# Highly potent macrocyclic BACE-1 inhibitors incorporating a hydroxyethylamine core: Design, synthesis and X-ray crystal structures of enzyme inhibitor complexes

Veronica Sandgren, Tatiana Agback, Per-Ola Johansson, Jimmy Lindberg, Ingemar Kvarnström, Bertil Samuelsson, Oscar Belda and Anders Dahlgren

### **Linköping University Post Print**

N.B.: When citing this work, cite the original article.

### **Original Publication:**

Veronica Sandgren, Tatiana Agback, Per-Ola Johansson, Jimmy Lindberg, Ingemar Kvarnström, Bertil Samuelsson, Oscar Belda and Anders Dahlgren, Highly potent macrocyclic BACE-1 inhibitors incorporating a hydroxyethylamine core: Design, synthesis and X-ray crystal structures of enzyme inhibitor complexes, 2012, Bioorganic & Medicinal Chemistry, (29), 14, 4377-4389.

http://dx.doi.org/10.1016/j.bmc.2012.05.039

Copyright: Elsevier

http://www.elsevier.com/

Postprint available at: Linköping University Electronic Press

http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-76172

Highly potent macrocyclic BACE-1 inhibitors incorporating a hydroxyethylamine core: Design, synthesis and X-ray crystal structures of enzyme inhibitor complexes

Veronica Sandgren,<sup>a</sup> Tatiana Agback,<sup>b</sup> Per-Ola Johansson,<sup>b</sup> Jimmy Lindberg,<sup>b</sup> Ingemar Kvarnström,<sup>a</sup> Bertil Samuelsson,<sup>b</sup> Oscar Belda,<sup>b,\*</sup> and Anders Dahlgren,<sup>a,\*</sup>

<sup>a</sup>Department of Chemistry, Linköping University, S-581 83 Linköping, Sweden <sup>b</sup>Medivir AB, Lunastigen 7, S-141 44 Huddinge, Sweden

\*Corresponding authors:

Phone +46 13 281000, fax: +46 13 281399; phone: +46 8 54683100, fax +46 8 54683199.

E-mail: andda@ifm.liu.se, oscar.belda@medivir.com

#### Abstract

A series of P1-P3 linked macrocyclic BACE-1 inhibitors containing a hydroxyethylamine (HEA) isostere scaffold has been synthesized. All inhibitors comprise a toluene or *N*-phenylmethanesulfonamide P2 moiety. Excellent BACE-1 potencies, both in enzymatic and cell-based assays, were observed in this series of target compounds, with the best candidates displaying cell-based IC<sub>50</sub> values in the low nanomolar range. As an attempt to improve potency, a phenyl substituent aiming at the S3 subpocket was introduced in the macrocyclic ring. X-ray analyses were performed on selected compounds, and enzyme-inhibitor interactions are discussed.

### Keywords

Alzheimer's disease; BACE-1 inhibition; macrocycles; hydroxyethylamine (HEA) isostere

#### 1. Introduction

Although it has been more than 100 years since the symptoms of Alzheimer's disease (AD) were first described, the underlying pathophysiological mechanisms behind the disease remain obscure. However, it is believed that the accumulation of the so called

amyloid- $\beta$  peptides (A $\beta$ ), leading to plaques and eventually neurofibrillary tangles in the brain, has a crucial role in the pathology of AD. The mechanism by which these amyloid- $\beta$  peptides are formed involves the enzymes  $\beta$ -secretase (BACE-1) followed by  $\gamma$ -secretase, which sequentially cleave the amyloid precursor protein (APP), generating the A $\beta$  peptides. Furthermore, it is known that BACE-1 catalyses the rate-limiting step in the sequence. This, together with the knowledge that BACE-1 knockout mice are viable and fertile, makes BACE-1 a promising therapeutic target.  $^{3,4}$ 

The development of low molecular weight, brain penetrating BACE-1 inhibitors has proven to be quite challenging.<sup>5,6</sup> However, it has been shown that the pharmacokinetics of standard linear inhibitors can be significantly improved by cyclization,<sup>7</sup> and this approach has indeed been incorporated in the search for promising drug candidates in the field of AD research. Different methodologies to obtain these macrocycles have been used, such as metathesis,<sup>8-12</sup> reductive amination,<sup>13</sup> peptide coupling<sup>9,14</sup> and macrolactonization.<sup>15</sup>

In this study, we describe the synthesis and biological evaluation of a series of macrocyclic BACE-1 inhibitors containing a hydroxyethylamine (HEA) isostere. The P1 and P3 moieties were connected by ring closing metathesis (RCM) (Figure 1). The P2 part of the target molecules incorporated a benzene ring with a sulfonamide functionality or a toluene core (see Table 1). These P2 moieties have emerged as promising building blocks in earlier reported studies. <sup>8,9,16</sup> Also, a phenyl substituent was introduced in the macrocyclic ring in an attempt to reach into the S3 subpocket (S3sp) of the enzyme and thus improve the activity even further.

#### 2. Results and discussion

#### 2.1. Chemistry

The target compounds **17** and **21-33** were prepared according to Schemes 1-4 (see also Table 1). Synthesis of key building block **3**, which was used in synthesis of all the target compounds, is outlined in Scheme 1. The alcohol **1**, synthesized from commercially available (-)-diethyl D-tartrate in four steps according to literature procedures, <sup>17-19</sup> was allylated with allyl bromide and Ag<sub>2</sub>O in toluene to give compound **2** in 78% yield. This reaction performed with NaH resulted in low yields. Reduction of the

S2
$$R^{2}$$

$$R^{3}$$

$$R^{3}$$

$$R^{3}$$

$$R^{3}$$

$$R^{4}$$

$$R^{4}$$

$$R^{4}$$

$$R^{5}$$

$$R^{4}$$

$$R^{5}$$

$$R^{4}$$

$$R^{5}$$

$$R^{4}$$

$$R^{5}$$

Figure 1. General structures of the macrocyclic target compounds (see Table 1).

azide group in compound **2** was achieved using 1,3-propanedithiol and TEA in methanol to provide the corresponding amine **3** in 73% yield.

$$N_3$$
 $N_3$ 
 $N_3$ 

**Scheme 1.** Synthesis of compound **3**, employed as a building block for synthesis of all the target compounds. Reagents and conditions: (i) allyl bromide, Ag<sub>2</sub>O, toluene, rt, 26 h, 78%; (ii) 1,3-propanedithiol, TEA, MeOH, rt, 22 h, 73%.

Synthesis of the building blocks (R)-9, (S)-9, (R)-10, and (S)-10 is described in Scheme 2. Commercially available (R)-(+)-styrene oxide was treated with lithium acetylide ethylenediamine complex to give compound (S)-4 in 95% yield. Mitsunobu conditions utilizing DPPA generated the azide (R)-5 with inversion of configuration in 97% yield. The azide group was subsequently converted to the corresponding amine (R)-6, using 1,3-propanedithiol and TEA in methanol, in 92% yield. The enantiomer (S)-6 was synthesized in the same manner from (S)-(-)-styrene oxide. The primary amines (R)-6 and (S)-6 were thereafter coupled with either commercially available 3-methoxycarbonyl-5-methylbenzoic acid or compound 11<sup>20</sup> (see Scheme 3) using HCTU and DIPEA in DMF to furnish the amides (R)-7, (R)-8, (S)-7, and (S)-8 in 73-89% yield.

Finally, alkyne reduction using Lindlar's catalyst afforded the four desired compounds (R)-9, (R)-10, (S)-9, and (S)-10 in 84-99% yield.

**Scheme 2.** Synthesis of building blocks **9-10**. Reagents and conditions: (i) lithium acetylide ethylenediamine complex, DMSO (dry), Ar, rt, 25 h, 95% and quantitative yield, respectively; (ii) PPh<sub>3</sub>, DIAD, DPPA, THF, -12 °C to rt, 25 h, 97% and 76%, respectively; (iii) 1,3-propanedithiol, TEA, MeOH, rt, 47 h, 92% and 87%, respectively; (iv) 3-methoxycarbonyl-5-methylbenzoic acid or compound **11**, HCTU, DIPEA, DMF, rt, 4 h, 73-89%; (v) Lindlar's catalyst, quinoline, MeOH, H<sub>2</sub>, rt, 1.5 h, 84-99%.

Target compound 17 was prepared as described in Scheme 3. Compounds 21-23 and 27-33 (Table 1) were synthesized using the same synthetic route and with similar yields (benzylamine was used instead of 3-isopropylbenzylamine in the final step for compounds 21 and 30, and 3-tert-butylbenzylamine was used for compound 22). Compound 12a was prepared in 83% yield from compound 11 in a peptide coupling step using 3-butenylamine hydrochloride, EDC, HOBt, and TEA. The ester functionality in 12a was hydrolyzed using aqueous LiOH in H<sub>2</sub>O/dioxane and the corresponding carboxylic acid was coupled with compound 3 using HATU and DIPEA in DMF to afford compound 13 in 90% yield over two steps. The acetal in 13 was then hydrolyzed (AcOH/H<sub>2</sub>O, 60 °C), resulting in the diol 14 (quant.). The subsequent ring closing metathesis step was achieved utilizing Hoveyda-Grubbs catalyst 2nd generation in refluxing DCM to give the desired 15-membered macrocycle 15 in 98% yield. The cyclization resulted in exclusive formation of the *trans* isomer, as confirmed by <sup>1</sup>H-NMR

analysis. Attempts to cyclisize compound 13 were unsuccessful, tentatively because of the more rigid structure found in 13. The primary hydroxyl group in 15 was selectively tosylated with *p*-TsCl, Bu<sub>2</sub>SnO, and TEA in dry DCM to generate compound 16 (64%). Finally, the tosylate 16 was reacted with 3-isopropylbenzylamine in refluxing ethanol, furnishing target compound 17 in 37% yield. The amide nitrogen in 12a was methylated using NaH and MeI in DMF to give compound 12b in 100% yield. This building block was used to synthesize target compound 27, which proved to be a separable mixture of *cis*-27 (regio chemistry at the macrocyclic double bond position) and trace amounts of a related compound that tentatively could be identified by NMR analysis as the *trans* isomer. Noteworthy, the methylated amide functionality in 27 seems to have changed the preferred regio chemistry in the macrocycle from *trans* to *cis*.

The synthesis of compounds **19** and **20** is outlined in Scheme 4. Compound **18** was synthesized in the same manner as compound **15** with the exception that 4-pentenylamine hydrochloride<sup>21</sup> was used instead of 3-butenylamine hydrochloride in the peptide coupling step (see Scheme 3). Hydrogenation of the double bond in compound **15** and **18** was achieved in the presence of Pd-C in methanol to give compounds **19** and **20** in 95% and 78% yield, respectively. To obtain the three saturated macrocyclic compounds **24-26**, the same synthetic route was used as for compound **17** (see Scheme 3) with the exception that benzylamine was used instead of 3-isopropylbenzylamine in the final step for compound **24**.

**Scheme 3.** Synthesis of target molecule **17**. Reagents and conditions: (i) 3-butenylamine hydrochloride, TEA, HOBt, EDC, DMF, 0 °C to rt, 19 h, 83%; (ii) NaH, MeI, DMF, rt, 23 h, 100%; (iii) a) LiOH, H<sub>2</sub>O/dioxane 1:2, 70 min, b) compound **3**, DIPEA, HATU, DMF, rt, 16 h, 90% over two steps; (iv) AcOH/H<sub>2</sub>O, 60 °C, 1 h 45 min, 100%; (v) Hoveyda-Grubbs catalyst 2nd generation, DCM (dry), N<sub>2</sub>, reflux, 20 h, 98%; (vi) *p*-TsCl, Bu<sub>2</sub>SnO, TEA, DCM (dry), rt, 23 h, 64%; (vii) 3-isopropylbenzylamine, EtOH 99.5%, reflux, 24 h, 37%.

**Scheme 4.** Synthesis of the saturated compounds **19** and **20** used to produce target compounds **24-26**. Reagents and conditions: (i) Pd-C 10%, H<sub>2</sub>, MeOH, rt, 3 h, 95% and 78%, respectively.

### 2.2. Biological data and structure activity relationships

Table 1 contains all target compounds presented in this paper. All the macrocycles are 15-membered, except **23** and **26**, which are 16-membered. The inhibitors were all collected and tested as *trans* isomers, except for compound **27**, which was tested as the *cis* isomer. Encouraged by the biological results for the first synthesized target in this series, compound **21**, further investigations were of interest. In order to reach further into the S2′ pocket of BACE-1, targets **17** and **22**, containing alkyl substituents on the benzylamine moiety, were synthesized. Both compounds showed similar IC<sub>50</sub> values in the enzymatic assay (14 and 15 nM, respectively), that is they were 20-fold more active than **21**. Even more significant, **17** displayed an IC<sub>50</sub> of 2.3 nM in the cell-based assay, with a 240-fold improvement compared to **21**, whereas compound **22** showed a 120-fold increase in cellular activity.

The 16-membered macrocycle **23** was synthesized to investigate the way ring-size of this series affects biological activity. This resulted in a 13-fold loss of activity in the enzymatic assay and a 30-fold loss in the cell-based assay compared to inhibitor **17**. All attempts to synthesize the corresponding 14-membered macrocycle were unsuccessful.

The somewhat more flexible saturated macrocycles **24-26** were prepared in order to see if the double bond affected potency. Compound **24** lost almost all its activity in both assays, while compound **25** kept the enzymatic activity but had a 20-fold drop in the cell-based assay compared to **17**. Interestingly, the 16-membered macrocycle **26** gained activities compared to its unsaturated analogue **23** and showed nearly similar potencies as inhibitor **17**. This result may be explained by the fact that this 16-membered

macrocycle's enhanced flexibility allowed it to mimic the conformation of its 15-membered unsaturated counterpart.

Compound 27 showed similar enzyme activity as 17, which possibly could indicate that substitution of the P2-P3 amide is non-critical for BACE-1 activity. Furthermore, since inhibitor 27 was tested as the *cis* isomer and the rest of the unsaturated macrocyclic targets were identified as *trans* (see above), the configuration of this double bond tentatively seems not to be crucial for activity in this series of compounds.

A phenyl substituent was introduced in the macrocyclic ring in compounds **28** and **29** in an attempt to reach into the S3sp and further optimize potency. The (*S*)-isomer **28** displayed almost 30-fold less enzyme activity and 110-fold less cellular activity compared to **17**. However, the (*R*)-isomer **29** yielded a small improvement of the enzymatic activity compared to inhibitor **17** and a 15-fold improvement of the cell activity.

Although some of the sulfonamide-based BACE-1 inhibitors displayed very high potencies all compounds exhibited poor cell-based Caco-2 permeability (around or less than  $1x10^{-6}$  cm/s). Other BACE-1 inhibitors with a sulfonamide group in the P2 position have been shown to lack permeability due to for example P-gp efflux. Replacement of the sulfonamide group with a less polar methyl substituent (compounds 30-33) led merely to decreased potencies and no permeability improvement could be observed.

Inhibition of cathepsin D for selected compounds at an inhibitor concentration of 10 μM: **17** (23%), **22** (10%), **28** (46%), **31** (30%), and **33** (14%).

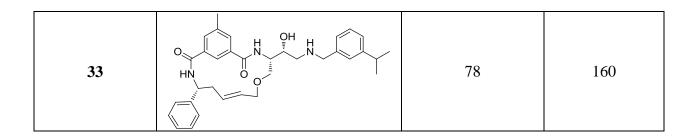
### 2.3. X-ray crystallography analysis

Compounds **28** and **33** were co-crystallized with BACE-1 (PDB ids: 4DPF and 4DPI, respectively). The general binding mode of the P1-P3 macrocyclic BACE-1 inhibitors was as anticipated for HEA-inhibitors (Figure 2). The protonated amino and hydroxyl groups form hydrogen bonds with the catalytic aspartates Asp32 and Asp228, respectively. The carbonyl of Gly34 also forms a hydrogen bond with the ionized amine. Moreover, the P2′ isopropyl substituted benzyl has a snug fit to the S2′

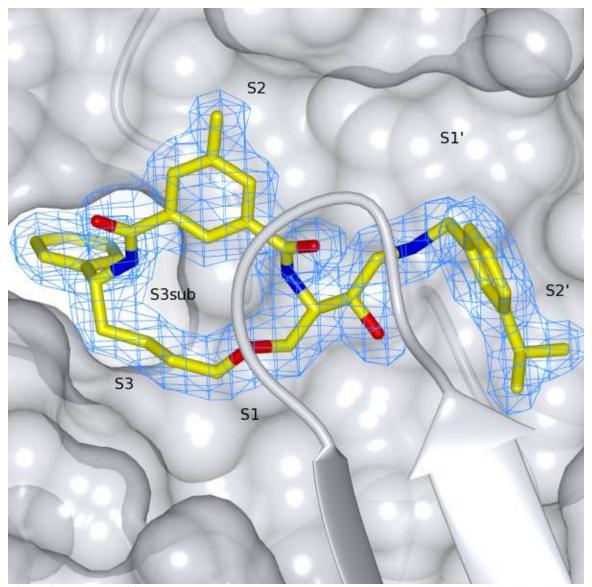
**Table 1.** BACE-1 inhibition data for compounds  $\bf 17$  and  $\bf 21\text{-}33$ .  $^a 16\text{-membered}$  macrocycle

Compound	Structure	IC <sub>50</sub> enzyme (nM)	IC <sub>50</sub> cell (nM)
21		270	560
17	O O O O O O O O O O O O O O O O O O O	14	2.3
22	O O O O O O O O O O O O O O O O O O O	15	4.6
23ª	O O O O O O O O O O O O O O O O O O O	180	73
24	O O O O O O O O O O O O O O O O O O O	5300	>10000
25	O O O O O O O O O O O O O O O O O O O	16	49

26ª	O O O O O O O O O O O O O O O O O O O	18	7.7
27		7.5	6.9
28	O O O O O O O O O O O O O O O O O O O	400	260
29	O O O O O O O O O O O O O O O O O O O	3.2	0.15
30	OHN OHN HX	>10000	>10000
31	OH HN OH HN N	500	220
32	OH HN OO O	4900	2300



pocket. The benzyl is sandwiched between Tyr198 and the peptide backbone of the flap, and the isopropyl extension is clamped in-between Ile126 (S2´) and Val69 (flap). This firm binding is reflected in the SAR, and underlines the importance of these additional hydrophobic interactions for compound activity (Table 1).

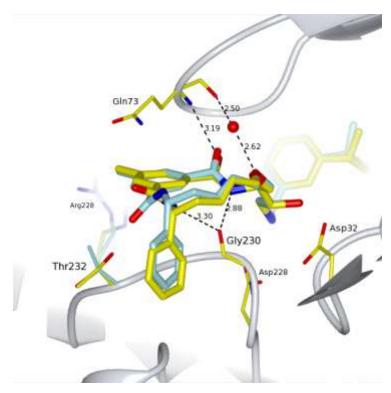


**Figure 2.** Compound **33** bound to the active site of BACE-1 (PDB id: 4DPI). The mode of binding of the compound is unambiguously shown in the electron density calculated at 1.9 Å resolution and contoured at  $1\sigma$ .

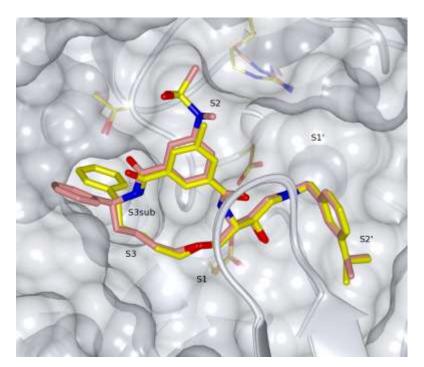
The co-crystal structure of **33** in complex with BACE-1 was solved to 1.9 Å resolution, and shows the complete P1-P3 macrocycle spanning the S1-S3 subsites (Figure 2). Similar to P1-P3 groups of linear HEA analogues, the P1-P3 macrocycle is coordinated between the flap and active site by a set of hydrogen bonds between the P1-P3-amides and the protein backbone (Gln73 NH and/or Gly230 CO). One additional hydrogen bond connecting the inhibitor and flap was observed between the ether-oxygen of the macrocycle and the backbone carbonyl of the flap residue, Gln73 bridged by structural water (Figure 3). Moreover, the P3-amide functionality of compound **33** is

observed in two distinct conformations, staggered and equiplanar with respect to the P2phenyl plane. In solution, the low energy state of the P3-amide is in staggered conformation. In the X-ray structure of 33 however, the equiplanar conformation is predominant. In this conformation, the amide NH donates a proton to the backbone CO of Gly230 in a hydrogen bond, and the amide CO is in range for an electrostatic interaction to the side chain of Gln73 in the flap. In the case of the staggered conformation, the amide CO is facing the side chain of Thr232 and the amide NH points towards Gln73 with no apparent interactions with the protein. This conformation is accommodated for the protein by a shift in the side chain rotamer of Thr232. Similarly, a P3-keto analogue of an unsubstituted macrocycle adopts the same set of two conformational isomers (unpublished observations). These observations point to the fact that the energetically unfavorable binding mode of the P3-amide is inherent to the P1-P3 macrocycle scaffold, rather than the macrocycle substituent. Computer modeling suggests that replacing the P3-amide with a secondary amine will release the strain on the P1-P3 macrocycle and maintain the hydrogen bond to Gly230, which may lead to better potency and permeability in this series of compounds.

The preference for the (R)- over the (S)-isomer was apparent from the structures of **28** and **33** with BACE-1, which is illustrated in the structural overlay of the two compounds (Figure 4). In the case of the (R)-isomer **33**, the phenyl protrudes from the macrocycle into the amphiphilic S3sp gaining hydrophobic interactions, whereas for the (S)-isomer **28** the macrocycle and phenyl adopt an energetically unfavorable binding conformation, where the phenyl protrudes on top of the S3sp facing the solvent. In order to improve potency for the (R)-isoform, the amphiphilic nature of the S3sp seen as a narrow polar entrance and a lipophilic interior needs to be reflected in the macrocycle substituent. The X-ray structure of **33** suggests extended aryls and heteroaryls as good substituents for future chemistry design.



**Figure 3.** The hydrogen bond coordination of the macrocycle of compound **33** between residues of S1-S3 and the flap. Shown are also the two conformational isomers of the compound and the two accompanying rotamers of Thr232; equiplanar (yellow) and staggered (light blue).



**Figure 4.** Structural overlay of compounds **28** (pink) and **33** (yellow) showing the preference for the (*R*)-isomer in S3sp interactions.

#### 3. Conclusion

In summary, a series of macrocyclic BACE-1 inhibitors containing a hydroxyethylamine (HEA) isostere scaffold have been designed and synthesized, some of which displayed excellent potency. The most potent inhibitor **29** displayed an IC<sub>50</sub> value of 3.2 nM in the enzymatic assay and 0.15 nM in the cell-based assay. Some additional target compounds also showed activity in the low nanomolar range. Furthermore, good selectivity over cathepsin D could generally be observed in this series of inhibitors. X-ray crystallography studies concluded that the macrocycle-attached phenyl group, *e.g.* in inhibitor **29**, did not fully reach into the S3 subpocket of BACE-1, which indicates that there is room for further potency optimizations.

Even though we could achieve low nanomolar BACE-1 activity and good selectivity, the compounds synthesized suffered from inadequate permeability tentatively preventing them from reaching the CNS. Future work should be aimed at designing inhibitors with enhanced brain permeability, while retaining the reported potencies.

### 4. Experimental section

### 4.1. BACE-1 enzyme assay

The BACE-1 assay was performed as previously described.<sup>27</sup>

### 4.2. BACE-1 cell assay

The cell-based assay was performed as previously described.<sup>28</sup>

#### 4.3. Caco-2 assay

The permeability measurements were performed as previously described.<sup>29</sup>

### 4.4. Cathepsin D assay

The cathepsin D enzyme assay was performed as previously described.<sup>27</sup>

#### 4.5. General

NMR-spectra were recorded on a Varian 300 MHz instrument using CDCl<sub>3</sub>, CD<sub>3</sub>OD or DMSO-d<sub>6</sub> as solvent. Thin layer chromatography (TLC) was carried out on Merck precoated 60 F<sub>254</sub> plates using UV-light and charring with ethanol/sulfuric acid/p-

anisaldehyde/acetic acid 90:3:2:1, or a solution of 0.5% ninhydrin in ethanol for visualization. Flash column chromatography was performed using silica gel 60 (0.040-0.063 mm, Merck). Organic phases were dried over anhydrous magnesium sulfate. Drying of solvents: DCM was refluxed over calcium hydride and distilled onto 4 Å molecular sieves, DMSO was stored overnight with 4 Å MS. Optical rotations were measured using a Perkin-Elmer 141 polarimeter at 22 °C. Gradient LC-MS was performed on a Gilson system (column: Phenomenex C18, 100 x 21 mm, 5 μm for preparative runs and xBridge<sup>TM</sup> C18, 50 x 4.6 mm, 2.5 μm for analytical runs; pump: Gilson gradient pump 322; UV/VIS-detector: Gilson 152; MS detector: Thermo Finnigan Surveyor MSQ; Gilson Fraction Collector FC204) using acetonitrile with 0.05% formic acid and deionized water with 0.05% formic acid as mobile phases. High resolution mass spectra (HRMS) were recorded on a Waters Synapt HDMS instrument equipped with an electrospray interface.

### 4.6. LC-MS purity measurements

### 4.6.1. Chromatography system A

Column: Phenomenex C18, 50x4.6 mm, 3µm; pump: Gilson gradient pump 322; UV/VIS-detector: Gilson 152; MS detector: Thermo Finnigan Surveyor MSQ; Software: Gilson Unipoint 4.0 and Xcalibur 1.3. Mobile phase A: 10 mM NH<sub>4</sub>OAc in water; mobile phase B: 10 mM NH<sub>4</sub>OAc in 90% acetonitrile; gradient: 20-100% B over 6 min at 1 mL/min followed by 100% of B for 3 min at 1 mL/min. Peaks were detected at 254 nm.

### 4.6.2. Chromatography system B

Column: XBridge C8, 50x4.6 mm, 2.5 µm; pump: Gilson gradient pump 322; UV/VIS-detector: Gilson 152; MS detector: Thermo Finnigan Surveyor MSQ; Software: Gilson Unipoint 4.0 and Xcalibur 1.3. Mobile phase A: 10 mM NH<sub>4</sub>OAc in water; mobile phase B: 10 mM NH<sub>4</sub>OAc in 90% acetonitrile; gradient: 35-100% B over 7 min at 1 mL/min followed by 100% of B for 3 min at 1 mL/min. Peaks were detected at 254 nm.

#### 4.7. Synthesis

### 4.7.1. (S)-4-((S)-2-(Allyloxy)-1-azidoethyl)-2,2-dimethyl-1,3-dioxolane (2).

Compound **1** (129 mg, 0.69 mmol) was dissolved in toluene (2.5 mL). Ag<sub>2</sub>O (326 mg, 1.4 mmol) and allyl bromide (350  $\mu$ L, 4.1 mmol) were added and the reaction mixture was stirred at room temperature for 26 h. The solution was filtered, concentrated and the crude remainder was purified using flash column chromatography (hexane/EtOAc 39:1) to give compound **2** (117 mg, 78%) as a colorless oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.32 (s, 3H), 1.42 (s, 3H), 3.49-3.55 (m, 1H), 3.61-3.70 (m, 2H), 3.88-3.93 (m, 1H), 4.00-4.07 (m, 4H), 5.16-5.31 (m, 2H), 5.82-5.95 (m, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  25.4, 26.7, 63.1, 66.7, 70.2, 72.5, 75.2, 109.9, 117.5, 134.3. MS calcd (M+H)<sup>+</sup>: 228.1; found 228.4.

### 4.7.2. (S)-2-(Allyloxy)-1-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)ethanamine (3).

Compound **2** (126 mg, 0.55 mmol) was dissolved in MeOH (3 mL). TEA (386  $\mu$ L, 2.77 mmol) and 1,3-propanedithiol (0.278 mL, 2.77 mmol) were added. The solution was stirred at room temperature for 22 h, diluted with water and MeOH, acidified with 1 M HCl and washed with Et<sub>2</sub>O. The water phase was made basic with 1 M NaOH and extracted with Et<sub>2</sub>O. The combined organic extracts were dried, filtered and concentrated to yield **3** (82 mg, 73%) as a colorless oil. [ $\alpha$ ]<sub>D</sub> +9.0 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.32 (s, 3H), 1.38 (s, 3H), 2.30 (bs, 2H), 3.03–3.09 (m, 1H), 3.39 (dd, J = 6.3, 9.3 Hz, 1H), 3.56 (dd, J = 3.6, 9.3 Hz, 1H), 3.84–3.91 (m, 1H), 3.97–4.03 (m, 4H), 5.12-5.26 (m, 2H), 5.80–5.93 (m, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  25.3, 26.7, 53.3, 66.3, 71.8, 72.3, 77.0, 109.0, 117.1, 134.6. MS calcd (M+H)<sup>+</sup>: 202.1; found 202.2.

### 4.7.3. (S)-1-Phenylbut-3-yn-1-ol ((S)-4).

Lithium acetylide ethylenediamine complex (2.24 g, 21.9 mmol) was added to dry DMSO (14 mL) and the solution was stirred for 30 minutes under Ar atmosphere. (*R*)-(+)-Styrene oxide (1.0 mL, 8.76 mmol) was added and the mixture was stirred for 25 h at room temperature under Ar atmosphere. The mixture was quenched with H<sub>2</sub>O and extracted with Et<sub>2</sub>O. The combined organic extracts were dried, filtered and concentrated. Purification using flash column chromatography (toluene/EtOAc 39:1) gave (*S*)-4 (1.22 g,

95%) as a colorless oil. [ $\alpha$ ]<sub>D</sub> -12.1 (c 0.14, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.10 (t, J = 2.4 Hz, 1H), 2.65 (dd, J = 2.5, 6.3 Hz, 2H), 4.84 (t, J = 6.3 Hz, 1H), 7.32–7.44 (m, 5H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  29.4, 71.0, 72.4, 80.8, 125.8, 128.0, 128.5, 142.6. MS calcd (M+H)<sup>+</sup>: 147.1; found 147.2.

### 4.7.4. (*R*)-1-Phenylbut-3-yn-1-ol ((*R*)-4).

Compound (*R*)-4 (340 mg, quant.) was synthesized from (*S*)-(-)-styrene oxide in the same manner as (*S*)-4 and was collected as a slightly yellow oil. [ $\alpha$ ]<sub>D</sub> +12.8 (c 0.15, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.07 (t, J = 2.6 Hz, 1H), 2.62 (dd, J = 2.5, 6.3 Hz, 2H), 2.80 (bs), 4.83 (t, J = 6.3 Hz, 1H), 7.29-7.39 (m, 5H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  29.3, 71.0, 72.3, 80.8, 125.8, 127.9, 128.4, 142.5. MS calcd (M+H)<sup>+</sup>: 147.1; found 147.2.

### 4.7.5. (R)-(1-Azidobut-3-yn-1-yl)benzene ((R)-5).

Compound (*S*)-4 (1.04 g, 7.09 mmol) and PPh<sub>3</sub> (2.79 g, 10.6 mmol) were dissolved in THF (25 mL) and the mixture was cooled to -12 °C. DIAD (2.1 mL, 10.7 mmol) was added and the mixture was stirred at -12 °C for 10 minutes. DPPA (2.3 mL, 10.7 mmol) was added and the solution was left to slowly reach room temperature for 25 h. The solution was then concentrated and purified using flash column chromatography (hexane/EtOAc 39:1). Compound (*R*)-5 (1.18 g, 97%) was collected as a colorless oil.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.03–2.07 (m, 1H), 2.57–2.74 (m, 2H), 4.66 (t, *J* = 6.9 Hz, 1H), 7.31–7.42 (m, 5H);  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  26.9, 64.5, 71.2, 80.0, 126.9, 128.8, 129.0, 138.3. MS calcd (M+H)<sup>+</sup>: 172.1; found 172.0.

### 4.7.6. (S)-(1-Azidobut-3-yn-1-yl)benzene ((S)-5).

Compound (*S*)-5 (299 mg, 76%) was synthesized from (*R*)-4 in the same manner as (*R*)-5 and was collected as a colorless oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.06 (t, *J* = 2.7 Hz, 1H), 2.59-2.75 (m, 2H), 4.67 (t, *J* = 6.9 Hz, 1H), 7.32-7.44 (m, 5H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  27.0, 64.6, 71.3, 80.0, 127.0, 128.8, 129.0, 128.3. MS calcd (M+H)<sup>+</sup>: 172.1; found 172.2.

### 4.7.7. (R)-1-Phenylbut-3-yn-1-amine ((R)-6).

1,3-Propanedithiol (3.5 mL, 34.8 mmol) was added to a solution of ( $\it{R}$ )-5 (1.2 g, 6.9 mmol) and TEA (4.8 mL, 34.7 mmol) in MeOH (15 mL). The solution was stirred at room temperature for 47 h, after which the reaction mixture was diluted with water and MeOH, acidified with 1 M HCl and washed with DCM. Thereafter, the water phase was made basic with 1 M NaOH and extracted with DCM. The combined organic extracts were dried, filtered and concentrated to give ( $\it{R}$ )-6 (930 mg, 92%) as a colorless oil.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.00 (bs, 2H), 2.04 (t,  $\it{J}$  = 2.4 Hz, 1H), 2.49 (ddd,  $\it{J}$  = 2.7, 7.8, 16.5 Hz, 1H), 2.60 (ddd,  $\it{J}$  = 2.7, 5.1, 16.8, Hz, 1H), 4.16 (dd,  $\it{J}$  = 5.1, 7.8 Hz, 1H), 7.21–7.36 (m, 5H);  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  29.7, 54.8, 70.6, 81.8, 126.3, 127.6, 128.6, 144.3. MS calcd (M+H) $^{+}$ : 146.1; found 146.5.

### 4.7.8. (S)-1-Phenylbut-3-yn-1-amine ((S)-6).

Compound (*S*)-6 (905 mg, 87%) was synthesized from (*S*)-5 in the same manner as compound (*R*)-6 and was collected as a colorless oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.79 (s, 2H), 2.03 (t, J = 2.7 Hz, 1H), 2.44 (ddd, J = 2.7, 7.8, 16.5 Hz, 1H), 2.55 (ddd, J = 2.7, 5.1, 16.5 Hz, 1H), 4.11 (dd, J = 5.1, 7.8 Hz, 1H), 7.21-7.26 (m, 1H), 7.28-7.37 (m, 4H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  29.5, 54.5, 70.5, 81.6, 126.1, 127.3, 128.3, 144.2. MS calcd (M+H)<sup>+</sup>: 146.1; found 145.8.

### 4.7.9. (R)-Methyl 3-methyl-5-((1-phenylbut-3-yn-1-yl)carbamoyl)benzoate ((R)-7).

Compound ( $\it{R}$ )-6 (270 mg, 1.86 mmol) and 3-methoxycarbonyl-5-methylbenzoic acid (506 mg, 1.76 mmol) were dissolved in DMF (8 mL). DIPEA (0.92 mL, 5.3 mmol) and HCTU (1.09 g, 2.6 mmol) were added and the mixture was stirred at room temperature for 4 h. The solution was diluted with brine and H<sub>2</sub>O and extracted with EtOAc. The combined organic extracts were dried, filtered and co-evaporated with toluene. The residue was purified using flash column chromatography (toluene/EtOAc 9:1) to give ( $\it{R}$ )-7 (650 mg, 89%) as a white solid.  $^1$ H-NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  2.45 (s, 3H), 2.52-2.53 (m, 1H), 2.70-2.79 (m, 1H), 2.81-2.88 (m, 1H), 3.90 (s, 3H), 5.21-5.28 (m, 1H), 7.25-7.30 (m, 1H), 7.36 (t,  $\it{J}$  = 7.2 Hz, 2H), 7.46-7.48 (m, 2H), 7.95 (s, 1H), 7.99 (s, 1H), 8.30 (s, 1H), 9.11 (d,  $\it{J}$  = 8.4 Hz, 1H);  $^{13}$ C-NMR (DMSO- $d_6$ , 75.5 MHz):  $\delta$ 

20.7, 25.3, 52.2, 52.4, 72.5, 81.7, 125.3, 126.6, 127.1, 128.2, 129.7, 132.1, 132.6, 134.8, 138.5, 142.0, 164.9, 165.9. MS calcd (M+H)<sup>+</sup>: 322.1; found 322.4.

### 4.7.10. (S)-Methyl 3-methyl-5-((1-phenylbut-3-yn-1-yl)carbamoyl)benzoate ((S)-7).

Compound (*S*)-7 (219 mg, 73%) was synthesized from (*S*)-6 in the same manner as compound (*R*)-7 and was collected as a white solid. <sup>1</sup>H-NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  2.40 (s, 3H), 2.67–2.84 (m, 3H), 3.86 (s, 3H), 5.21 (m, 1H), 7.21–7.26 (m, 1H), 7.30-7.35 (m, 2H), 7.42-7.45 (m, 2H), 7.91 (s, 1H), 7.95 (s, 1H), 8.27 (s, 1H), 9.09 (d, J = 8.4 Hz, 1H); <sup>13</sup>C-NMR (DMSO- $d_6$ , 75.5 MHz):  $\delta$  20.7, 25.4, 52.2, 52.5, 72.5, 81.8, 125.3, 126.7, 128.2, 129.7, 132.1, 132.6, 134.8, 138.5, 142.0, 165.0, 165.9. MS calcd (M+H)<sup>†</sup>: 322.1; found 322.3.

### 4.7.11. (*R*)-Methyl 3-(*N*-methylmethylsulfonamido)-5-((1-phenylbut-3-yn-1-yl)carbamoyl)benzoate ((*R*)-8).

Compound (R)-8 (649 mg, 89%) was synthesized from (R)-6 in the same manner as compound (R)-7, using compound 11 instead of 3-methoxycarbonyl-5-methylbenzoic acid, and was collected as a white powder.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.06–2.08 (m, 1H), 2.81–2.88 (m, 2H), 2.86 (s, overlap, 3H), 3.32 (s, 3H), 3.89 (s, 3H), 5.31–5.42 (m, 1H), 7.15–7.44 (m, 5H), 8.08 (s, 1H), 8.13 (s, 1H), 8.30 (s, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  25.6, 35.8, 37.9, 52.2, 52.7, 71.8, 80.0, 126.1, 126.7, 128.0, 128.8, 129.2, 130.0, 131.7, 135.9, 140.3, 142.4, 164.9, 165.6. MS calcd (M+H) $^{+}$ : 415.1; found 415.4.

### 4.7.12. (S)-Methyl 3-(N-methylmethylsulfonamido)-5-((1-phenylbut-3-yn-1-yl)carbamoyl)benzoate ((S)-8).

Compound (*S*)-8 (381 mg, 89%) was synthesized from (*S*)-6 in the same manner as (*R*)-8 and was collected as a white solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.08 (t, J = 2.6 Hz, 1H), 2.79–2.96 (m, 2H), 2.87 (s, overlap, 3H), 3.34 (s, 3H), 3.91 (s, 3H), 5.35–5.42 (m, 1H), 7.01 (d, J = 8.1 Hz, 1H), 7.26–7.32 (m, 1H), 7.34-7.39 (m, 2H), 7.42-7.45 (m, 2H), 8.06-8.07 (m, 1H), 8.14-8.15 (m, 1H), 8.28-8.29 (m, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  25.6, 35.9, 38.0, 52.1, 52.8, 72.0, 79.9, 126.1, 126.8, 128.1, 128.9, 129.2, 130.0, 131.8, 135.9, 140.2, 142.4, 164.9, 165.6. MS calcd (M+H)<sup>+</sup>: 415.1; found 415.4.

### 4.7.13. (*R*)-Methyl 3-methyl-5-((1-phenylbut-3-en-1-yl)carbamoyl)benzoate ((*R*)-9).

Compound ( $\it{R}$ )-7 (27 mg, 0.08 mmol) was dissolved in MeOH (7 mL). Lindlar's catalyst (4 mg, ~5% Pd on CaCO<sub>3</sub>) and quinoline (20 µL, 0.17 mmol) were added and the mixture was stirred at room temperature for 1.5 h under H<sub>2</sub> (1 atm). The mixture was diluted with Et<sub>2</sub>O and washed with H<sub>2</sub>O. The organic phase was dried, filtered through Celite and concentrated. Compound ( $\it{R}$ )-9 (27 mg, 99%) was collected as a white solid.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.42 (s, 3H), 2.70 (t,  $\it{J}$  = 6.9 Hz, 2H), 3.91 (s, 3H), 5.10-5.20 (m, 2H), 5.26-5.33 (m, 1H), 5.70-5.84 (m, 1H), 6.50 (bs, 1H), 7.24-7.29 (m, 1H), 7.34-7.36 (m, 4H), 7.83 (s, 1H), 7.96 (s, 1H), 8.14 (s, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  21.3, 40.6, 52.4, 53.1, 118.6, 124.7, 126.7, 127.6, 128.8, 130.5, 132.8, 133.2, 134.1, 135.0, 139.2, 141.6, 166.0, 166.7. MS calcd (M+H)<sup>+</sup>: 324.2; found 324.3.

### 4.7.14. (S)-Methyl 3-methyl-5-((1-phenylbut-3-en-1-yl)carbamoyl)benzoate ((S)-9).

Compound (*S*)-9 (213 mg, 99%) was synthesized from (*S*)-7 in the same manner as (*R*)-9 and was collected as a white powder.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.36 (s, 3H), 2.66-2.71 (m, 2H), 3.88 (s, 3H), 5.08-519 (m, 2H), 5.28 (m, 1H), 5.69-5.83 (m, 1H), 6.80 (d, J = 8.1 Hz, 1H), 7.24-7.29 (m, 1H), 7.31-7.37 (m, 4H), 7.80 (s, 1H), 7.92 (s, 1H), 8.16 (s, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  21.2, 40.4, 52.3, 53.1, 118.3, 124.8, 126.6, 127.5, 128.7, 130.4, 132.7, 133.0, 134.2, 134.9, 139.0, 141.6, 166.0, 166.6. MS calcd (M+H) $^{+}$ : 324.2; found 324.4.

### 4.7.15. (*R*)-Methyl 3-(*N*-methylmethylsulfonamido)-5-((1-phenylbut-3-en-1-yl)carbamoyl)benzoate ((*R*)-10).

Compound ( $\it R$ )-10 (326 mg, 84%) was synthesized from ( $\it R$ )-8 in the same manner as ( $\it R$ )-9 and was collected as a white solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.63–2.72 (m, 2H), 2.87 (s, 3H), 3.32 (s, 3H), 3.89 (s, 3H), 5.08–5.29 (m, 3H), 5.70–5.83 (m, 1H), 6.87 (d,  $\it J$  = 8.0 Hz, 1H), 7.22–7.35 (m, 5H), 8.04 (m, 1H), 8.12 (m, 1H), 8.24 (m, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  35.8, 37.9, 40.4, 52.7, 53.5, 118.6, 126.0, 126.7,

127.6, 128.7, 129.1, 130.0, 131.6, 134.1, 136.1, 141.4, 142.4, 164.7, 165.6. MS calcd (M+H)<sup>+</sup>: 417.1; found 417.4.

### 4.7.16. (S)-Methyl 3-(N-methylmethylsulfonamido)-5-((1-phenylbut-3-en-1-yl)carbamoyl)benzoate ((S)-10).

Compound (*S*)-10 (1.5 g, 92%) was synthesized from (*S*)-8 in the same manner as (*R*)-9 and was collected as a white solid.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.67–2.73 (m, 2H), 2.87 (s, 3H), 3.33 (s, 3H), 3.90 (s, 3H), 5.10–5.22 (m, 2H), 5.23-5.30 (m, 1H), 5.70–5.84 (m, 1H), 6.87 (d, J = 7.8 Hz, 1H), 7.22–7.38 (m, 5H), 8.06-8.07 (m, 1H), 8.12 (bs, 1H), 8.26 (bs, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  35.8, 38.0, 40.4, 52.8, 53.6, 118.6, 125.4, 126.0, 126.7, 127.7, 128.8, 129.2, 130.1, 131.6, 134.1, 136.1, 141.4, 142.4, 164.8, 165.7. MS calcd (M+H)<sup>+</sup>: 417.1; found 417.3.

### 4.7.17. Methyl 3-(but-3-en-1-ylcarbamoyl)-5-(*N*-methylmethyl-sulfonamido)benzoate (12a).

Compound **11** (414 mg, 1.44 mmol) was dissolved in DMF (5 mL). 3-Butenylamine hydrochloride (186 mg, 1.73 mmol), TEA (500  $\mu$ L, 3.60 mmol) and HOBt (234 mg, 1.73 mmol) were added and the solution was cooled to 0 °C. EDC (332 mg, 1.73 mmol) was added and the reaction mixture was allowed to attain room temperature and was stirred for 19 hours. Brine was added and the solution was extracted with EtOAc. The combined organic extracts were dried, filtered and concentrated. The residue was purified using flash column chromatography (toluene/EtOAc 3:1) to give **12a** (406 mg, 83%) as a white solid.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.40 (quartet, J = 6.9 Hz, 2H), 2.88 (s, 3H), 3.37 (s, 3H), 3.51-3.58 (m, 2H), 3.94 (s, 3H), 5.11-5.19 (m, 2H), 5.76-5.90 (m, 1H), 6.38 (bs, 1H), 8.03 (t, J = 1.8 Hz, 1H), 8.14-8.15 (m, 1H), 8.24-8.25 (m, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  33.8, 35.9, 38.0, 39.3, 52.8, 117.7, 126.1, 129.1, 129.6, 131.9, 135.1, 136.4, 142.4, 165.5, 165.7. MS calcd (M+H) $^{+}$ : 341.1; found 341.3.

### 4.7.18. Methyl 3-(but-3-en-1-yl(methyl)carbamoyl)-5-(*N*-methylmethyl-sulfonamido)benzoate (12b).

Compound **12a** (429 mg, 1.3 mmol) was dissolved in DMF (6 mL). NaH (60%) (66 mg, 1.7 mmol) and MeI (237  $\mu$ L, 3.8 mmol) were added and the mixture was stirred

at room temperature for 23 h. The reaction was quenched with water and extracted with EtOAc three times. The organic phases were dried, filtered and evaporated. This gave compound **12b** (448 mg, 100 %) as a slightly yellow powder. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz): δ 2.23-2.36 (m, 2H), 2.82 (s, 3H), 2.88 (s, 3H), 3.23-3.27 (m, 1H), 3.29 (s, 3H), 3.53-3.54 (m, 1H), 3.87 (s, 3H), 4.96-5.12 (m, 2H), 5.51-5.79 (m, 1H), 7.58 (s, 1H), 7.91 (s, 1H), 7.96 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz): δ 29.6, 35.7, 37.8, 46.9, 50.7, 52.5, 117.0, 117.9, 126.6, 129.5, 131.6, 135.0, 138.1, 141.9, 162.5, 165.5. MS calcd (M+H)<sup>+</sup>: 355.1; found 355.3.

### 4.7.19. $N^1$ -((S)-2-Allyloxy-1-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethyl)- $N^3$ -but-3-enyl-5-(methanesulfonyl-methyl-amino)-isophthalamide (13).

Compound 12a (429 mg, 1.26 mmol) was dissolved in H<sub>2</sub>O (8 mL) and dioxane (16 mL). Aqueous LiOH (1.5 mL, 40 mg/ml, 60 mg, 2.5 mmol) was added and the reaction mixture was stirred at room temperature for 1 h and 10 min. The solution was co-evaporated with toluene and the crude material was re-dissolved in DMF (12 mL). Compound 3 (250 mg, 1.24 mmol), DIPEA (658 µL, 3.78 mmol) and HATU (719 mg, 1.89 mmol) were added and the reaction mixture was stirred at room temperature for 16 h. H<sub>2</sub>O was added and the solution was extracted with EtOAc. The combined organic phases were washed with brine, dried, filtered and concentrated. The residue was purified using flash column chromatography (toluene/EtOAc 1:1). Compound 13 (571 mg, 90% over two steps) was collected as an orange oil. <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 300 MHz): δ 1.32 (s, 3H), 1.41 (s, 3H), 2.33-2.40 (m, 2H), 2.95 (s, 3H), 3.34 (s, 3H), 3.44 (t, J = 6.9 Hz, 2H), 3.71 (d, J = 4.5 Hz, 2H), 3.90 (dd, J = 5.4, 8.7 Hz, 1H), 4.00-4.03 (m, 2H), 4.06 (dd, J = 4.5 Hz, 1H)6.0, 8.7 Hz, 1H), 4.24-4.37 (m, 2H), 5.02-5.30 (m, 4H), 5.78-5.97 (m, 2H), 7.99 (bs, 2H), 8.18 (m, 1H); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 75.5 MHz): δ 25.6, 27.1, 34.7, 35.9, 38.3, 40.5, 53.9, 68.1, 70.1, 73.1, 76.2, 110.8, 117.1, 117.4, 125.8, 128.9, 129.0, 135.9, 136.6, 137.2, 137.3, 143.7, 168.2, 168.5. MS calcd (M+H)<sup>+</sup>: 510.2; found 510.5.

### 4.7.20. $N^1$ -((2S,3S)-1-(Allyloxy)-3,4-dihydroxybutan-2-yl)- $N^3$ -(but-3-en-1-yl)-5-(N-methylmethylsulfonamido)isophthalamide (14).

Compound **13** (42 mg, 0.08 mmol) was dissolved in AcOH (1.3 mL) and H<sub>2</sub>O (0.5 mL) and heated to 60 °C. The reaction mixture was stirred for 1 h and 45 min, coevaporated with toluene and concentrated. The residue was purified using flash column chromatography (MeOH/EtOAc 1:12) to give **14** (39 mg, 100%) as a yellow oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.36 (quartet, J = 6.9 Hz, 2H), 2.89 (s, 3H), 3.33 (s, 3H), 3.46-3.52 (m, 2H), 3.66-3.70 (m, 3H), 3.75-3.77 (m, 1H), 3.90 (dd, J = 3.9, 9.9 Hz, 1H), 4.04 (d, J = 5.7 Hz, 2H), 4.15-4.22 (m, 1H), 5.07-5.31 (m, 4H), 5.73-5.94 (m, 2H), 6.82 (bs, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.90 (s, 2H), 8.03 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  33.7, 35.7, 38.0, 39.5, 52.0, 63.2, 68.5, 71.2, 72.6, 117.6, 118.2, 124.1, 127.5, 127.9, 134.1, 135.1, 136.1, 142.3, 165.5, 166.7. MS calcd (M+H)<sup>+</sup>: 470.2; found 470.4.

## 4.7.21. *N*-[(*E*)-(*S*)-4-((*S*)-1,2-Dihydroxy-ethyl)-2,13-dioxo-6-oxa-3,12-diaza-bicyclo[12.3.1]-octadeca-1(18),8,14,16-tetraen-16-yl]-*N*-methyl-methanesulfonamide (15).

Compound **14** (119 mg, 0.25 mmol) was dissolved in dry DCM (200 mL) and the solution was degassed with N<sub>2</sub>. Hoveyda-Grubbs catalyst 2nd generation (37 mg, 0.06 mmol) was added and the reaction mixture was refluxed overnight under N<sub>2</sub> atmosphere. The solution was subsequently concentrated and the crude material was purified using flash column chromatography (MeOH/EtOAc 1:12). Compound **15** (109 mg, 98%) was collected as a white powder. <sup>1</sup>H-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 300 MHz):  $\delta$  2.27-2.37 (m, 2H), 2.86 (s, 3H), 3.24-3.26 (m, 1H), 3.28 (s, 3H), 3.51-3.55 (m, 1H), 3.58-3.64 (m, 3H), 3.77 (d, J = 4.8 Hz, 2H), 3.94-4.00 (m, 3H), 5.65 (dt, J = 6.9, 15.3 Hz, 1H), 5.87 (dt, J = 6.9, 15.3 Hz, 1H), 7.62 (bs, 1H), 7.80 (s, 2H), 7.93 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 75.5 MHz):  $\delta$  32.3, 35.1, 37.2, 37.4, 52.2, 63.3, 68.9, 71.3, 71.5, 126.3, 127.2, 127.3, 130.4, 131.7, 136.0, 136.4, 142.5, 167.4, 169.2. MS calcd (M+H)<sup>+</sup>: 442.2; found 442.4.

### 4.7.22. Toluene-4-sulfonic acid (*S*)-2-hydroxy-2-[(*E*)-(*S*)-16-(methanesulfonyl-methyl-amino)-2,13-dioxo-6-oxa-3,12-diazabicyclo[12.3.1]octadeca-1(18),8,14,16-tetraen-4-yl]-ethyl ester (16).

Compound **15** (145 mg, 0.33 mmol) was dissolved in dry DCM (5 mL). *p*-TsCl (75 mg, 0.39 mmol), Bu<sub>2</sub>SnO (2 mg, 0.007 mmol) and TEA (55  $\mu$ L, 0.39 mmol) were added and the mixture was stirred at room temperature for 23 hours. DCM was added and the solution was washed with brine three times. The organic phase was dried, filtered and concentrated. The crude product was purified using flash column chromatography (toluene/EtOAc 1:2 to 1:9) to give compound **16** (125 mg, 64%) as a colorless oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.28-2.37 (m, 2H), 2.41 (s, 3H), 2.82 (s, 3H), 3.16-3.23 (m, 1H), 3.27 (s, 3H), 3.55-3.66 (m, 2H), 3.75-3.80 (m, 1H), 3.91-3.95 (m, 3H), 4.00 (d, *J* = 6.6 Hz, 2H), 4.05-4.11 (m, 1H), 5.63-5.73 (m, 1H), 5.82-5.91 (m, 1H), 6.74 (bs, 1H), 7.15 (d, *J* = 6.9 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.79 (bs, 2H), 7.81 (bs, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  21.7, 32.6, 36.4, 37.5, 37.9, 53.3, 68.0, 69.4, 71.6, 71.9, 125.4, 127.0, 128.0, 130.1, 131.1, 132.2, 132.3, 136.4, 136.7, 142.7, 145.3, 166.1, 168.4. MS calcd (M+H)<sup>+</sup>: 596.2; found 596.5.

## 4.7.23. N-{(E)-(S)-4-[(R)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-2,13-dioxo-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),8,14,16-tetraen-16-yl}-N-methyl-methanesulfonamide (17).

Compound **16** (80 mg, 0.14 mmol) and 3-isopropylbenzylamine (57 mg, 0.38 mmol) were dissolved in EtOH (99.5%, 5 mL) and the reaction mixture was refluxed for 24 hours. The mixture was concentrated and the crude product was purified using flash column chromatography (MeOH/EtOAc 1:9 + 1% TEA). Compound **17** (29 mg, 37%) was collected as a white powder. [ $\alpha$ ]<sub>D</sub> +40.0 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.24 (d, J = 6.60 Hz, 6H), 2.12-2.46 (m, 5H), 2.90 (s, 3H), 3.35 (s, 3H), 3.52-3.55 (m, 1H), 3.61-3.82 (m, 3H), 3.86 (s, 2H), 3.94-4.23 (m, 4H), 5.71 (m, 1H), 5.88-6.01 (m, 1H), 6.40 (bs, 1H), 7.00-7.07 (m, 1H), 7.11-7.14 (m, 2H), 7.17 (s, 1H), 7.24-7.26 (m, 1H), 7.84 (s, 1H), 7.90-7.96 (m, 2H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  23.8, 23.9, 34.1, 36.3, 37.6, 38.0, 44.0, 45.9, 52.8, 52.9, 63.2, 69.3, 71.8, 125.6, 126.4, 127.4, 127.7, 128.4, 129.2, 129.8, 131.1, 132.5, 132.7, 136.2, 137.0, 137.1, 150.1, 162.1, 166.2. HRMS calcd

 $(M+H)^+$ : 573.2741; found 573.2728. LC-MS purity system A:  $t_R = 4.58$  min, 100%; system B:  $t_R = 1.64$  min, 100%.

## 4.7.24. *N*-[(*E*)-(*S*)-4-((*S*)-1,2-Dihydroxy-ethyl)-2,14-dioxo-6-oxa-3,13-diaza-bicyclo[13.3.1]nonadeca-1(19),8,15,17-tetraen-17-yl]-*N*-methyl-methanesulfonamide (18).

Compound **18** was synthesized according to the synthetic route for **15** using 4-pentenylamine hydrochloride instead of 3-butenylamine hydrochloride in the peptide coupling step and was collected as a white powder.  $^{1}$ H-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 300 MHz):  $\delta$  1.81-1.93 (m, 2H), 2.31-2.39 (m, 2H), 2.96 (s, 3H), 3.38 (s, 3H), 3.40-3.41 (m, 2H), 3.60 (dd, J = 4.2, 9.6 Hz, 1H), 3.66-3.73 (m, 2H), 3.77-3.83 (m, 1H), 3.88-3.94 (m, 1H), 4.03 (dd, J = 3.9, 9.6 Hz, 1H), 4.09-4.16 (m, 2H), 5.73-5.86 (m, 1H), 5.98 (dt, J = 6.9, 15.6 Hz, 1H), 7.89-7.90 (m, 1H), 7.95 (s, 1H), 8.01-8.02 (m, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 75.5 MHz):  $\delta$  27.6, 31.8, 35.0, 37.1, 41.2, 51.4, 63.3, 68.2, 70.4, 71.7, 123.9, 126.5, 127.4, 127.5, 133.9, 135.4, 135.9, 142.5, 166.2, 167.9. MS calcd (M+H)<sup>+</sup>: 456.4; found 456.4.

## 4.7.25. *N*-[(*S*)-4-((*S*)-1,2-Dihydroxy-ethyl)-2,13-dioxo-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),14,16-trien-16-yl]-*N*-methyl-methanesulfonamide (19).

Compound **15** (113 mg, 0.26 mmol) was dissolved in MeOH (8 mL) and Pd-C 10% (40 mg) was added. The mixture was stirred at room temperature under  $H_2$  (1 atm) for 3 h. The solution was then filtered through Celite and concentrated. The crude material was purified using flash column chromatography (EtOAc to EtOAc/MeOH 12:1) to yield compound **19** (108 mg, 95%) as a colorless oil.  $^1$ H-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 300 MHz):  $\delta$  0.89-0.97 (m, 2H), 1.12-1.18 (m, 2H), 1.36-1.41 (m, 2H), 1.72 (bs, 2H), 2.96 (s, 3H), 3.34-3.36 (m, 2H), 3.38 (s, 3H), 3.39-3.41 (m, 2H), 3.45-3.56 (m, 2H), 3.67-3.70 (m, 3H), 3.81-3.97 (m, 1H), 7.85 (s, 1H), 7.92 (s, 1H), 8.00-8.02 (m, 1H), 8.08 (s, 1H) 8.21-8.22 (m, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 75.5 MHz):  $\delta$  21.4, 28.3, 29.1, 34.8, 37.0, 39.2, 51.9, 63.2, 68.8, 70.0, 70.9, 126.7, 127.6, 135.5, 137.2, 142.6, 168.1, 168.9. MS calcd (M+H) $^+$ : 444.2; found 444.4.

## 4.7.26. *N*-[(*S*)-4-((*S*)-1,2-Dihydroxy-ethyl)-2,14-dioxo-6-oxa-3,13-diaza-bicyclo[13.3.1]nonadeca-1(19),15,17-trien-17-yl]-*N*-methyl-methanesulfonamide (20).

Compound **20** (106 mg, 78%) was synthesized from **18** in the same manner as **19** and was collected as white crystals.  $^{1}$ H-NMR (CD<sub>3</sub>OD, 300 MHz):  $\delta$  1.55-1.65 (m, 4H), 1.66-1.76 (m, 4H), 2.95 (s, 3H), 3.35-3.38 (m, 2H), 3.36 (s, overlapped, 3H), 3.40-3.50 (m, 2H), 3.52-3.60 (m, 2H), 3.63 (d, J = 3.3 Hz, 1H), 3.65-3.71 (m, 1H), 3.75-3.81 (m, 1H), 3.90-3.94 (m, 1H), 7.90-7.93 (m, 2H), 7.95-7.96 (m, 1H);  $^{13}$ C-NMR (CD<sub>3</sub>OD, 75.5 MHz):  $\delta$  30.0, 30.2, 30.4, 32.1, 35.9, 38.3, 42.3, 53.1, 65.0, 70.3, 72.1, 73.0, 126.4, 128.6, 128.7, 137.2, 138.2, 144.5, 169.0, 169.2. MS calcd (M+H)<sup>+</sup>: 458.2; found 458.5.

## 4.7.27. *N*-[(*E*)-(*S*)-4-((*R*)-2-Benzylamino-1-hydroxy-ethyl)-2,13-dioxo-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(17),8,14(18),15-tetraen-16-yl]-*N*-methyl-methanesulfonamide (21).

Compound **21** was synthesized according to the synthetic route for **17**, using benzylamine in the final step, and was collected as a colorless oil (42% for the final step). [ $\alpha$ ]<sub>D</sub> +54.3 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.22-2.52 (m, 2H), 2.73-2.78 (m, 1H), 2.87 (s, 3H), 3.26-3.31 (m, 2H), 3.33 (s, 3H), 3.45-3.52 (m, 1H), 3.60-3.74 (m, 3H), 3.84 (d, J = 8.7 Hz, 2H), 3.87-4.11 (m, 3H), 5.68-5.75 (m, 1H), 5.86-5.94 (m, 1H), 6.58 (bs, 1H), 7.04 (d, J = 7.5 Hz, 1H), 7.19-7.33 (m, 5H), 7.84 (s, 1H), 7.89 (s, 2H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  32.7, 36.5, 37.5, 38.0, 51.0, 53.6, 54.0, 69.2, 71.9, 124.8, 127.1, 127.3, 127.4, 127.6, 128.5, 128.6, 128.7, 129.0, 131.4, 132.4, 136.9, 138.6, 143.2, 165.8, 168.0. HRMS calcd (M+H)<sup>+</sup>: 531.2272; found 531.2293. LC-MS purity system A:  $t_R$  = 3.38 min, 96%; system B:  $t_R$  = 0.72 min, 96%.

## 4.7.28. N-{(E)-(S)-4-[(R)-2-(3-tert-Butyl-benzylamino)-1-hydroxy-ethyl]-2,13-dioxo-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(17),8,14(18),15-tetraen-16-yl}-N-methyl-methanesulfonamide (22).

Compound **22** was synthesized according to the synthetic route for **17**, using 3-*tert*-butylbenzylamine in the final step, and was collected as a white powder (34% for the final step). [ $\alpha$ ]<sub>D</sub> +8.0 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.30 (s, 9H), 2.28-

2.49 (m, 2H), 2.72-2.87 (m, 2H), 3.23 (s, 2H), 3.30-3.38 (m, 4H), 3.42-3.53 (m, 3H), 3.57-3.62 (m, 2H), 3.81-3.87 (m, 1H), 3.93-4.00 (m, 1H), 4.02-4.22 (m, 2H), 4.44 (bs, 1H), 5.60-5.74 (m, 1H), 5.85-5.95 (m, 1H), 6.45 (bs, 1H), 7.01 (bs, 1H), 7.10-7.17 (m, 1H), 7.23-7.32 (m, 3H), 7.50-7.53 (m, 1H), 7.81 (s, 1H), 7.91 (s, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  31.5, 34.8, 36.1, 37.8, 46.9, 53.7, 54.3, 57.5, 62.5, 69.3, 71.4, 73.0, 123.9, 124.2, 124.9, 125.4, 125.8, 128.3, 129.0, 131.9, 132.1, 133.2, 134.9, 137.4, 142.2, 151.5, 161.4, 165.2. HRMS calcd (M+H)<sup>+</sup>: 587.2898; found 587.2929. LC-MS purity system A:  $t_R = 3.89 \text{ min}$ , 96%; system B:  $t_R = 2.40 \text{ min}$ , 95%.

## 4.7.29. N-{(E)-(S)-4-[(R)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-2,14-dioxo-6-oxa-3,13-diaza-bicyclo[13.3.1]nonadeca-1(18),8,15(19),16-tetraen-17-yl}-N-methyl-methanesulfonamide (23).

Compound **23** was synthesized according to the synthetic route for **17**, using 4-pentenylamine hydrochloride in the first peptide coupling step, and was collected as a white powder (13% for the final step). [ $\alpha$ ]<sub>D</sub> -2.2 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.22 (s, 3H), 1.26 (s, 3H), 1.83 (bs, 2H), 2.33 (bs, 2H), 2.85-2.94 (m, 4H), 3.32-3.37 (m, 5H), 3.45-3.53 (m, 2H), 3.57-3.67 (m, 3H), 3.78 (s, 2H), 3.84-3.89 (m, 2H), 3.98-4.15 (m, 1H), 5.70-6.00 (m, 2H), 7.10-7.13 (m, 2H), 7.17 (s, 1H), 7.20-7.23 (m, 1H), 7.52 (s, 1H), 7.63 (s, 1H), 7.90 (s, 1H), 7.94 (s, 1H), 8.00 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  23.9, 24.0, 28.0, 29.7, 32.3, 36.3, 37.9, 41.9, 51.7, 60.5, 63.3, 68.9, 71.6, 72.0, 122.3, 124.9, 125.4, 125.7, 126.6, 127.3, 128.3, 128.7, 128.9, 134.7, 135.8, 136.0, 143.3, 149.8, 165.0, 167.5. HRMS calcd (M+H)<sup>+</sup>: 587.2898; found 587.2910. LC-MS purity system A:  $t_R$  = 4.21 min, 98%; system B:  $t_R$  = 2.76 min, 97%.

## 4.7.30. N-[(S)-4-((R)-2-Benzylamino-1-hydroxy-ethyl)-2,13-dioxo-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),14,16-trien-16-yl]-N-methyl-methanesulfonamide (24).

Compound **24** was synthesized from **19** according to the synthetic route for **17**, using benzylamine in the final step, and was collected as a colorless oil (51% for the final step). [ $\alpha$ ]<sub>D</sub> -9.0 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 300 MHz):  $\delta$  1.06-1.14 (m, 2H), 1.24-1.40 (m, 4H), 1.66 (bs, 2H), 2.93 (s, 3H), 3.15-3.22 (m, 1H), 3.29-3.34 (m, 5H),

3.38-3.47 (m, 2H), 3.51-3.62 (m, 5H), 3.74-3.82 (m, 2 H), 7.21-7.35 (m, 5H), 7.81-7.87 (m, 2H), 8.01 (s, 1H);  $^{13}$ C-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 75.5 MHz):  $\delta$  22.8, 29.2, 35.1, 37.2, 39.3, 43.4, 51.4, 53.2, 67.6, 68.7, 70.1, 72.1, 126.8, 127.0, 127.3, 127.3, 127.6, 127.9, 128.0, 128.2, 128.2, 128.3, 135.8, 137.3, 142.6, 167.6, 168.9. HRMS calcd (M+H)<sup>+</sup>: 533.2428; found 533.2437. LC-MS purity system A:  $t_R$  = 3.53 min, 96%; system B:  $t_R$  = 0.93 min, 97%.

## 4.7.31. N-{(S)-4-[(R)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-2,13-dioxo-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),14,16-trien-16-yl}-N-methyl-methanesulfonamide (25).

Compound **25** was synthesized from **19** according to the synthetic route for **17** and was collected as a colorless oil (77% for the final step). [ $\alpha$ ]<sub>D</sub> +10.0 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 300 MHz):  $\delta$  1.23-1.27 (m, 12H), 1.70 (bs, 2H), 2.76-2.80 (m, 1H), 2.86-2.93 (m, 2H), 2.96 (s, 3H), 3.35-3.37 (m, 1H), 3.38 (s, 3H), 3.45-3.62 (m, 3H), 3.66-3.74 (m, 2H), 3.78-3.84 (m, 3H), 3.86-3.90 (m 1H), 7.14-7.20 (m, 2H), 7.23-7.29 (m, 2H), 7.85 (s, 1H), 7.92 (s, 1H), 8.07 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD, 75.5 MHz):  $\delta$  22.7, 23.2, 28.4, 29.1, 33.7, 35.0, 37.2, 39.2, 45.1, 51.5, 53.4, 68.7, 70.0, 71.8, 124.3, 124.8, 125.0, 125.2, 127.0, 128.0, 128.1, 135.8, 137.2, 138.4, 142.6, 148.9, 167.6, 168.9. HRMS calcd (M+H)<sup>+</sup>: 575.2898; found 575.2899. LC-MS purity system A:  $t_R$  = 4.54 min, 97%; system B:  $t_R$  = 2.07 min, 97%.

## 4.7.32. N-{(S)-4-[(R)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-2,14-dioxo-6-oxa-3,13-diaza-bicyclo[13.3.1]nonadeca-1(19),15,17-trien-17-yl}-N-methyl-methanesulfonamide (26).

Compound **26** was synthesized from **20** according to the synthetic route for **17** and was collected as a colorless oil (17% for the final step). [ $\alpha$ ]<sub>D</sub> +31.1 (c 0.1, MeOH). <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 300 MHz):  $\delta$  1.11-1.15 (m, 2H), 1.24 (d, J = 6.9 Hz, 6H), 1.52-1.76 (m, 8H), 2.77-2.90 (m, 2H), 2.91-2.97 (m, 1H), 2.96 (s, overlapped, 3H), 3.35-3.38 (m, 1H), 3.37 (s, overlapped, 3H), 3.44-3.55 (m, 4H), 3.63-3.71 (m, 2H), 3.86-3.92 (m, 2H), 3.94-4.06 (m, 1H), 7.16-7.19 (m, 2H), 7.24-7.31 (m, 2H), 7.90-7.92 (m, 2H), 7.94-7.96 (m, 1H); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 75.5 MHz):  $\delta$  24.4, 30.1, 30.2, 30.3, 32.1, 35.3, 36.0, 38.3,

42.3, 52.6, 54.0, 54.6, 69.2, 70.2, 73.0, 126.4, 127.1, 127.5, 128.2, 128.6, 129.0, 129.8, 137.0, 138.3, 144.6, 150.7, 153.9, 169.0, 169.3. HRMS calcd  $(M+H)^+$ : 589.3054; found 589.3075. LC-MS purity system A:  $t_R = 5.07 \text{ min}$ , 96%; system B:  $t_R = 2.86 \text{ min}$ , 97%.

## 4.7.33. N-{(Z)-(S)-4-[(R)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-12-methyl-2,13-dioxo-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),8,14,16-tetraen-16-yl}-N-methyl-methanesulfonamide (27).

Compound **27** was synthesized from **12b** according to the synthetic route for **17** and was collected as a colorless oil (5% for the final step). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz): δ 1.25 (bs, 6H), 2.34 (bs, 1H), 2.52 (bs, 1H), 2.77-2.79 (m, 1H), 2.82-3.06 (m, 5H), 3.08 (bs, 3H), 3.33 (s, 3H), 3.65-3.79 (m, 5H), 3.82 (s, 2H), 3.88-4.13 (m, 3H), 5.81-5.84 (m, 2H), 6.64 (bs, 1H), 7.11-7.20 (m, 3H), 7.27 (bs, 1H), 7.54 (bs, 1H), 7.67 (s, 1H), 7.87 (bs, 1H). HRMS calcd (M+H)<sup>+</sup>: 587.2898; found 587.2916.

## 4.7.34. *N*-{(*E*)-(4*S*,11*S*)-4-[(*R*)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-2,13-dioxo-11-phenyl-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),8,14,16-tetraen-16-yl}-*N*-methyl-methanesulfonamide (28).

Compound **28** was synthesized from (*S*)-**10** according to the synthetic route for **17** and was collected as a white solid (34% for the final step). [ $\alpha$ ]<sub>D</sub> +52.7 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.25 (d, J = 6.9 Hz, 6H), 2.34 (bs, 1H), 2.71 (s, 3H), 2.75–2.95 (m, 5H), 3.17 (s, 3H), 3.47–3.79 (m 3H), 3.84 (s, 2H), 3.92–4.13 (m, 3H), 4.99–5.06 (m, 1H), 5.79–5.89 (m, 1H), 6.05–6.13 (m, 1H), 7.10–7.35 (m, 11H), 7.63 (s, 1H), 7.73 (s, 1H), 7.84 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  24.1, 34.2, 36.3, 40.7, 46.6, 53.2, 53.5, 53.9, 63.5, 69.7, 71.8, 72.2, 124.7, 125.0, 125.4, 125.9, 126.0, 126.2, 126.6, 126.9, 127.6, 128.6, 128.8, 131.6, 136.9, 139.1, 142.0, 143.1, 149.3, 166.2, 170.0. HRMS calcd (M+H)<sup>+</sup>: 649.3054; found 649.3037. LC-MS purity system A:  $t_R$  = 5.66 min, 96%; system B:  $t_R$  = 3.92 min, 97%.

## 4.7.35. N-{(E)-(4S,11R)-4-[(R)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-2,13-dioxo-11-phenyl-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),8,14,16-tetraen-16-yl}-N-methyl-methanesulfonamide (29).

Compound **29** was synthesized from (*R*)-**10** according to the synthetic route for **17** and was collected as a white solid (91% for the final step). [ $\alpha$ ]<sub>D</sub> +8.9 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.26 (d, J = 6.9 Hz, 6H), 1.95 (bs, 1H), 2.59–2.97 (m, 5H), 3.34 (s, 3H), 3.62-3.66 (m, 2H), 3.67–3.73 (m, 1H), 3.82-3.89 (m, 1H), 3.85 (s, overlapped, 3H), 3.90-3.96 (m, 1H), 4.00-4.08 (m, 1H), 4.11-4.14 (m, 1H), 4.19-4.25 (m, 1H), 5.14-5.20 (m, 1H), 5.73–5.83 (m, 1H), 5.99–6.09 (m, 1H), 6.82 (d, J = 6.9 Hz, 1H), 7.06–7.33 (m, 10H), 7.88 (s, 1H), 7.95 (s, 1H), 8.01 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  24.0, 34.1, 36.4, 37.9, 40.4, 46.4, 52.2, 52.7, 53.3, 68.3, 68.9, 71.9, 123.7, 124.6, 125.0, 125.3, 126.0, 126.1, 126.9, 127.4, 128.6, 128.7, 130.6, 132.0, 136.5, 141.4, 143.4, 149.3, 165.1, 167.2. HRMS calcd (M+H)<sup>+</sup>: 649.3054; found 649.47. LC-MS purity system A:  $t_R$  = 5.75 min, 97%; system B:  $t_R$  = 4.13 min, 96%.

### 4.7.36. (*E*)-(*S*)-4-((*R*)-2-Benzylamino-1-hydroxy-ethyl)-16-methyl-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),8,14,16-tetraene-2,13-dione (30).

Compound **30** was synthesized from 3-methoxycarbonyl-5-methylbenzoic acid according to the synthetic route for **17**, using benzylamine in the final step, and was collected as a colorless oil (48% for the final step). [ $\alpha$ ]<sub>D</sub> +31.1 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  2.28-2.51 (m, overlapped, 3H), 2.44 (s, overlapped, 3H), 2.72 (dd, J = 3.6, 12.4 Hz, 1H), 2.89 (dd, J = 5.2, 12.4 Hz, 1H), 3.30-3.48 (m, 2H), 3.53-3.65 (m, 3H), 3.69-3.74 (m, 1H), 3.85 (d, J = 11.8 Hz, 2H), 3.95-4.00 (m, 1H), 4.14-4.21 (m, 1H), 5.68-5.78 (m, 1H), 5.92-6.01 (m, 1H), 6.20 (bs, 1H), 6.75 (d, J = 7.1 Hz, 1H), 7.22-7.34 (m, 5H), 7.66 (s, 1H), 7.74 (s, 1H), 7.81 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  21.5, 32.7, 37.4, 50.9, 53.1, 54.1, 69.1, 69.4, 71.9, 122.6, 127.2, 127.3, 128.4, 128.6, 131.4, 131.7, 132.5, 135.6, 140.6, 166.8, 168.6. HRMS calcd (M+H)<sup>+</sup>: 438.2393; found 438.2404. LC-MS purity system A: t<sub>R</sub> = 3.67 min, 97%; system B: t<sub>R</sub> = 0.85 min, 98%.

## 4.7.37. (*E*)-(*S*)-4-[(*R*)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-16-methyl-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),8,14,16-tetraene-2,13-dione (31).

Compound **31** was synthesized from 3-methoxycarbonyl-5-methylbenzoic acid according to the synthetic route for **17** and was collected as a white solid (66% for the final step). [ $\alpha$ ]<sub>D</sub> +82.0 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.23 (d, J = 7.1 Hz, 6H), 2.30-2.46 (m, 2H), 2.37 (s, 3H), 2.76 (dd, J = 3.8, 12.6 Hz, 1H), 2.84-2.95 (m, 2H), 3.16-3.76 (m, 7H), 3.79 (d, J = 13.2 Hz, 1H), 3.86 (d, J = 13.2 Hz, 1H), 3.90-4.05 (m, 2H), 4.06-4.18 (m, 1H), 5.70 (dt, J = 7.5, 14.9 Hz, 1H), 5.94 (dt, J = 7.5, 14.9 Hz, 1H), 6.46 (t, J = 4.9 Hz, 1H), 6.93 (d, J = 7.4 Hz, 1H), 7.09-7.30 (m, 4H), 7.61 (s, 1H), 7.64 (s, 1H), 7.72 (s, 1H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  21.4, 24.1, 24.2, 32.7, 34.2, 37.5, 51.1, 53.3, 54.0, 69.1, 69.4, 71.9, 123.1, 125.5, 125.9, 126.7, 128.6, 131.2, 131.3, 131.5, 132.5, 135.4, 135.7, 139.0, 140.2, 149.3, 167.1, 169.0. HRMS calcd (M+H)<sup>+</sup>: 480.2857; found 480.2872. LC-MS purity system A:  $t_R$  = 4.62 min, 96%; system B:  $t_R$  = 3.97 min, 95%.

## 4.7.38. (*E*)-(4*S*,11*S*)-4-[(*R*)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-16-methyl-11-phenyl-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),8,14,16-tetraene-2,13-dione (32).

Compound **32** was synthesized from (*S*)-**9** according to the synthetic route for **17** and was collected as a white solid (61% for the final step). [ $\alpha$ ]<sub>D</sub> +101.4 (c 0.1, MeOH). <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 300 MHz):  $\delta$  1.23 (d, J = 7.1 Hz, 3H), 1.25 (d, J = 6.9 Hz, 3H), 2.33 (s, 3H), 2.46-2.72 (m, 2H), 2.75-2.98 (m, 3H), 3.58-3.90 (m, 4H), 3.92-4.09 (m, 3H), 4.13-4.24 (m, 1H), 4.97 (dd, J = 4.4, 11.4 Hz, 1H), 5.79-5.94 (m, 1H), 6.00-6.15 (m, 1H), 7.05-7.50 (m, 11H), 7.91 (s, 1H); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 75.5 MHz):  $\delta$  21.3, 24.5, 35.3, 41.9, 53.2, 54.4, 55.0, 55.8, 70.9, 71.8, 73.3, 126.5, 126.9, 127.2, 127.4, 127.8, 128.1, 129.5, 129.6, 130.9, 131.0, 132.2, 133.0, 137.6, 137.8, 139.6, 140.3, 144.4, 150.4, 170.9, 173.0. HRMS calcd (M+H)<sup>+</sup>: 556.3170; found 556.3190. LC-MS purity system A:  $t_R$  = 5.85 min, 96%; system B:  $t_R$  = 4.21 min, 95%.

4.7.39. (*E*)-(4*S*,11*R*)-4-[(*R*)-1-Hydroxy-2-(3-isopropyl-benzylamino)-ethyl]-16-methyl-11-phenyl-6-oxa-3,12-diaza-bicyclo[12.3.1]octadeca-1(18),8,14,16-tetraene-2,13-dione (33).

Compound **33** was synthesized from (*R*)-**9** according to the synthetic route for **17** and was collected as a white solid (30% for the final step). [ $\alpha$ ]<sub>D</sub> +28.9 (c 0.1, MeOH). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.24 (d, J = 7.1 Hz, 3H), 1.25 (d, J = 6.9 Hz, 3H), 2.42 (s, 3H), 2.54-2.67 (m, 1H), 2.67-2.82 (m, 2H), 2.83-2.97 (m, 2H), 3.37 (s, 1H), 3.61 (dd, J = 4.1, 8.5 Hz, 1H), 3.64-3.72 (m, 1H), 3.82 (s, 2H), 3.85-4.05 (m, 2H), 4.07-4.16 (m, 1H), 4.17-4.26 (m, 1H), 5.12-5.24 (m, 1H), 5.76 (dt, J = 7.3, 14.6 Hz, 1H), 6.03 (dt, J = 7.3, 14.6 Hz, 1H), 6.70 (d, J = 7.1 Hz, 1H), 7.00 (d, J = 8.2 Hz, 1H), 7.07-7.38 (m, 10H), 7.74 (s, 1H), 7.81 (s, 2H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  21.5, 24.2, 24.3, 34.2, 40.7, 51.0, 52.0, 52.6, 54.2, 68.7, 69.3, 72.1, 122.2, 125.3, 125.8, 126.0, 126.5, 127.4, 128.6, 128.8, 130.8, 131.8, 132.0, 132.2, 135.4, 135.5, 139.9, 140.7, 141.7, 149.3, 166.4, 167.8. HRMS calcd (M+H)<sup>+</sup>: 556.3170; found 556.3167. LC-MS purity system A:  $t_R$  = 5.96 min, 97%; system B:  $t_R$  = 4.37 min, 96%.

### **Acknowledgements**

We gratefully thank the following personnel at Medivir AB: Kurt Benkestock for helping with the HRMS measurements, Elizabeth Hamelink and Cathrine Åhgren for providing the inhibition data. Finally, we would like to acknowledge Medivir AB for performing the X-ray crystallography work and for financial support.

#### References and notes

- (1) Hardy, J.; Selkoe, D. J. *Science* **2002**, 297, 353-6.
- (2) Hunt, C. E.; Turner, A. J. Febs J **2009**, 276, 1845-59.
- (3) Luo, Y.; Bolon, B.; Kahn, S.; Bennett, B. D.; Babu-Khan, S.; Denis, P.; Fan, W.; Kha, H.; Zhang, J.; Gong, Y.; Martin, L.; Louis, J. C.; Yan, Q.; Richards, W. G.; Citron, M.; Vassar, R. *Nat Neurosci* **2001**, *4*, 231-2.
- (4) Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S. B.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappenman, K. E.; Kawabe, T. T.; Kola, I.; Kuehn, R.; Lee, M.; Liu, W.; Motter, R.; Nichols, N. F.; Power, M.; Robertson, D. W.; Schenk, D.; Schoor, M.; Shopp, G. M.; Shuck, M. E.; Sinha, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. *Hum Mol Genet* **2001**, *10*, 1317-24.
- (5) Beher, D.; Graham, S. L. *Expert Opin Investig Drugs* **2005**, *14*, 1385-409.

- (6) Silvestri, R. *Med Res Rev* **2009**, 29, 295-338.
- (7) Loughlin, W. A.; Tyndall, J. D.; Glenn, M. P.; Fairlie, D. P. *Chem Rev* **2004**, *104*, 6085-117.
- (8) Lerchner, A.; Machauer, R.; Betschart, C.; Veenstra, S.; Rueeger, H.; McCarthy, C.; Tintelnot-Blomley, M.; Jaton, A. L.; Rabe, S.; Desrayaud, S.; Enz, A.; Staufenbiel, M.; Paganetti, P.; Rondeau, J. M.; Neumann, U. *Bioorg Med Chem Lett* **2010**, *20*, 603-7.
- (9) Stachel, S. J.; Coburn, C. A.; Sankaranarayanan, S.; Price, E. A.; Wu, G.; Crouthamel, M.; Pietrak, B. L.; Huang, Q.; Lineberger, J.; Espeseth, A. S.; Jin, L.; Ellis, J.; Holloway, M. K.; Munshi, S.; Allison, T.; Hazuda, D.; Simon, A. J.; Graham, S. L.; Vacca, J. P. *J Med Chem* **2006**, *49*, 6147-50.
- (10) Ghosh, A. K.; Devasamudram, T.; Hong, L.; DeZutter, C.; Xu, X.; Weerasena, V.; Koelsch, G.; Bilcer, G.; Tang, J. *Bioorg Med Chem Lett* **2005**, *15*, 15-20.
- (11) Rojo, I.; Martin, J. A.; Broughton, H.; Timm, D.; Erickson, J.; Yang, H. C.; McCarthy, J. R. *Bioorg Med Chem Lett* **2006**, *16*, 191-5.
- (12) Machauer, R.; Laumen, K.; Veenstra, S.; Rondeau, J. M.; Tintelnot-Blomley, M.; Betschart, C.; Jaton, A. L.; Desrayaud, S.; Staufenbiel, M.; Rabe, S.; Paganetti, P.; Neumann, U. *Bioorg Med Chem Lett* **2009**, *19*, 1366-70.
- (13) Huang, Y.; Strobel, E. D.; Ho, C. Y.; Reynolds, C. H.; Conway, K. A.; Piesvaux, J. A.; Brenneman, D. E.; Yohrling, G. J.; Moore Arnold, H.; Rosenthal, D.; Alexander, R. S.; Tounge, B. A.; Mercken, M.; Vandermeeren, M.; Parker, M. H.; Reitz, A. B.; Baxter, E. W. *Bioorg Med Chem Lett* **2010**, *20*, 3158-60.
- (14) Lindsley, S. R.; Moore, K. P.; Rajapakse, H. A.; Selnick, H. G.; Young, M. B.; Zhu, H.; Munshi, S.; Kuo, L.; McGaughey, G. B.; Colussi, D.; Crouthamel, M. C.; Lai, M. T.; Pietrak, B.; Price, E. A.; Sankaranarayanan, S.; Simon, A. J.; Seabrook, G. R.; Hazuda, D. J.; Pudvah, N. T.; Hochman, J. H.; Graham, S. L.; Vacca, J. P.; Nantermet, P. G. *Bioorg Med Chem Lett* 2007, 17, 4057-61.
- (15) Moore, K. P.; Zhu, H.; Rajapakse, H. A.; McGaughey, G. B.; Colussi, D.; Price, E. A.; Sankaranarayanan, S.; Simon, A. J.; Pudvah, N. T.; Hochman, J. H.; Allison, T.; Munshi, S. K.; Graham, S. L.; Vacca, J. P.; Nantermet, P. G. *Bioorg Med Chem Lett* 2007, 17, 5831-5.
- (16) Maillard, M. C.; Hom, R. K.; Benson, T. E.; Moon, J. B.; Mamo, S.; Bienkowski, M.; Tomasselli, A. G.; Woods, D. D.; Prince, D. B.; Paddock, D. J.; Emmons, T. L.; Tucker, J. A.; Dappen, M. S.; Brogley, L.; Thorsett, E. D.; Jewett, N.; Sinha, S.; John, V. *J Med Chem* 2007, 50, 776-81.
- (17) He, L.; Byun, H.; Bittman, R. Tetrahedron Letters **1998**, 39, 2071-2074.
- (18) Calderon, F.; Doyaguez, E. G.; Fernandez-Mayoralas, A. *J Org Chem* **2006**, *71*, 6258-61.
- (19) Mori, K.; Kinsho, T. *Liebigs Annalen der Chemie* **1991**, 1991, 1309–1315.
- (20) Stachel, S. J.; Coburn, C. A.; Steele, T. G.; Jones, K. G.; Loutzenhiser, E. F.; Gregro, A. R.; Rajapakse, H. A.; Lai, M. T.; Crouthamel, M. C.; Xu, M.; Tugusheva, K.; Lineberger, J. E.; Pietrak, B. L.; Espeseth, A. S.; Shi, X. P.; Chen-Dodson, E.; Holloway, M. K.; Munshi, S.; Simon, A. J.; Kuo, L.; Vacca, J. P. *J Med Chem* **2004**, *47*, 6447-50.
- 4-Pentenylamine hydrochloride was synthesized according to a published procedure: Bäck, M.; Johansson, P. O.; Wångsell, F.; Thorstensson, F.;

Kvarnström, I.; Ayesa, S.; Wahling, H.; Pelcman, M.; Jansson, K.; Lindström, S.; Wallberg, H.; Classon, B.; Rydergård, C.; Vrang, L.; Hamelink, E.; Hallberg, A.; Rosenquist, A.; Samuelsson, B. Bioorg Med Chem 2007, 15, 7184-202. 4-Penten-1-ol (1.20 mL, 11.61 mmol), Et<sub>3</sub>N (4.9 mL, 34.83 mmol) and DMAP (0.142 g, 1.16 mmol) were dissolved in DCM (40 mL) and the solution was cooled to 0 °C. Methanesulfonyl chloride (1.35 mL, 17.42 mmol) was added and the solution was stirred at 0 °C for two hours. The reaction mixture was washed with water and the water phase was extracted with EtOAc. The organic phases were pooled, dried and evaporated carefully (volatile product) which gave the crude compound as an orange oil. The crude intermediate was dissolved in MeOH (90 mL) and conc. NH<sub>3</sub> (103 mL) was added. The solution was stirred at room temperature for 48 h. After careful evaporation to eliminate the ammonia, more water was added and the solution was washed with EtOAc twice. The water phase was acidified with HCl (1 M) and the solution was evaporated. This gave 4pentenylamine hydrochloride (1.41 g, 100% over two steps) as a white salt. <sup>1</sup>H-NMR (D<sub>2</sub>O, 300 MHz):  $\delta$  1.51-1.59 (m, 2H), 1.87-1.96 (m, 2H), 2.78 (t, J = 7.28Hz, 2H), 4.81-4.92 (m, 2H), 5.59-5.68 (m, 1H);  $^{13}$ C-NMR (D<sub>2</sub>O, 75.5 MHz):  $\delta$ 25.9, 29.9, 38.8, 115.9, 137.6.

- (22) Data not shown.
- (23) Beswick, P.; Charrier, N.; Clarke, B.; Demont, E.; Dingwall, C.; Dunsdon, R.; Faller, A.; Gleave, R.; Hawkins, J.; Hussain, I.; Johnson, C. N.; MacPherson, D.; Maile, G.; Matico, R.; Milner, P.; Mosley, J.; Naylor, A.; O'Brien, A.; Redshaw, S.; Riddell, D.; Rowland, P.; Skidmore, J.; Soleil, V.; Smith, K. J.; Stanway, S.; Stemp, G.; Stuart, A.; Sweitzer, S.; Theobald, P.; Vesey, D.; Walter, D. S.; Ward, J.; Wayne, G. *Bioorg Med Chem Lett* **2008**, *18*, 1022-6.
- (24) Sealy, J. M.; Truong, A. P.; Tso, L.; Probst, G. D.; Aquino, J.; Hom, R. K.; Jagodzinska, B. M.; Dressen, D.; Wone, D. W.; Brogley, L.; John, V.; Tung, J. S.; Pleiss, M. A.; Tucker, J. A.; Konradi, A. W.; Dappen, M. S.; Toth, G.; Pan, H.; Ruslim, L.; Miller, J.; Bova, M. P.; Sinha, S.; Quinn, K. P.; Sauer, J. M. *Bioorg Med Chem Lett* **2009**, *19*, 6386-91.
- (25) Probst, G. D.; Bowers, S.; Sealy, J. M.; Stupi, B.; Dressen, D.; Jagodzinska, B. M.; Aquino, J.; Gailunas, A.; Truong, A. P.; Tso, L.; Xu, Y. Z.; Hom, R. K.; John, V.; Tung, J. S.; Pleiss, M. A.; Tucker, J. A.; Konradi, A. W.; Sham, H. L.; Jagodzinski, J.; Toth, G.; Brecht, E.; Yao, N.; Pan, H.; Lin, M.; Artis, D. R.; Ruslim, L.; Bova, M. P.; Sinha, S.; Yednock, T. A.; Gauby, S.; Zmolek, W.; Quinn, K. P.; Sauer, J. M. *Bioorg Med Chem Lett* **2010**, *20*, 6034-9.
- (26) Clarke, B.; Cutler, L.; Demont, E.; Dingwall, C.; Dunsdon, R.; Hawkins, J.; Howes, C.; Hussain, I.; Maile, G.; Matico, R.; Mosley, J.; Naylor, A.; O'Brien, A.; Redshaw, S.; Rowland, P.; Soleil, V.; Smith, K. J.; Sweitzer, S.; Theobald, P.; Vesey, D.; Walter, D. S.; Wayne, G. *Bioorg Med Chem Lett* **2010**, *20*, 4639-44.
- (27) Bäck, M.; Nyhlén, J.; Kvarnström, I.; Appelgren, S.; Borkakoti, N.; Jansson, K.; Lindberg, J.; Nyström, S.; Hallberg, A.; Rosenquist, Å.; Samuelsson, B. *Bioorg Med Chem* **2008**, *16*, 9471-86.
- (28) Wångsell, F.; Gustafsson, K.; Kvarnström, I.; Borkakoti, N.; Edlund, M.; Jansson, K.; Lindberg, J.; Hallberg, A.; Rosenquist, Å.; Samuelsson, B. *Eur J Med Chem* **2010**, *45*, 870-82.

(29) Artursson, P.; Karlsson, J. Biochem Biophys Res Commun 1991, 175, 880-5.

### **Supporting Information**

Highly potent macrocyclic BACE-1 inhibitors incorporating a hydroxyethylamine core: Design, synthesis and X-ray crystal structures of enzyme inhibitor complexes

Veronica Sandgren,<sup>a</sup> Tatiana Agback,<sup>b</sup> Per-Ola Johansson,<sup>b</sup> Jimmy Lindberg,<sup>b</sup> Ingemar Kvarnström,<sup>a</sup> Bertil Samuelsson,<sup>b</sup> Oscar Belda,<sup>b,\*</sup> and Anders Dahlgren,<sup>a,\*</sup>

<sup>a</sup>Department of Chemistry, Linköping University, S-581 83 Linköping, Sweden <sup>b</sup>Medivir AB, Lunastigen 7, S-141 44 Huddinge, Sweden

### Synthesis of 3-isopropylbenzylamine and 3-tert-butylbenzylamine

HO R 
$$Tf_2O$$
 Pyridine  $F$   $O$  Pyridine  $F$  Pyridine  $F$   $O$  Pyridine  $F$   $O$  Pyridine  $F$   $O$  Pyridine  $F$ 

Compounds **S1** and **S3** were synthesized according to a published procedure. Compound **S2** was synthesized according to a published procedure.

**3-Isopropylbenzonitrile** (**S4**). Compound **S2** (1.46 g, 5.43 mmol) was dissolved in DMF (9 mL). Zn(CN)<sub>2</sub> (0.770 g, 6.52 mmol) and Pd(PPh<sub>3</sub>)<sub>4</sub> (0.310 g, 0.270 mmol) were added and the solution was degassed with Ar. The solution was then stirred at 80 °C under Aratmosphere for 24 hours. H<sub>2</sub>O was added and the solution was extracted with EtOAc. The combined organic phases were dried, filtered and evaporated, and the crude product was purified using flash column chromatography (hexane with 2 % EtOAc) to give compound **S4** (0.55 g, 70 %) as a colorless oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz): δ 1.24 (s, 3H), 1.27 (s, 3H), 2.87-3.00 (m, 1H), 7.32-7.50 (m, 4H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz): δ 23.7, 34.0, 112.4, 119.3, 129.2, 129.7, 130.2, 131.3, 150.2.

*tert*-Butyl 3-(*tert*-butyl)benzylcarbamate (S5). Compound S3 (0.20 g, 1.28 mmol) was dissolved in dry MeOH (10 mL) and Boc<sub>2</sub>O (0.61 g, 2.80 mmol) and NiCl<sub>2</sub>·6H<sub>2</sub>O (0.04 g,

0.17 mmol) were added. The solution was cooled to 0°C and NaBH<sub>4</sub> (0.35 g, 9.12 mmol) was added in small portions over 30 minutes, followed by stirring at room temperature for 5 h. TEA (0.18 mL, 1.28 mmol) was added and the solution was stirred for an additional 30 minutes, followed by evaporation. The residue was dissolved in EtOAc and washed with NaHCO<sub>3</sub> (aq, sat). The organic phase was dried, filtered and evaporated. The crude product was purified using flash column chromatography (100% toluene) which provided compound **S5** (0.22 g, 64%) as white crystals.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  1.33 (s, 9H), 1.48 (s, 9H), 4.31 (d, J = 5.5 Hz, 2H), 4.82 (bs, 1H), 7.08-7.11 (m, 1H), 7.29-7.32 (m, 3H).  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz):  $\delta$  28.5, 31.4, 34.8, 45.1, 79.4, 124.4, 124.6, 124.7, 128.4, 138.7, 151.6, 156.0. MS (M+H) $^{+}$  calcd: 264.2; found: 264.1.

*tert*-Butyl 3-isopropylbenzylcarbamate (S6). Compound S6 (colorless oil, 70%) was synthesized from S4 in the same manner as S5.  $^{1}$ H-NMR (CDCl<sub>3</sub>, 300 MHz): δ 1.24 (s, 3H), 1.27 (s, 3H), 1.47 (s, 9H), 2.89 (m, 1H), 4.29 (d, J = 5.8 Hz, 2H), 7.08-7.14 (m, 3H), 7.22-7.28 (m, 1H).  $^{13}$ C-NMR (CDCl<sub>3</sub>, 75.5 MHz) δ 24.1, 28.5, 34.2, 45.0, 79.4, 125.0, 125.5, 125.7, 128.7, 139.0, 149.3, 156.0. MS (M+H) $^{+}$  calcd: 250.2; found: 250.6.

**3-tert-Butylbenzylamine** (**S7**). Compound **S5** (0.11 g, 0.46 mmol) was dissolved in DCM (5 mL). Et<sub>3</sub>SiH (0.15 mL, 0.91 mmol) and TFA (2 mL) were added and the solution was stirred at room temperature for 3 h. The reaction mixture was co-evaporated with toluene and the crude product **S7** was used in the next step without any further purification.

**3-isopropylbenzylamine** (S8). Compound S8 was synthesized from S6 in the same manner as S7 and was used in the next step without further purification.

- 1. Betschart, C.; Lerchner, A.; Machauer, R.; Rueger, H.; Tintelnot-Blomley, M.; Veenstra, S. J. PCT Int. Appl. Patent US2008/132477 A1.
- 2. Petrassi, H. M.; Klabunde, T.; Sacchettini, J.; Kelly, J. W.; *J. Am. Chem. Soc.* **2000**, *122*, 2178 2192.