Gammons, O. Fedorov, D. Ivison, C. Du, T. Clark, C. Hopkins, M. Hagiwara, A. D. Dick, R. Cox, S. J. Harper, J. C. Hancox, S. Knapp, D. O. Bates, *Investigative Ophthalmology & Visual Science* **2013**, *54*, 6052-6062; b) O. Fedorov, K. Huber, A. Eisenreich, P. Filippakopoulos, O. King, A. N. Bullock, D. Szklarczyk, L. J. Jensen, D. Fabbro, J. Trappe, U. Rauch, F. Bracher, S. Knapp, *Chemistry & Biology* **2011**, *18*, 67-76.

[39] L. R. Vidler, N. Brown, S. Knapp, S. Hoelder, *Journal of Medicinal Chemistry* **2012**, *55*, 7346-7359.

[40] S. Muller, S. Knapp, *Medicinal Chemical Communications* **2014**, *5*, 288-296.

[41] A. Huston, C. H. Arrowsmith, S. Knapp, M. Schapira, *Nature Chemical Biology* **2015**, *11*, 542-545.

[42] P. Filippakopoulos, S. Picaud, O. Fedorov, M. Keller, M. Wrobel, O. Morgenstern, F. Bracher, S. Knapp, *Bioorganic & Medicinal Chemistry* **2012**, *20*, 1878-1886.

[43] SGC, *http://www.thesgc.org/chemical-probes/I-CBP112/teaser* **2015**.

[44] T. P. C. Rooney, P. Filippakopoulos, O. Fedorov, S. Picaud, W. A. Cortopassi, D. A. Hay, S. Martin, A. Tumber, C. M. Rogers, M. Philpott, M. Wang, A. L. Thompson, T. D. Heightman, D. C. Pryde, A. Cook, R. S. Paton, S. Müller, S. Knapp, P. E. Brennan, S. J. Conway, *Angewandte Chemie International Edition* **2014**, *53*, 6126-6130.

[45] A. Unzue, M. Xu, J. Dong, L. Wiedmer, D. Spiliotopoulos, A. Caflisch, C. Nevado, *Journal of Medicinal Chemistry* **2015**.

[46] D. A. Hay, O. Fedorov, S. Martin, D. C. Singleton, C. Tallant, C. Wells, S. Picaud, M. Philpott, O. P. Monteiro, C. M. Rogers, S. J. Conway, T. P. C. Rooney, A. Tumber, C. Yapp, P. Filippakopoulos,

M. E. Bunnage, S. Müller, S. Knapp, C. J. Schofield, P. E. Brennan, *Journal of the American Chemical Society* **2014**, *136*, 9308-9319.

[47] A. Hammitzsch, C. Tallant, O. Fedorov, A. O'Mahony, P. E. Brennan, D. A. Hay, F. O. Martinez, M. H. Al-Mossawi, J. de Wit, M. Vecellio, C. Wells, P. Wordsworth, S. Müller, S. Knapp, P. Bowness, *Proceedings of the National Academy of Sciences* **2015**, *112*, 10768-10773.

[48] S. Picaud, O. Fedorov, A. Thanasopoulou, K. Leonards, K. Jones, J. Meier, H. Olzscha, O. Monteiro, S. Martin, M. Philpott, A. Tumber, P. Filippakopoulos, C. Yapp, C. Wells, K. H. Che, A. Bannister, S. Robson, U. Kumar, N. Parr, K. Lee, D. Lugo, P. Jeffrey, S. Taylor, M. L. Vecellio, C. Bountra, P. E. Brennan, A. O'Mahony, S. Velichko, S. Müller, D. Hay, D. L. Daniels, M. Urh, N. B. La Thangue, T. Kouzarides, R. Prinjha, J. Schwaller, S. Knapp, *Cancer Research* **2015**, *75*, 5106-5119.

[49] L. R. Swett, R. G. Stein, E. T. Kimura, *Journal of Medicinal Chemistry* **1972**, *15*, 42-45.

[50] J. K. Mishra, K. Samanta, M. Jain, M. Dikshit, G. Panda, *Bioorganic & Medicinal Chemistry Letters* **2010**, *20*, 244-247.

[51] a) K. Samanta, B. Chakravarti, J. K. Mishra, S. K. D. Dwivedi, L. V. Nayak, P. Choudhry, H. K. Bid, R. Konwar, N. Chattopadhyay, G. Panda, *Bioorganic & Medicinal Chemistry Letters* **2010**, *20*, 283-287; b) N. B. Aay, O. J.; Bussenius, J.; Curtis, J. K.; Defina, S. C.; Dubenko, L.; Harris, J. R.; Jackson-Ugueto, E. E.; Kim, A. I.; Manalo, J. L.; Pack, M.; Rice, K. D.; Tsang, T. H.; Wang, L., *WO Patent 2010/138490 A1* **2010**; c) S. Naganathan, D. L. Andersen, N. G. Andersen, S. Lau, A. Lohse, M. D. Sørensen, *Organic Process Research & Development* **2015**, *19*, 721-734.

[52] B. M. Fox, H. P. Beck, P. M. Roveto, F. Kayser, Q. Cheng, H. Dou, T. Williamson, J. Treanor, H. Liu, L. Jin, G. Xu, J. Ma, S. Wang, S. H. Olson, *Journal of Medicinal Chemistry* **2015**, *58*, 5256-5273.

[53] S. L. Ramurthy, X.; Subramanian, S.; Rico, A. C.; Wang, X. M.; Jain R.; Murray, J. M.; Basham, S. E.; Warne, R. L.; Shu, W.; Zhou, Y.; Dove, J.; Aikawa, M.; Amiri, P., *WO Patent 2008079988 A2* **2008**.

[54] a) L. Katz, L. S. Karger, W. Schroeder, M. S. Cohen, *The Journal of Organic Chemistry* **1953**, *18*, 1380-1402; b) H. Sugiyama, M. Yoshida, K. Mori, T. Kawamoto, S. Sogabe, T. Takagi, H. Oki, T. Tanaka, H. Kimura, Y. Ikeura, *Chemical and Pharmaceutical Bulletin* **2007**, *55*, 613-624.

[55] a) N. O. Kaneko, T.; Sakai, T.; Oota, H., *US Patent 5416066* **1995**; b) C. Liang, L. Wang, H. Y. Yun, X. Zheng, WO Patent 20130020993, **2013**.

[56] a) J. A. Ellman, *Accounts of Chemical Research* **1996**, *29*, 132-143; b) N. Kaur, D. Kishore, *Synthetic Communications* **2014**, *44*, 1375-1413.

[57] N. Sakai, A. Watanabe, R. Ikeda, Y. Nakaike, T. Konakahara, *Tetrahedron* **2010**, *66*, 8837-8845.

[58] J.-Y. Wang, X.-F. Guo, D.-X. Wang, Z.-T. Huang, M.-X. Wang, *ChemInform* **2008**, *39*, 1979–1982.

[59] T. VoCam-Van, M. U. Luescher, J. W. Bode, *Nature Chemistry* **2014**, *6*, 310-314.

[60] M. Rigamonti, G. Prestat, G. Broggini, G. Poli, *Journal of Organometallic Chemistry* **2014**, *760*, 149-155.

[61] V. R. Jumde, E. Cini, A. Porcheddu, M. Taddei, *European Journal of Organic Chemistry* **2015**, *2015*, 1068-1074.

[62] a) R. L. Clark, K. C. Carter, A. B. Mullen, G. D. Coxon, G. Owusu-Dapaah, E. McFarlane, M. D. Duong Thi, M. H. Grant, J. N. A. Tettey, S. P. Mackay, *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, 624-627; b) O. Lack, R. E. Martin, *Tetrahedron Letters* **2005**, *46*, 8207-8211; c) G. L. Grunewald, V. H. Dahanukar, P. Ching, K. R. Criscione, *Journal of Medicinal Chemistry* **1996**, *39*, 3539-3546; d) C. Z. Ding, R. Batorsky, R. Bhide, H. J. Chao, Y. Cho, S. Chong, J. Gullo-Brown, P. Guo, S. H. Kim, F. Lee, K. Leftheris, A. Miller, T. Mitt, M. Patel, B. A. Penhallow, C. Ricca, W. C. Rose, R. Schmidt, W. A. Slusarchyk, G. Vite, N. Yan, V. Manne, J. T. Hunt, *Journal of Medicinal Chemistry* **1999**, *42*, 5241-5253; e) M. J. Kukla, H. J. Breslin, C. J. Diamond, P. P. Grous, C. Y. Ho, M. Miranda, J. D. Rodgers, R. G. Sherrill, E. De Clercq, R. Pauwels, K. Andries, L. J. Moens, M. A. C. Janssen, P. A. J. Janssen, *ChemInform* **1992**, *23*.

[63] P. E. Maligres, M. M. See, D. Askin, P. J. Reider, *Tetrahedron Letters* **1997**, *38*, 5253-5256.

[64] A. K. Singh, L. D. S. Yadav, *Synthesis* **2012**, *44*, 591-599.

[65] a) T. Kan, T. Fukuyama, *Chemical Communications* **2004**, 353-359; b) T. Fukuyama, C.-K. Jow, M. Cheung, *Tetrahedron Letters* **1995**, *36*, 6373-6374.
[66] O. Mitsunobu, *Synthesis* **1981**, *1981*, 1-28.
[67] Y. Tajima, M. Kobayashi, K. Noguchi, K. Tanaka, *European Journal of Organic Chemistry* **2013**, *2013*, 5266-5271.
[68] M. Righi, A. Bedini, G. Piersanti, F. Romagnoli, G. Spadoni, *The Journal of Organic Chemistry* **2011**, *76*, 704-707.
[69] a) J. A. Ruell, E. De Clercq, C. Pannecouque, M. Witvrouw, T. L. Stup, J. A. Turpin, R. W. Buckheit, M. Cushman, *The Journal of Organic Chemistry* **1999**, *64*, 5858-5866; b) Y. Xiang, B. Hirth, G. Asmussen, H.-P. Biemann, K. A. Bishop, A. Good, M. Fitzgerald, T. Gladysheva, A. Jain, K. Jancsics, J. Liu, M. Metz, A. Papoulis, R. Skerlj, J. D. Stepp, R. R. Wei, *Bioorganic & Medicinal Chemistry Letters* **2011**, *21*, 3050-3056.
[70] F. H. Niesen, H. Berglund, M. Vedadi, *Nature Protocols* **2007**, *2*, 2212-2221.
[71] M.-C. Lo, A. Aulabaugh, G. Jin, R. Cowling, J. Bard, M. Malamas, G. Ellestad, *Analytical Biochemistry* **2004**, *332*, 153-159.
[72] R. M. Eglen, T. Reisine, P. Roby, N. Rouleau, C. Illy, R. Bossé, M. Bielefeld, *Current Chemical Genomics* **2008**, *1*, 2-10.
[73] SGC, *http://www.thesgc.org/screening-platforms* **2015**.
[74] G. A. Holdgate, W. H. J. Ward, *Drug Discovery Today* **2005**, *10*, 1543-1550.
[75] B. L. Sprague, J. G. McNally, *Trends in Cell Biology* **2005**, *15*, 84-91.
[76] M. Philpott, C. M. Rogers, C. Yapp, C. Wells, J.-P. Lambert, C. Strain-Damerell, N. A. Burgess-Brown, A.-C. Gingras, S. Knapp, S. Müller, *Epigenetics & Chromatin* **2014**, *7*, 14-14.
[77] A. M. Smyth, L. Yang, K. J. Martin, C. Hamilton, W. Lu, M. A. Cousin, C. Rickman, R. R. Duncan, *Journal of Biological Chemistry* **2013**, *288*, 5102-5113.
[78] a) M. Philpott, J. Yang, T. Tumber, O. Fedorov, S. Uttarkar, P. Filippakopoulos, S. Picaud, T. Keates, I. Felletar, A. Ciulli, S. Knapp, T. D. Heightman, *Molecular BioSystems* **2011**, *7*, 2899-2908; b) C. A. Day, L. J. Kraft, M. Kang, A. K. Kenworthy, *Current protocols in cytometry* **2012**.
[79] T. Mosmann, *Journal of Immunological Methods* **1983**, *65*, 55-63.
[80] a) J. V. Hatton, R. E. Richards, *Molecular Physics* **1960**, *3*, 253-263; b) H. Möhrle, H. Ehrhardt, R. Kilian, P. Spillmann, *Archiv der Pharmazie* **1970**, *303*, 531-544.
[81] W. Walter, H.-W. Meyer, A. Lehmann, *Justus Liebigs Annalen der Chemie* **1974**, *1974*, 765-775.
[82] T. H. Siddall, W. E. Stewart, F. D. Knight, *The Journal of Physical Chemistry* **1970**, *74*, 3580-3583.
[83] M. Geffe, L. Andernach, O. Trapp, T. Opatz, *Beilstein Journal of Organic Chemistry* **2014**, *10*, 701-706.
[84] M. Fardis, H. Jin, X. Chen, M. Tsiang, J. Chen, C. Kim, M. Wright, HIV-1 Integrase, **2011**, pp. 239-254.
[85] a) A. Hammitzsch, C. Tallant, O. Fedorov, A. O'Mahony, P. E. Brennan, D. A. Hay, F. O. Martinez, M. H. Al-Mossawi, J. de Wit, M. Vecellio, C. Wells, P. Wordsworth, S. Muller, S. Knapp, P. Bowness, *Proceedings of the National Academy of Sciences of the United States of America* **2015**, *112*, 10768-10773; b) D. A. Hay, O. Fedorov, S. Martin, D. C. Singleton, C. Tallant, C. Wells, S. Picaud, M. Philpott, O. P. Monteiro, C. M. Rogers, S. J. Conway, T. P. Rooney, A. Tumber, C. Yapp, P. Filippakopoulos, M. E. Bunnage, S. Muller, S. Knapp, C. J. Schofield, P. E. Brennan, *J Am Chem Soc* **2014**, *136*, 9308-9319.
[86] M. Kosugi, T. Sumiya, Y. Obara, M. Suzuki, H. Sano, T. Migita, *Bulletin of the Chemical Society of Japan* **1987**, *60*, 767-768.
[87] F. Bracher, D. Hildebrand, *Liebigs Annalen der Chemie* **1993**, *1993*, 837-839.
[88] R. M. Dodson, L. C. King, *Journal of the American Chemical Society* **1945**, *67*, 2242-2243.
[89] a) B. K. Min, H.-J. Lee, Y. S. Choi, J. Park*, C.-J. Yoon, J.-A. Yu, *Journal of Molecular Structure* **1998**, *471*, 283-288; b) H.-J. Lee, Y.-S. Choi, K.-B. Lee, J. Park, C.-J. Yoon, *The Journal of Physical Chemistry A* **2002**, *106*, 7010-7017.
[90] a) S. Purser, P. R. Moore, S. Swallow, V. Gouverneur, *Chemical Society Reviews* **2008**, *37*, 320-330; b) K. Müller, C. Faeh, F. Diederich, *Science* **2007**, *317*, 1881-1886.

[91] E. P. Gillis, K. J. Eastman, M. D. Hill, D. J. Donnelly, N. A. Meanwell, *Journal of medicinal chemistry* **2015**, *58*, 8315-8359.

[92] J. Wrobel, A. Dietrich, B. J. Gorham, K. Sestanj, *The Journal of Organic Chemistry* **1990**, *55*, 2694-2702.

[93] J. Tucker, S. Sviridov, L. Brodsky, C. Burkhart, A. Purmal, K. Gurova, A. Gudkov, WO Patent 2010042445 A1, **2010**.

[94] K. C. K. Swamy, N. N. B. Kumar, E. Balaraman, K. V. P. P. Kumar, *Chemical Reviews* **2009**, *109*, 2551-2651.

[95] a) I. Sakamoto, H. Kaku, T. Tsunoda, *Chemical & pharmaceutical bulletin* **2003**, *51*, 474-476; b) T. Tsunoda, C. Nagino, M. Oguri, S. Itô, *Tetrahedron Letters* **1996**, *37*, 2459-2462.

[96] T. A. Popp, E. Uhl, D. N. Ong, S. Dittrich, F. Bracher, *Tetrahedron* **2016**.

[97] a) M. T. Hovey, C. T. Check, A. F. Sipher, K. A. Scheidt, *Angewandte Chemie International Edition* **2014**, *53*, 9603-9607; b) F. Jahani, M. Tajbakhsh, H. Golchoubian, S. Khaksar, *Tetrahedron Letters* **2011**, *52*, 1260-1264.

[98] S. D. Lepore, Y. He, *The Journal of Organic Chemistry* **2003**, *68*, 8261-8263.

[99] J. I. Andrés, J. M. Alonso, J. Fernández, L. Iturrino, P. Martínez, T. F. Meert, V. K. Sipido, *Bioorganic & Medicinal Chemistry Letters* **2002**, *12*, 3573-3577.

[100] a) J. P. Patel, A.-H. Li, H. Dong, V. L. Korlipara, M. J. Mulvihill, *Tetrahedron Letters* **2009**, *50*, 5975-5977; b) C. Z. Ding, J. T. Hunt, K. Leftheris, R. S. Bhide, *US Patent 6458783 B1* **2002**.

[101] a) E. L. Larghi, T. S. Kaufman, *Tetrahedron Letters* **1997**, *38*, 3159-3162; b) A. J. Birch, A. H. Jackson, P. V. R. Shannon, *Journal of the Chemical Society, Perkin Transactions 1* **1974**, 2185–2190.

[102] Y. Ida, A. Matsubara, T. Nemoto, M. Saito, S. Hirayama, H. Fujii, H. Nagase, *Bioorganic & Medicinal Chemistry* **2012**, *20*, 5810-5831.

[103] B.-C. Chen, J. E. Sundeen, P. Guo, M. S. Bednarz, R. Zhao, *Tetrahedron Letters* **2001**, *42*, 1245-1246.

[104] R. F. Borch, A. I. Hassid, *The Journal of Organic Chemistry* **1972**, *37*, 1673-1674.

[105] S. Picaud, O. Fedorov, A. Thanasopoulou, K. Leonards, K. Jones, J. Meier, H. Olzscha, O. Monteiro, S. Martin, M. Philpott, A. Tumber, P. Filippakopoulos, C. Yapp, C. Wells, K. H. Che, A. Bannister, S. Robson, U. Kumar, N. Parr, K. Lee, D. Lugo, P. Jeffrey, S. Taylor, M. L. Vecellio, C. Bountra, P. E. Brennan, A. O'Mahony, S. Velichko, S. Muller, D. Hay, D. L. Daniels, M. Urh, N. B. La Thangue, T. Kouzarides, R. Prinjha, J. Schwaller, S. Knapp, *Cancer Res* **2015**, *75*, 5106-5119.

[106] S. Herman, US Patent 2465489 A, **1949**.

[107] a) H. T. Clarke, H. B. Gillespie, S. Z. Weisshaus, *Journal of the American Chemical Society* **1933**, *55*, 4571-4587; b) W. Eschweiler, *Berichte der deutschen chemischen Gesellschaft* **1905**, *38*, 880-882.

[108] B. L. Sondengam, J. H. Hémo, G. Charles, *Tetrahedron Letters* **1973**, *14*, 261-263.

[109] S. Kim, C. H. Oh, J. S. Ko, K. H. Ahn, Y. J. Kim, *The Journal of Organic Chemistry* **1985**, *50*, 1927-1932.

[110] J. L. Castro, R. Baker, A. R. Guiblin, S. C. Hobbs, M. R. Jenkins, M. G. N. Russell, M. S. Beer, J. A. Stanton, K. Scholey, *Journal of Medicinal Chemistry* **1994**, *37*, 3023-3032.

[111] J. K. Horner, W. A. Skinner, *Canadian Journal of Chemistry* **1966**, *44*, 315-319.

[112] S. Bhattacharyya, *Synthetic Communications* **1995**, *25*, 2061-2069.

[113] X. Jiang, A. Tiwari, M. Thompson, Z. Chen, T. P. Cleary, T. B. K. Lee, *Organic Process Research & Development* **2001**, *5*, 604-608.

[114] K. Honarnejad, A. Daschner, A. P. Gehring, A. Szybinska, A. Giese, J. Kuznicki, F. Bracher, J. Herms, *Translational Psychiatry* **2014**, *4*, e489.

[115] W.-C. Shieh, S. Dell, A. Bach, O. Repič, T. J. Blacklock, *The Journal of Organic Chemistry* **2003**, *68*, 1954-1957.

[116] M. Righi, F. Topi, S. Bartolucci, A. Bedini, G. Piersanti, G. Spadoni, *The Journal of Organic Chemistry* **2012**, *77*, 6351-6357.

[117] a) D. A. Pearson, M. Blanchette, M. L. Baker, C. A. Guindon, *Tetrahedron Letters* **1989**, *30*, 2739-2742; b) A. E. Lanzilotti, R. Littell, W. J. Fanshawe, T. C. McKenzie, F. M. Lovell, *The Journal of Organic Chemistry* **1979**, *44*, 4809-4813.
[118] M. Z. Chen, G. C. Micalizio, *Organic Letters* **2009**, *11*, 4982-4985.
[119] K. V. Butler, J. Kalin, C. Brochier, G. Vistoli, B. Langley, A. P. Kozikowski, *Journal of the American Chemical Society* **2010**, *132*, 10842-10846.
[120] A. Mehta, R. Jaouhari, T. J. Benson, K. T. Douglas, *Tetrahedron Letters* **1992**, *33*, 5441-5444.
[121] V. Diep, J. J. Dannenberg, R. W. Franck, *The Journal of Organic Chemistry* **2003**, *68*, 7907-7910.
[122] F. Bracher, D. Hildebrand, *Liebigs Annalen der Chemie* **1992**, *1992*, 1315-1319.
[123] F. Bracher, *Archiv der Pharmazie* **1992**, *325*, 645-648.
[124] T. A. Popp, F. Bracher, *Synthesis* **2015**, *47*, 3333-3338.
[125] P. Filippakopoulos, J. Qi, S. Picaud, Y. Shen, W. B. Smith, O. Fedorov, E. M. Morse, T. Keates, T. T. Hickman, I. Felletar, M. Philpott, S. Munro, M. R. McKeown, Y. Wang, A. L. Christie, N. West, M. J. Cameron, B. Schwartz, T. D. Heightman, N. La Thangue, C. A. French, O. Wiest, A. L. Kung, S. Knapp, J. E. Bradner, *Nature* **2010**, *468*, 1067-1073.
[126] M. Philpott, J. Yang, T. Tumber, O. Fedorov, S. Uttarkar, P. Filippakopoulos, S. Picaud, T. Keates, I. Felletar, A. Ciulli, S. Knapp, T. D. Heightman, *Mol Biosyst* **2011**, *7*, 2899-2908.
[127] S. L. Ramurthy, X.; Subramanian, S.; Rico, A. C.; Wang, X. M.; Jain R.; Murray, J. M.; Basham, S. E.; Warne, R. L.; Shu, W.; Zhou, Y.; Dove, J.; Aikawa, M.; Amiri, P., **2008**.
[128] E. M. Briggs, G. W. Brown, J. Jiricny, M. F. Meidine, *Synthesis* **1980**, *1980*, 295-296.
[129] D. Seymour, K. B. Wolfstirn, *Journal of the American Chemical Society* **1948**, *70*, 1177-1179.
[130] TCI, *http://www.tcichemicals.com/eshop/en/gb/commodity/D0760* **2016**.
[131] H. Nishi, T. Furukawa, K. Kitahara, S. Tokita, *NIPPON KAGAKU KAISHI* **1985**, *1985*, 255-259.
[132] M. Uchiyama, Y. Matsumoto, S. Nakamura, T. Ohwada, N. Kobayashi, N. Yamashita, A. Matsumiya, T. Sakamoto, *Journal of the American Chemical Society* **2004**, *126*, 8755-8759.