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Investigation of α-phenylnorstatine and α-benzylnorstatine as transition state isostere motifs in the search for new BACE-1 inhibitors

Fredrik Wångsell [a], Patrik Nordeman [a], Jonas Sävmarker [a], Rikard Emanuelsson [a], Katarina Jansson [b], Jimmy Lindberg [b], Åsa Rosenquist [b,c], Bertil Samuelsson [b,d], Mats Larhed [a,*]

[a] Organic Pharmaceutical Chemistry, Department of Medicinal Chemistry, Uppsala University, BMC, Box 574, SE-751 23 Uppsala, Sweden
[b] Medivir AB, Lunastigen 7, SE-141 44 Huddinge, Sweden
[c] Department of Chemistry, Linköping University, SE-581 83 Linköping, Sweden
[d] Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-106 91 Stockholm, Sweden

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ABSTRACT

Inhibition of the BACE-1 protease enzyme has over the recent decade developed into a promising drug strategy for Alzheimer therapy. In this report, more than 20 new BACE-1 protease inhibitors based on α-phenylnorstatine, α-benzylnorstatine, *iso*-serine, and β-alanine moieties have been prepared. The inhibitors were synthesized by applying Fmoc solid phase methodology and evaluated for their inhibitory properties. The most potent inhibitor, *tert*-alcohol containing (*R*)-**12** (IC_{50} = 0.19 μM) was co-crystallized in the active site of the BACE-1 protease, furnishing a novel binding mode in which the N-terminal amine makes a hydrogen bond to one of the catalytic aspartic acids.

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1. Introduction

Inhibition of the human aspartic protease BACE-1 (β-secretase or memapsin-2) has emerged as a promising therapeutic target for the treatment of Alzheimer's disease (AD).[1,2] AD is the most common form of dementia among the elderly and it is believed that approximately 37 million people worldwide have the disease.[3,4] Up to today the available treatment can only partly reduce the symptoms and do not affect the underlying progression of the disease.[5] Two hallmarks for AD are accumulation and aggregation of insoluble extracellular Aβ-plaques, along with intracellular neurofibrillary tangles in the brain.[6] The human aspartic protease BACE-1 is the initial protease that process amyloid precursor protein (APP) in the pathway leading to Aβ proteins.[7] Thus, inhibition of BACE-1 has emerged as a promising pharmaceutical target for combating AD. Peptide based inhibitors in which the scissile bond has been replaced with a transition state (TS) isostere have proven to be effective inhibitors of this protease.[8,9] A key structural element in most of the successful TS-isosteres is a *sec*-alcohol moiety that interacts with the catalytic aspartic acids (Asp32 and Asp228) of the BACE-1 protease via hydrogen bonds. Hence, a number of potent BACE-1 inhibitors have been reported that contain a *sec*-alcohol functionality, for example, norstatine, statine, hydroxyethylene, and hydroxyethylamine structures as non-cleavable TS-moieties.[2,10,11] Kiso and co-workers have recently reported a series of potent BACE-1 inhibitors containing a phenyl norstatine moiety as the TS-isostere, for example, inhibitor **A** (Fig. 1).[12–14]

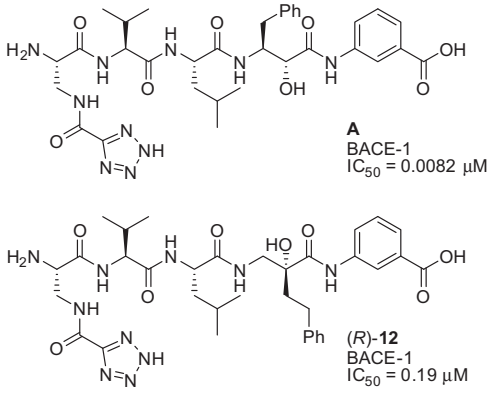

Figure 1. Previously reported phenylnorstatine (*sec*-alcohol) inhibitor **A**[12], together with one of the novel α-benzylnorstatine (*tert*-alcohol) inhibitors, (*R*)-**12**, discussed in this report.

* Corresponding author. Tel.: +46 18 471 4667; fax: +46 18 471 4474.
E-mail address: mats.larhed@orgfarm.uu.se (M. Larhed).

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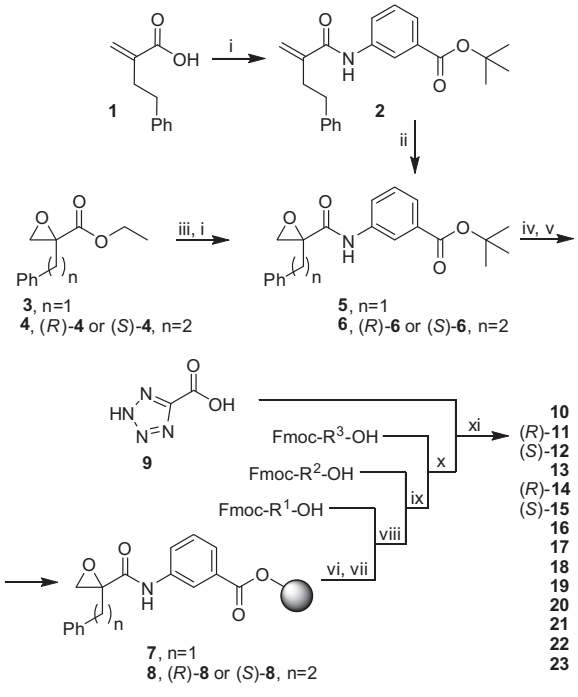
In the literature there are few examples reported where *tert*-alcohols have been used as parts of TS-isosteres in protease inhibitors.[15,16] Previous research on HIV-1 protease inhibitors comprising a *tert*-alcohol TS-mimetic indicates the possibility to retain high potency, while improving the membrane permeability.[17–19] Inspired by these results we sought to develop and evaluate a series of new *tert*-alcohol containing BACE-1 inhibitors[20,21] utilizing either a α-phenylnorstatine or a α-benzylnorstatine moiety as the central core.

2. Results and discussion

2.1. Chemistry

The synthesis of the final compounds containing the α-phenylnorstatine ($n = 1$) or the α-benzylnorstatine ($n = 2$) TS-mimic is depicted in Scheme 1. The pivotal intermediates **5**, **6**, (*R*)-**6**, and (*S*)-**6** were synthesized using two different protocols, starting from the alkene **1**, epoxide **3**, **4**, (*R*)-**4** or (*S*)-**4**. The acrylic acid **1** was synthesized from diethyl-2-phenethylmalonate in a two step protocol according to a literature procedure.[22]

Next, **1** was attached to *tert*-butyl-3-aminobenzoate by peptide coupling to render **2** in 40% yield. Epoxidation of the alkene **2** with *m*CPBA furnished the racemic epoxide **6** in a yield of 80%. The epoxides **3** and **4** were synthesized in a similar fashion[23] and access to the enantiomers (*R*)-**4** and (*S*)-**4** were achieved by chiral preparative HPLC. Hydrolysis of the esters **3**, **4**, (*R*)-**4**, and (*S*)-**4** followed by peptide coupling with *tert*-butyl-3-aminobenzoate gave the racemic compounds **5** and **6**, or the pure enantiomers (*R*)-**6** and (*S*)-**6** (37–46%). The esters were hydrolyzed to the corresponding acids and attached to the resin (2-chlorotrityl chloride resin) to furnish **7**, **8**, (*R*)-**8**, and (*S*)-**8**. The epoxide was opened with ammonia in DMF and diversified with different Fmoc protected amino acids by solid phase chemistry. Finally the tetrazole **9**[24] was attached and the final BACE-1 inhibitors **10**–**23** were cleaved of the resin and purified by HPLC in a yield of 3–17% (calculated from **5**, **6**, (*R*)-**6** or (*S*)-**6**).



Scheme 1. Reagents and conditions: (i) *tert*-butyl-3-aminobenzoate, HATU, DIPEA, CH_2Cl_2 (dry), 40 °C, 37–46%; (ii) *m*CPBA, CH_2Cl_2, reflux, 80%; (iii) KOH, EtOH (99%) rt, quant.; (iv) TFA, CH_2Cl_2, rt, quant.; (v) 2-chlorotrityl chloride resin, DIPEA, CH_2Cl_2 (dry); (vi) NH_3 (g) DMF; (vii) Fmoc-L-Leu-OH or Fmoc-L-Cha-OH, PyBOP, DIPEA, DMF; (viii) (1) 20% piperidine in DMF; (2) Fmoc-L-Val-OH, Fmoc-L-β-Leu-OH, Fmoc-L-Nle-OH or Fmoc-L-Phe-OH, PyBOP, DIPEA, DMF; (ix) (1) 20% piperidine in DMF; (2) Boc-L-DAP(Fmoc)-OH, Boc-D-DAP(Fmoc)-OH, Fmoc-L-Ser(^{t}Bu)-OH or Fmoc-D-Ser(^{t}Bu)-OH, PyBOP, DIPEA, DMF; (x) **9**, PyBOP, DIPEA, DMF, DMSO, H_2O; (xi) TFA (95% aq), Et_3SiH.

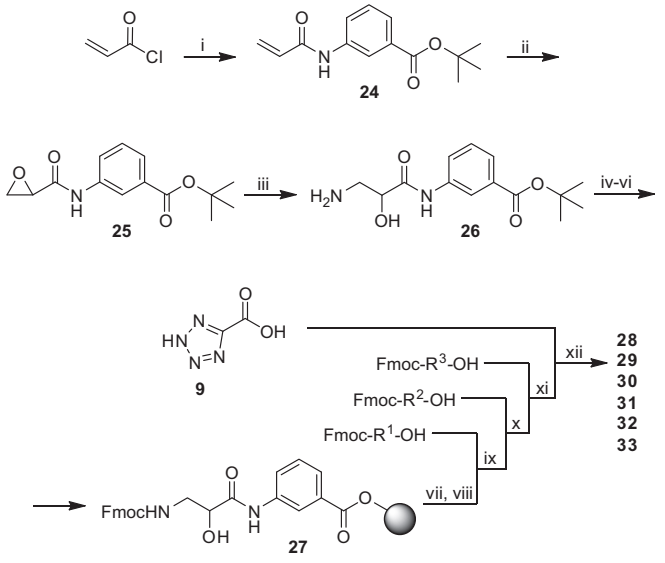
Inhibitors with secondary alcohols were also synthesized in order to evaluate the importance of the phenyl- and benzyl-group (Scheme 2). The commercially available acryloyl chloride was reacted with *tert*-butyl-3-aminobenzoate to give the alkene **24** (82%). The alkene was treated with *m*CPBA which gave epoxide **25** (racemate) and after subsequent opening with 25% aqueous ammonia the primary amine (**26**) was protected with Fmoc-Cl.[25] After removal of the *tert*-butyl protecting group the corresponding acid was coupled to the resin (2-chlorotrityl chloride resin) to give **27**. The immobilized **27** was treated with 20% piperidine in DMF to give the corresponding amine. Using Fmoc protected amino acids the inhibitors **28**–**33** were produced and purified with HPLC (9–16% by yield calculated from structure **26**).

To further evaluate the α-phenylnorstatine and the α-benzylnorstatine scaffold, inhibitors without the *tert*-alcohol and the aromatic tether were synthesized (Scheme 3). Readily available Fmoc-3-Abz-OH was attached to the resin to furnish **34**. The inhibitors **35–38** were synthesized by solid phase chemistry as described above in a yield of 12–35% (calculated from Fmoc-3-Abz-OH).

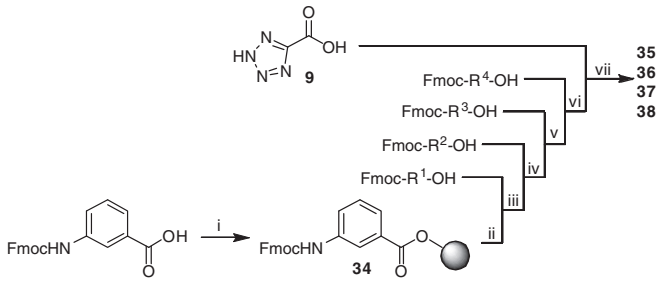
Two compounds (**39**, **40**) with reduced C-terminal were prepared using solid phase synthesis, beginning from Fmoc protected *iso*-serine and leucine (Scheme 4). The protected acid was first coupled to the resin (2-chlorotrityl chloride resin) with subsequent removal of the Fmoc group by 20% piperidine in DMF. After the subsequent coupling cycles the peptide was liberated 95% aqueous TFA with 5% Et_3SiH, and purified with HPLC (**39**, 12% and **40**, 23% yield from respective acid before coupling).

2.2. X-ray crystal structure results

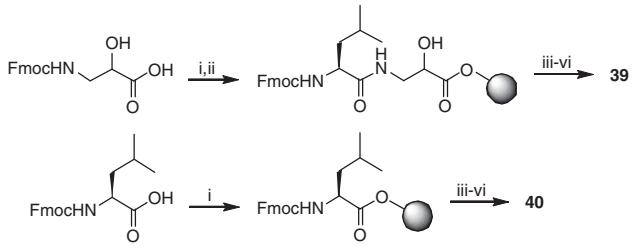
One inhibitor, (*R*)-**12**, was co-crystallized together with BACE-1 providing an X-ray crystal structure (PDB-code: 3KYR, Fig. 2). Anal-



Scheme 2. Reagents and conditions: (i) *tert*-butyl-3-aminobenzoate, CH_2Cl_2 (dry), 0 °C, 82%; (ii) *m*CPBA, CH_2Cl_2, reflux, 42%; (iii) NH_3 (25% aq), 60 °C, quant.; (iv) Fmoc-Cl, $NaHCO_3$, dioxane, 0 °C, 95%; (v) TFA, CH_2Cl_2, rt, quant.; (vi) 2-chlorotrityl chloride resin, DIPEA, CH_2Cl_2 (dry); (vii) 20% piperidine in DMF; (viii) Fmoc-L-Leu-OH or Fmoc-L-Val-OH, PyBOP, DIPEA, DMF; (ix) (1) 20% piperidine in DMF; (1) Fmoc-L-Val-OH, PyBOP, DIPEA, DMF; (x) (1) 20% piperidine in DMF; (2) Boc-L-DAP(Fmoc)-OH, Boc-D-DAP(Fmoc)-OH or Fmoc-L-Ser(^{t}Bu)-OH, PyBOP, DIPEA, DMF; (xi) **9**, PyBOP, DIPEA, DMF, DMSO, H_2O; (xii) TFA (95% aq), Et_3SiH.



Scheme 3. Reagent and conditions: (i) 2-chlorotrityl chloride resin, DIPEA, CH_2Cl_2 (dry); (ii) (1) 20% piperidine in DMF; (2) Fmoc-β-Ala-OH, PyBOP, DIPEA, DMF; (iii) (1) 20% piperidine in DMF; (2) Fmoc-L-Leu-OH, PyBOP, DIPEA, DMF; (iv) (1) 20% piperidine in DMF; (2) Fmoc-L-Val-OH, PyBOP, DIPEA, DMF; (v) (1) 20% piperidine in DMF; (2) Boc-L-DAP(Fmoc)-OH, Boc-D-DAP(Fmoc)-OH, Fmoc-L-Ser(^{t}Bu)-OH or Fmoc-D-Ser(^{t}Bu)-OH, PyBOP, DIPEA, DMF; (vi) **9**, PyBOP, DIPEA, DMF, DMSO, H_2O; (vii) TFA (95% aq), Et_3SiH.



Scheme 4. Reagent and conditions: (i) 2-chlorotrityl chloride resin, DIPEA, CH_2Cl_2 (dry); (ii) (1) 20% piperidine in DMF; (2) Fmoc-L-Leu-OH, PyBOP, DIPEA, DMF; (iii) (1) 20% piperidine in DMF; (2) Fmoc-L-Val-OH, PyBOP, DIPEA, DMF; (iv) (1) 20% piperidine in DMF; (2) Boc-L-DAP(Fmoc)-OH, PyBOP, DIPEA, DMF; (v) **9**, PyBOP, DIPEA, DMF, DMSO, H_2O; (vi) TFA (95% aq), Et_3SiH.

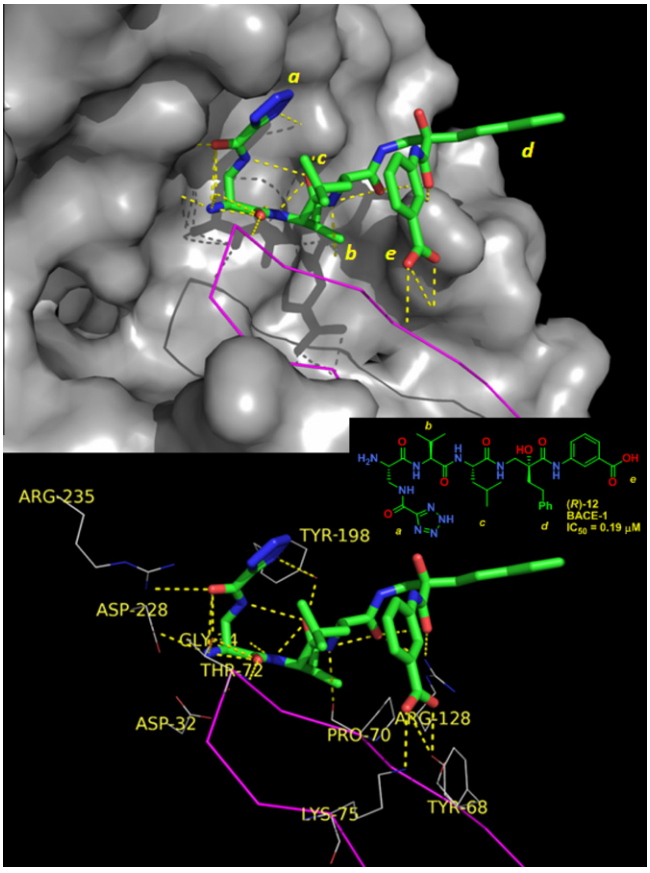


Figure 2. X-ray crystal structure of inhibitor (*R*)-**12** (green) and BACE-1 (PDB-code: 3KYR) showing the polar key interactions between the inhibitor and enzyme. Flap residues (66–77) are marked in magenta.

ysis of the crystal structure revealed a number of interactions. The N-terminal amine, and not the tertiary alcohol, makes a hydrogen bond to Asp228. Further the tetrazole carbonyl (*a*) is in proximity to Arg235 and one of the nitrogens in the tetrazole makes a hydrogen bond to Tyr198. The N-terminal carbonyl in the inhibitor interacts with the flap residue Thr72. Additionally the Val-NH (*b*) coordinates to Gly34 and the Val-carbonyl with Thr198. As deduced from the X-ray crystallography, the Leu-NH (*c*) hydrogen bonds to Pro70 (flap) and the Leu-carbonyl is closely situated to Thr198. The *C*-terminal carboxylic functionality (*e*) in (*R*)-**12** makes polar interactions with Thr68 and Lys75 and the carbonyl next to the *tert*-alcohol makes a hydrogen bond to Arg128. Several internal interactions in (*R*)-**12** lock the inhibitor in its arrangement. Interestingly, the phenylethyl side chain (*d*) of the α-benzylnorstatine isostere extends beyond the protein surface; therefore this moiety might not be necessary for protein interaction.

2.3. Biological evaluation

The structures of all the inhibitors together with the inhibitory data (BACE-1 IC_{50}) are summarized in Table 1. Compared to **A**[12] (IC_{50} = 8.2 nM, Fig. 1) the interchange from the phenylnorstatine (*sec*-alcohol) TS-mimic to the α-phenylnorstatine (*tert*-alcohol) containing core structure gave a compound with declined inhibitory properties (**10**, epimeric mixture (1:1), IC_{50} = 0.43 μM). Elongation of the tether by one carbon (α-benzylnorstatine) gave an equipotent inhibitor (**11**, IC_{50} = 0.40 μM) and the corresponding diastereomers (*R*)-**12** and (*S*)-**13** provided IC_{50} values of 0.19 and 1.4 μM, respectively. As mentioned vide supra, (*R*)-**12** was co-crystallized together with the BACE-1 enzyme. The disclosed information from this X-ray structure was used in the search for inhibitors with more desirable inhibitory properties. By replacing the leucine (*c*) to the larger Chg, a slight change in activity (**14**, IC_{50} = 0.68 μM) was observed. The alteration from the valine (*b*) to Phe, Nle or Leu resulted in complete loss in inhibitory properties: the inhibitors showed no inhibition against the BACE-1 protease, exemplified by comparing **11** with (*R*)-**15** and (*S*)-**16** (Phe, IC_{50} >10 μM), **17** (Nle, IC_{50} >10 μM) and **18** (Leu, IC_{50} >10 μM). Introduction of β-Leu (**19**, IC_{50} >10 μM) as well as reversing the stereochemistry (L- to D- in the *a*-position, cf. **11** and **20**, IC_{50} >10 μM) in the N-terminal resulted in inactive inhibitors. Important interactions with the enzyme were apparently lost by removal of the tetrazole moiety (*a*) (**21**, IC_{50} >10 μM). The X-ray crystallographic data revealed that the N-terminal (R-NH_2) was positioned close to one of the catalytic aspartic residues (Asp228), and that the *tert*-hydroxy group not served as a functional TS-isostere. From these findings inhibitors with a serine analogue in the N-terminal were evaluated with the aim of providing closer contact with the aspartic residues. This attempt resulted in the inactive inhibitors **22** and **23** (L- and D-serine, IC_{50} >10 μM). Notably, a one carbon contraction in the tetrazole moiety (*a*) could also explain the loss in activity. Further conclusions from the X-ray crystal structure of (*R*)-**12** disclosed that the aromatic tether (*d*) was exposed to the solvent and the *tert*-alcohol moiety did not contributed with important interactions with the BACE-1 enzyme (Fig. 2). On the basis of this molecular insight, the inhibitors lacking the *tert*-alcohol were designed and produced. Removing the phenyl- and benzylnorstatine gave access to *iso*-serine comprising *sec*-alcohols **28–33**. Compound **28** (IC_{50} = 0.44 μM) showed almost equal inhibitory properties as the inhibitors **10** and **11**, proving that the substituents in *d*-position do not participate in the enzyme binding. By shortening (*c*) from leucine to valine a loss of activity was observed (**29**, IC_{50} = 3 μM). Removing the tetrazole moiety or incorporating the serine analogue

Table 1
BACE-1 inhibition data of the inhibitors

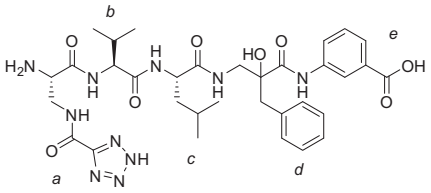
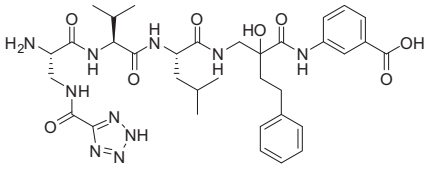
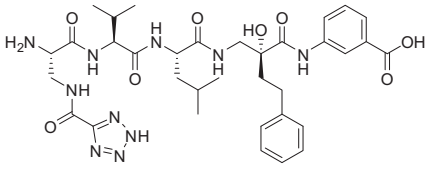
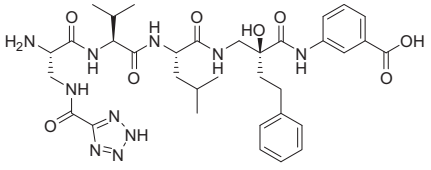
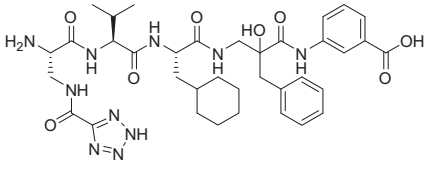
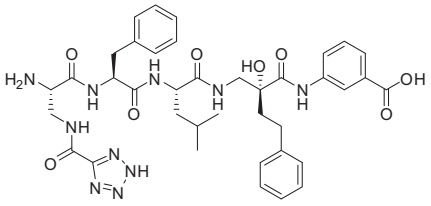
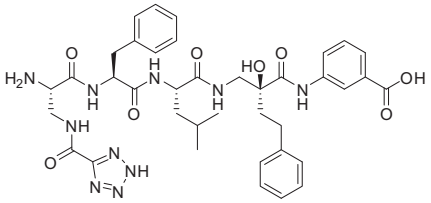
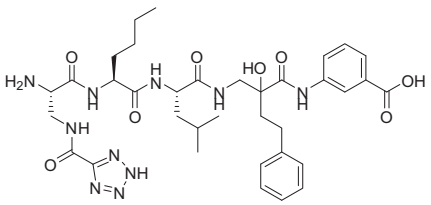
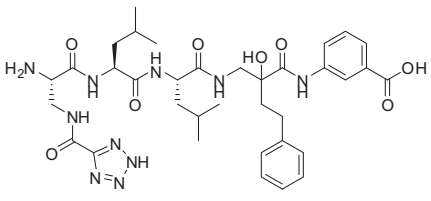
Compd	Structure	IC_{50} (μM)
10		0.43[a]
11		0.40[a]
(*R*)-**12**		0.19
(*S*)-**13**		1.4
14		0.68[a]
(*R*)-**15**		>10
(*S*)-**16**		>10
17		>10[a]
18		>10[a]

Table 1 (*continued*)

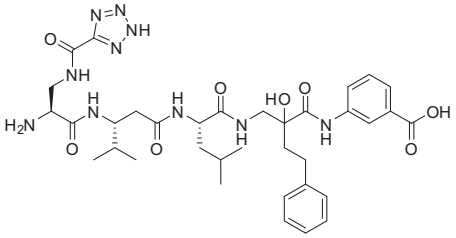
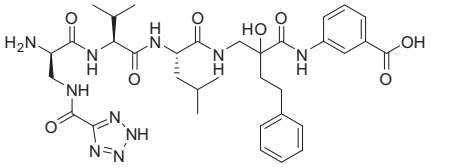
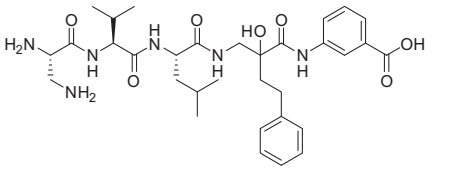
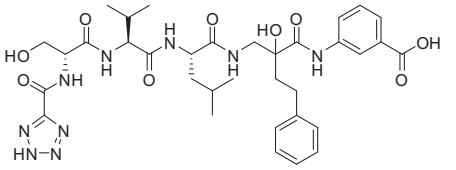
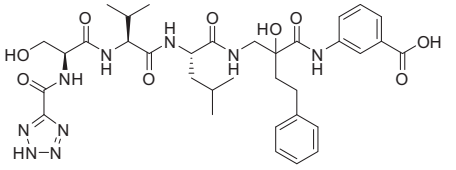
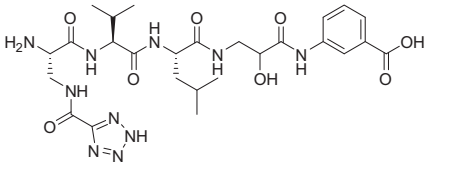
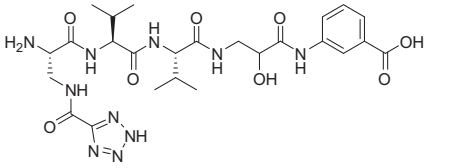
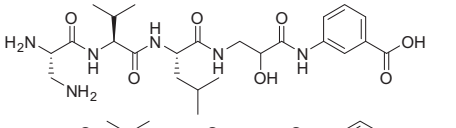
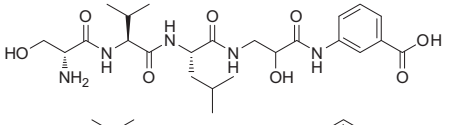
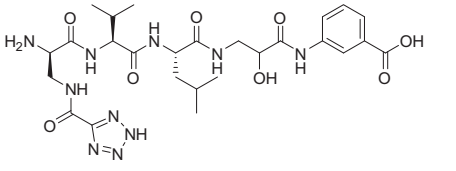
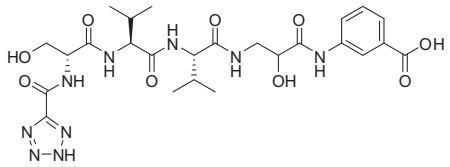
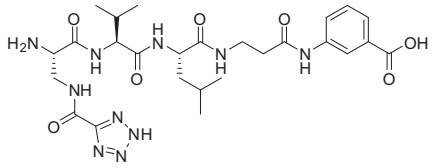
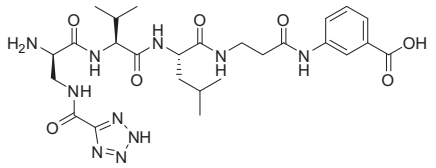
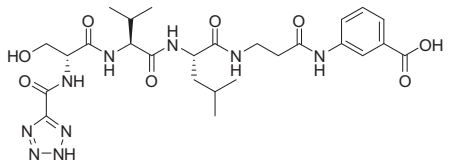
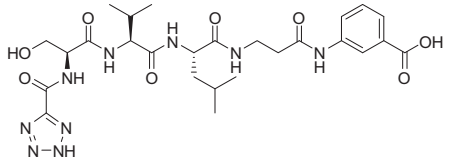
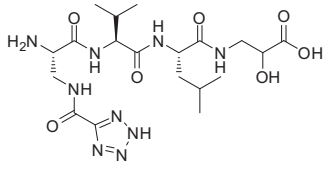
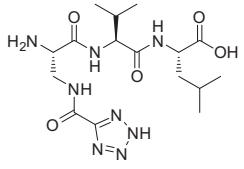
Compd	Structure	IC_{50} (μM)
19		>10[a]
20		>10[a]
21		>10[a]
22		>10[a]
23		>10[a]
28		0.44[a]
29		3[a]
30		>10[a]
31		>10[a]
32		>10[a]

Table 1 (*continued*)

Compd	Structure	IC_{50} (μM)
33		>10[a]
35		0.87
36		>10
37		>10
38		>10
39		6.1[a]
40		4.4

[a] Diastereomeric mixture (1:1).

also resulted in reduced activity (**30–33**, IC_{50} >10 μM). To further expand the investigation, compounds lacking both hydroxyl group and phenyl- and benzylstatine moiety were synthesized. Interestingly, one of these compounds, **35**, showed inhibitory properties (IC_{50} = 0.87 μM), supporting the X-ray enzyme–ligand complex structure. The inhibitors comprising the Ser moiety as well as the D-configuration in the N-terminal residue furnished inactive inhibitors (**36**, **37** and **38**, IC_{50} >10 μM). Deletion of the benzoic acid group (*e*) and the α-phenylethyl moiety (*d*) and/or the *tert*/*sec*-alcohol functionality resulted in less potent compounds **39** and **40** (6.1 and 4.4 μM).

3. Conclusion

The *tert*-alcohol containing α-phenylnorstatine or α-benzylnorstatine moieties were evaluated as the central core structures in the search for drug-like BACE-1 inhibitors. Also inhibitors lacking the *tert*-alcohol moiety were produced, which showed promising activity against the BACE-1 enzyme. More than twenty inhibitors were synthesized and the most potent inhibitor, (*R*)-**12** (IC_{50} = 0.19 μM), was co-crystallized (PDB-code: 3KYR) together with the BACE-1 enzyme. From the solved structure a novel binding mode for this class of inhibitors were disclosed, in which the N-terminal amine and not the *tert*-hydroxy group served as the TS-isostere. Subtraction of the hydroxyl group also furnished active compound **35** which strengthens the theory of the new binding mode. Removal of the benzoic acid moiety decreased the activity 10-fold (**39**, **40**). All these findings might be of importance for further drug discovery research. Nevertheless, the search for potent BACE-1 inhibitors continues to be a difficult challenge.

4. Experimental

4.1. General

Standard ^1H NMR (399.9 MHz or 300.0 MHz if stated) and ^{13}C NMR (100.6 MHz or 75.4 MHz if stated) spectra were recorded using $(CD_3)_2SO$, $CDCl_3$ or methanol-d_4 (CD_3OD) as δ values (ppm) referenced to TMS (0.00 ppm) or the solvent residual signal. In the recorded NMR spectra of diastereomeric mixtures the presumed diastereomeric peaks were put into brackets with the general formula [nn.n & nn.n] for ^{13}C NMR and [n.nn & n.nn, (x, yH)] for ^1H NMR, respectively. TLC was carried out on Merck precoated 60 F_{254} aluminum plates (0.2 mm) using UV-light (254 nm) and charring with ethanol/sulfuric acid/acetic acid/*p*-anisaldehyde (90:3:1:2) or a solution of 0.5% ninhydrin in ethanol (95%) for visualization. Flash column chromatography was performed using Silica Gel 60 (0.040–0.063 mm, Merck). Analytical LC/MS was performed on a Gilson HPLC system with a Finnigan AQA quadropole mass spectrometer using a C18 (5 μm 4.6 × 100 mm) column, with a gradient of CH_3CN in 0.05% aqueous HCOOH as mobile phase at a flow rate of 4 mL/min. Dry dichloromethane (CH_2Cl_2) was achieved by refluxing the solvent over calcium hydride and distilled onto 4 Å molecular sieves. Optical rotations were obtained on a Perkin–Elmer 241 polarimeter. Specific optical rotations ($[\alpha]_D^T$) are reported in deg/dm at ambient temperature (T) in the specified solvent and the concentration (c) is given as g/100 mL. Evaporations were performed under diminished pressure (7–10 mbar) at a water bath temperature of 35 °C. Organic extracts were dried over magnesium sulfate monohydrate and filtered. Starting materials and reagents were purchased from commercial suppliers and used without further purification. Compound **24**[26] is known and the spectroscopic data are in agreement with the literature values. Tetrazole **9** was synthesized according to the literature procedure.[24]

4.1.1. General procedure for the loading of substrate onto resin

A tube was charged with 2-chlorotrityl chloride resin (substitution: 1.41 mmol/g resin, the polymer matrix was copoly(styrene-1% DVB), 100–200 mesh) (1.5 equiv relative to the loading acid substrate) and dry CH_2Cl_2 (substrate concentration 0.1 M) and *N*,*N*-diisopropylethylamine (DIPEA) (4 equiv) was added and the tube was capped. After the mixture had been agitated for 3 h, unreacted linkers was capped with MeOH (0.5 mL) during 15 min before the resin was filtered off and washed with several portions of DMF, CH_2Cl_2, and finally MeOH. The resin was dried in vacuo overnight yielding the corresponding substrate attached to the 2-chlorotrityl chloride resin.

4.1.2. General procedure for the epoxide opening with ammonia on solid support

To a falcon tube charged with the epoxide attached to the resin, DMF was added and the solution was cooled to 0 °C. NH_3 (g) was added via a needle into the solution. After bubbling NH_3 (g) for 15 min the tube was capped and agitated for 4 h at ambient tem-

perature. The solution was then re-cooled and the procedure repeated until the reaction went to completion. Monitoring and analysis were performed by analytical LC/MS after removal of a small portion of the resin and subsequent release of the product by addition of TFA (100 μL, 95% aq) and triethylsilane (Et_3SiH) (10 μL). The resin was filtered off and washed with several portions of DMF, CH_2Cl_2 and finally MeOH. Thereafter, the resin was dried in vacuo overnight yielding the corresponding amine compound attached to the resin.

4.1.3. General procedure for the synthesis of BACE-1 inhibitors on solid support

The substrate attached to the resin (mmol calculated from the substrate loading procedure) was weighted into a 2 mL syringe fitted with porous polyethylene filter. The Fmoc group was removed (if necessary) by treatment with 20% piperidine in DMF (2 × 1.5 mL, 10 + 15 min) and the resin was washed with DMF (4 × 1.5 mL, 4 × 1 min). Coupling of the appropriate (Fmoc protected) amino acid was performed by agitation overnight in DMF (2 mL), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (2 equiv) and DIPEA (6 equiv). The resin was washed with DMF (4 × 1.5 mL, 4 × 1 min) and subsequently Fmoc-deprotected and washed as described above. Finally, after three or four coupling procedures, the tetrazole derivative (**9**) was attached as described in the General procedure for attaching the tetrazole carboxylate. After completion of the coupling cycles, the resin was washed with several portions of DMF, CH_2Cl_2, and finally MeOH before it was dried in vacuo. The peptide was cleaved of the resin by treatment with TFA (95% aq, 2.5 mL) and Et_3SiH (100 μL) for 2 h in a capped tube. The resin was filtered off and washed with TFA (3 × 0.5 mL). The filtrate was collected in a centrifuge tube and concentrated in a stream of N_2 (g). Cold diethyl ether (12 mL) was used to precipitate the product as a TFA-salt, which was collected by centrifugation, washed with cold diethyl ether (2 × 3 mL) and dried.

4.1.4. General procedure for the attachment of the tetrazole carboxylate

The dilithium or dipotassium salt (generally the dilithium salt was more soluble) of the tetrazole carboxylate[24] (2 equiv relative to substrate loading) was dissolved in DMF (0.75 mL), DMSO (0.75 mL), DIPEA (6 equiv) and minimum amount of water (~150–200 μL). The solution was gently heated and subject to ultrasonic treatment to dissolve the tetrazole salt. PyBOP (2 equiv) was separately dissolved in minimum amount of DMF (~200 μL) and both solutions added to the syringe via the needle containing the resin and agitated overnight at ambient temperature. The resin was filtered off and washed with several portions of DMF, CH_2Cl_2, and finally MeOH. Thereafter, the resin was dried in vacuo overnight yielding the corresponding product.

4.1.5. General procedure for the purification of the compounds (*R*)-4 and (*S*)-4

Chiral LC–MS was performed on the Gilson HPLC system in straight-phase using 2-Propanol and iso-Hexane using isocratic elution (iso-hexane/2-propanol, 99.25:0.75) and Reprosil Chiral NR columns 8 μm 250 × 4.6 mm and 8 μm 250 × 20 mm (Dr. Maisch GmbH) with flow rate of 10 mL/min. Several repetitions were needed in order to achieve sufficient separation.

4.1.6. General procedure for the purification of the final inhibitors

The TFA-salt of the crude BACE-1 inhibitor was dissolved in MeCN/H_2O (2 mL, 3:1). Preparative HPLC was performed using 5–65% gradient (MeCN containing 0.09% TFA and H_2O containing 0.09% TFA) over 60 min with UV-detection at 254 nm. Analysis of the fractions were performed using analytical HPLC, 30–60% over 5 min at a wavelength of 220 nm.

4.1.7. General procedure for the HPLC purity analysis of the final inhibitors

Purity analysis was performed on two different HPLC systems with UV- and ELSD-detector (Sedex 55), respectively or by using different columns (C4 and C18). The purity for the corresponding final compound together with the retention time (rt, min) is reported. HPLC analysis with UV (254 nm) detection was performed on a Gilson-Finnegan ThermoQuest AQA system equipped with a C18 (50 × 4.6 mm) and C4 (ACT C4 4.6 × 50 mm, 5 μm) column, using CH_3CN (or MeOH if stated) in 0.05% aqueous HCOOH as mobile phase at a flow rate of 4 mL/min, 10 min gradient from 10% to 100% MeCN (or MeOH if stated). HPLC analysis with ELSD detection was performed on a Gilson-Finnigan MSQ system equipped with a C18 (50 × 4.6 mm) column, using CH_3CN (or MeOH if stated) in 0.05% aqueous HCOOH as mobile phase at a flow rate of 4 mL/min, 10 min gradient from 10% to 100% MeCN (or MeOH if stated).

4.1.7.1. 3-(2-Methylene-4-phenyl-butyrylamino)-benzoic acid *tert*-butyl ester (2). The acid **1** (0.50 g, 2.84 mmol), prepared according to literature procedure[21] starting from diethyl 2-phenethylmalonate instead of diethyl 2-benzylmalonate, was dissolved in CH_2Cl_2. *tert*-Butyl-3-aminobenzoate (0.55 g, 2.84 mmol), DIPEA (1.52 mL, 8.52 mmol) and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetra-methyluronium hexafluorophosphate (HATU) (1.18 g, 3.12 mmol) was added. The reaction mixture was stirred at 40 °C in 14 h. The reaction mixture was concentrated and subsequent purification by flash column chromatography (*iso*-hexane/ethyl acetate, 9:1) afforded the desired compound **2** (0.36 g, 36%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.60 (s, 9H), 2.71 (d, J = 8.4 Hz, 1H), 2.72 (d, J = 9.4 Hz, 1H), 2.82 (d, J = 8.4 Hz, 1H), 2.83 (d, J = 9.4 Hz, 1H), 5.37 (s, 1H), 5.74 (s, 1H), 7.17–7.21 (m, 3H), 7.25–7.30 (m, 2H), 7.32 (m, 1H), 7.72 (m, 1H), 7.94 (m, 1H), 8.06 (m, 1H), 8.08 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.1, 34.2, 34.5, 81.2, 118.9, 121.1, 124.4, 125.3, 126.1, 128.4, 128.5, 128.9, 132.7, 138.0, 141.1, 145.2, 165.3, 167.2; MS *m/z* 352.1 [$(M+H)^+$ calcd for: $C_{22}H_{26}NO_3^+$ 352.19].

4.1.7.2. 3-[(2-Benzyl-oxiranecarbonyl)-amino]-benzoic acid *tert*-butyl ester (5). To the ester **3**[23] (136 mg, 0.66 mmol) was 0.5 M (99% EtOH) KOH (1.32 mmol, 2.63 mL) added at 0 °C. The mixture was allowed to attain room temperature, stirred for 2 h, and the mixture was concentrated. The residue was dissolved in CH_2Cl_2 (2 mL), water (2 mL), and 1 M (aq) HCl (0.5 mL) was added. The reaction mixture was extracted with CH_2Cl_2 (3 × 2 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated to afford the corresponding acid which was used in the next step without further purification. The acid was dissolved in dry CH_2Cl_2 (3.3 mL) and 3-amino-benzoic acid *tert*-butyl ester (127 mg, 0.658 mmol), DIPEA (344 μL, 1.97 mmol) and HATU (275 mg, 0.724 mmol) was added. After 3 h at 30 °C the crude mixture was concentrated. Purification by flash column chromatography (*iso*-hexane/ethyl acetate, 6:1) yielded the desired **5** (103 mg, 44%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.56 (s, 9H), 2.85 (d, J = 4.7 Hz, 1H), 2.93 (d, J = 4.7 Hz, 1H), 3.02 (d, J = 14.7 Hz, 1H), 3.67 (d, J = 14.7 Hz, 1H), 7.18–7.29 (m, 5H), 7.34 (t, J = 8.0 Hz, 1H), 7.70 (dt, J = 7.8, 1.4 Hz, 1H), 7.82–7.88 (m, 2H), 8.09 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.1, 35.9, 52.7, 59.9, 81.3, 120.4, 123.6, 125.5, 127.0, 128.4, 129.0, 129.8, 132.8, 135.1, 136.9, 165.1, 167.7; MS *m/z* 354.1 [$(M+H)^+$ calcd for $C_{21}H_{24}NO_4^+$ 354.42].

4.1.7.3. 3-[(2-Phenethyl-oxiranecarbonyl)-amino]-benzoic acid *tert*-butyl ester (6, (*R*)-6, and (*S*)-6). From **4**, (*R*)-**4** or (*S*)-**4**: compound **6** (103 mg, yield 44%), (*R*)-**6** (98 mg, yield 37%) and (*S*)-**6** (121 mg, yield 46%) were prepared from **4** (136 mg,

0.658 mmol), (*R*)-**4** (160 mg, 0.727 mmol) or (*S*)-**4** (158 mg, 0.716 mmol) according to the same procedure as for compound **5**. Compound **6**: colorless oil. From **2**: The alkene **2** (0.105 g, 0.299 mmol) was dissolved in CH_2Cl_2 (5 mL), and *m*CPBA (70%, 221 mg, 0.896 mmol) was added. The stirred solution was heated at reflux temperature. After 4 h, additional *m*CPBA (111 mg, 0.448 mmol) was added, and the reaction mixture was stirred for 20 h. The reaction mixture was concentrated and dissolved in CH_2Cl_2 (20 mL), washed with 10% (aq) Na_2SO_3 (25 mL) and satd (aq) $NaHCO_3$ (3 × 25 mL). The organic layer was dried with $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (*iso*-hexane/ethyl acetate, 6:1) yielded **6** (0.087 g, 80%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ 1.60 (s, 9H), 1.87 (m, 1H), 2.72–2.86 (m, 3H), 2.96 (s, 2H), 7.16–7.31 (m, 5H), 7.40 (t, J = 7.8 Hz, 1H), 7.76 (dt, J = 7.8, 1.2 Hz, 1H), 7.91–7.95 (m, 2H), 8.20 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.1, 30.9, 32.7, 53.3, 59.4, 81.3, 120.4, 123.6, 125.5, 126.1, 128.4 (2C), 129.0, 132.8, 136.9, 140.7, 165.1, 167.9; MS *m/z* 368.2 [(M+H)$^+$ calcd for: $C_{22}H_{26}NO_4^+$ 368.19].

4.1.7.4. 3-Acryloylamino-benzoic acid *tert*-butyl ester (24). *tert*-Butyl-3-aminobenzoate (1.06 g, 5.5 mmol) was dissolved in dry CH_2Cl_2 (30 mL) under nitrogen atmosphere and stirred at 0 °C. Acryloyl chloride (0.9 mL, 11 mmol, 2 equiv) was slowly added and the solution was stirred for 2 h. The cloudy mixture was then washed two times with brine (2 × 20 mL), dried over $MgSO_4$, filtered and concentrated to give product **24** as white powder (1.11 g, 82%) and used without further purification. ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 9H), 5.80 (d, J = 10.2 Hz, 1H), 6.26 (dd, J = 16.8, 10.2 Hz, 1H), 6.46 (d, J = 16.8 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.92–7.24 (m, 1H), 7.99–8.03 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 28.3, 81.5, 120.7, 124.2, 124.7, 125.7, 128.4, 129.3, 131.1, 133.0, 137.9, 163.7, 165.4; MS *m/z* 495.70 [(2M+H)$^+$ calcd for $C_{14}H_{18}NO_3^+$ 248.13].

4.1.7.5. 3-(Oxiranecarbonyl-amino)-benzoic acid *tert*-butyl ester (25). Compound **24** (1.11 g, 4.5 mmol) was dissolved in CH_2Cl_2 (20 mL) and *m*CPBA (12.6 g, 45 mmol) was added to the solution. The mixture was refluxed for 24 h, then additional *m*CPBA (2.8 g, 10 mmol) was added and the reaction was then further refluxed over night. The yellow solution was washed with 10% (aq) $Na_2S_2O_3$ (50 mL), washed three times with satd $NaHCO_3$ (30 mL) solution and then one time with brine (20 mL). The aqueous $NaHCO_3$ and brine was extracted once with CH_2Cl_2 and all the organic layers were dried over $MgSO_4$, filtered and concentrated. The mixture was purified by flash column chromatography (*iso*-hexane/ethyl acetate 2.5:1.5) yielding **25** (506 mg, 42%) as white powder. ^{1}H NMR ($CDCl_3$) δ 1.57 (s, 9H), 2.92 (dd, J = 5.4, 2.6 Hz, 1H), 3.09 (t, J = 5.6 Hz, 1H), 3.57 (dd, J = 4.6, 2.6, 1H), 7.38 (t, J = 8.3 Hz, 1H), 7.74 (d, J = 8.3 Hz, 1H), 7.96–7.98 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 28.3, 47.9, 50.1, 81.5 120.6, 123.9, 125.9, 129.3, 133.1, 136.9, 165.2, 166.7; MS *m/z* 527.45 [(2M+H)$^+$ calcd for $C_{14}H_{18}NO_4^+$ 264.12].

4.1.7.6. 3-(3-Amino-2-hydroxy-propionylamino)-benzoic acid *tert*-butyl ester (26). Compound **25** (100 mg, 3.8 mmol) was dissolved in *tert*-BuOH (10 mL) and slowly added to a 25% aqueous ammonia solution (100 mL) stirred at 60 °C over a period of 30 min. The reaction was left for 6 h and then the yellow solution was evaporated and dried in vacuo. The amine could be retrieved in quantitative yield and was used in the next step without purification. ^{1}H NMR (DMSO) δ 1.54 (s, 9H), 2.73 (dd, J = 12.7, 6.0 Hz, 1H), 2.85 (d, J = 12.7 Hz, 1H), 3.96–4.00 (m, 1H), 7.41 (t, J = 8.0, 1H), 7.58 (t, J = 7.4 Hz, 1H), 7.93 (d, J = 8.6 Hz, 1H), 8.31–8.34 (m, 1H); ^{13}C NMR (DMSO) δ 27.8, 45.7, 73.7, 80.7, 120.2, 123.9, 124.0, 128.8, 131.8, 138.9, 164.9, 172.4; MS *m/z* 281.25 [(M+H)$^+$ calcd for $C_{14}H_{21}N_2O_3^+$ 281.15].

4.1.7.7. 3-{3-[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-2-benzyl-2-hydroxy-propionylamino}-benzoic acid (10). Compound **10** (16 mg, 15% yield) was prepared according to the General procedures from **5** (51 mg, 0.15 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **10**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.73–0.81 (m, 6H), 0.93–0.98 (m, 6H), 1.41–1.65 (m, 3H), 2.09 (m, 1H), 2.93 (m, 1H), 3.15 (m, 1H), 3.47 (m, 1H), 3.72 (m, 1H), 3.84 (m, 1H), 3.96 (m, 1H), 4.15–4.22 (m, 2H), 4.41 (m, 1H), 7.12–7.26 (m, 4H), 7.35 (m, 1H), 7.63 (m, 1H), 7.71 (m, 1H), 8.11–8.33 (m, 2H); ^{13}C NMR (CD_3OD) δ [18.6 & 18.7], [19.7 & 19.8], 22.1, [23.1 & 23.2], [25.7 & 25.8], [31.5 & 31.6], 41.4, 42.0, [43.9 & 44.0], [53.3 & 53.6], 54.4, [61.0 & 61.2], [80.5 & 80.9], [122.9 & 123.1], [126.0 & 126.2], [126.7 & 126.8], 127.8, [128.9 & 129.0], [129.7 & 129.8], 131.5, 132.5, [137.0 & 137.1], 139.0, 154.73, [159.8 & 159.9], [168.5 & 168.7], 169.4, [173.4 & 173.5], [173.9 & 174.3], [176.1 & 176.7]; HRMS *m/z* 709.3422 [(M+H)$^+$ calcd for $C_{33}H_{45}N_{10}O_8^+$ 709.3416]; HPLC purity: >99% (UV, rt 2.34), 98.9% (ELSD, rt 2.42); BACE-1 IC_{50} = 0.43 μM.

4.1.7.8. 3-(2-{[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid (11). Compound **11** (16 mg, 14% yield) was prepared according to the General procedures from **6** (70 mg, 0.19 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **11**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.72–0.80 (m, 6H), 0.92–0.97 (m, 6H), 1.41–1.64 (m, 3H), 1.90 (m, 1H), 2.04–2.24 (m, 2H), 2.51 (m, 1H), 2.84 (m, 1H), [3.34 & 3.88 (d, J = 13.8 Hz, 1H), 3.55 & 3.61 (d, J = 13.8 Hz, 1H)], 3.82- 4.00 (m, 2H), 4.17 (m, 1H)], 4.22 (m, 1H), 4.41 (m, 1H), 7.10 (m, 1H), 7.14–7.22 (m, 4H), 7.41 (m, 1H), 7.76 (m, 1H), 7.85 (m, 1H), 8.32 (m, 1H); ^{13}C NMR (CD_3OD) δ [18.6 & 18.7], [19.7 & 19.8], [22.0 & 22.1], [23.0 & 23.1], [25.7 & 25.8], 30.6, [31.5 & 31.6], 40.3, 41.4, [41.8 & 42.0], [53.3 & 53.5], 54.3, [61.1 & 61.3], [79.7 & 80.1], [122.8 & 123.0], [125.9 & 126.1], [126.7 & 126.8], 126.9, 129.3, 129.4, [129.8 & 129.9], [132.5 & 132.6], [139.2 & 139.3], [142.9 & 143.0], 153.9, 158.3, [168.5 & 168.6], 169.4, [173.3 & 173.4], [174.5 & 174.9], [175.9 & 176.5]; HRMS *m/z* 723.3578 [(M+H)$^+$ calcd for $C_{34}H_{47}N_{10}O_8^+$ 723.3573]; HPLC purity: 97.2% (UV, rt 2.54), >99% (ELSD, rt 2.69); BACE-1 IC_{50} = 0.40 μM.

4.1.7.9. 3-(*R*)-(2-{[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid ((*R*)-12). Compound (*R*)-**12** (17.1 mg, 17% yield) was prepared according to the General procedures from (*R*)-**6** (50 mg, 0.14 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound (*R*)-**12**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.73 (d, J = 6.2 Hz, 3H), 0.74 (d, J = 6.2 Hz, 3H), 0.95 (d, J = 8.0 Hz, 6H), 1.39–1.61 (m, 3H), 1.89 (m, 1H), 2.07 (m, 1H), 2.20 (m, 1H), 2.51 (dt, J = 12.5, 5.3 Hz, 1H), 2.84 (dt, J = 13.5, 4.5 Hz, 1H), 3.35 (m, 1H), 3.80–4.00 (m, 3H), 4.14–4.23 (m, 2H), 4.41 (m, 1H), 7.10 (m, 1H), 7.15–7.24 (m, 4H), 7.41 (m, 1H), 7.77 (m, 1H), 7.87 (m, 1H), 8.33 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.7, 19.7, 22.1, 23.1, 25.7, 30.7, 31.5, 40.2, 41.4, 41.8, 53.5, 54.3, 61.2, 79.7, 122.8, 125.9, 126.7, 126.9, 129.4 (2C), 129.9, 132.6, 139.2, 143.0, 154.5, 159.6, 168.7, 169.5, 173.5, 174.5, 175.9; $[\alpha]_D^{23}$ = −12.6 (*c* 0.76, MeOH); HRMS *m/z* 723.3578 [(M+H)$^+$ calcd for $C_{34}H_{47}N_{10}O_8^+$ 723.3573]; HPLC purity: >99% (UV, rt 2.58), >99% (ELSD, rt 2.68); BACE-1 IC_{50} = 0.19 μM.

4.1.7.10. 3-(*S*)-(2-{[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid ((*S*)-13). Compound (*S*)-**13** (21 mg, 17% yield) was prepared according to the General procedures from (*S*)-**6** (63 mg, 0.17 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound (*S*)-**13**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.77 (d, J = 6.3 Hz, 3H), 0.78 (d, J = 6.3 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 6.8 Hz, 3H), 1.49 (m, 1H), 1.55–1.66 (m, 2H), 1.92 (m, 1H), 2.05 (m, 1H), 2.16 (m, 1H), 2.51 (dt, J = 13.6, 4.9 Hz, 1H), 2.83 (dt, J = 12.7, 4.4 Hz, 1H), 3.55 (d, J = 13.7 Hz, 1H), 3.62 (d, J = 13.7 Hz, 1H), 3.87 (dd, J = 14.6, 5.6 Hz, 1H), 3.95 (dd, J = 14.6, 5.6 Hz, 1H), 4.16 (d, J = 6.8 Hz, 1H), 4.22 (dd, J = 5.4, 4.4 Hz, 1H), 4.41 (m, 1H), 7.10 (m, 1H), 7.14–7.23 (m, 4H), 7.43 (m, 1H), 7.79 (m, 1H), 7.84 (m, 1H), 8.32 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.6, 19.7, 22.0, 23.2, 25.8, 30.7, 31.6, 40.3, 41.4, 42.0, 53.3, 54.3, 61.1, 80.1, 123.0, 126.1, 126.8, 126.9, 129.3, 129.4, 129.9, 132.6, 139.2, 143.0, 154.6, 159.7, 168.5, 169.5, 173.4, 174.9, 176.5; $[\alpha]_D^{23}$ = −46.8 (c 1.00, MeOH); HRMS m/z 723.3578 [(M+H)$^+$ calcd for $C_{34}H_{47}N_{10}O_8^+$ 723.3573]; HPLC purity: 97.3% (UV, rt 2.72), >99% (ELSD, rt 2.61); BACE-1 IC_{50} = 1.4 μM.

4.1.7.11. 3-{3-[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-3-cyclohexyl-propionylamino]-2-benzyl-2-hydroxy-propionylamino}-benzoic acid (14). Compound **14** (16.6 mg, 14% yield) was prepared according to the General procedures from **5** (54 mg, 0.15 mmol) in the following order: Fmoc-L-Cha-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **14**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.69–0.89 (m, 2H), 0.93–0.99 (m, 6H), 1.04–1.17 (m, 3H), 1.30 (m, 1H), 1.43–1.70 (m, 7H), 2.08 (m, 1H), 2.93 (m, 1H), 3.14 (m, 1H), 3.39 (m, 1H), 3.64 (m, 1H), 3.79–3.99 (m, 2H), 4.14–4.24 (m, 2H), 4.44 (m, 1H), 7.11–7.25 (m, 5H), 7.35 (m, 1H), 7.51–7.79 (m, 2H), 8.12 (m, 1H); ^{13}C NMR (CD_3OD) δ [18.6 & 18.7], [19.6 & 19.7], 27.0, [27.2 & 27.4], [31.6 & 31.7], 33.3, 34.6, [35.1 & 35.2], [40.4 & 40.6], 41.4, 44.0, [52.6 & 52.8], 54.4, [61.1 & 61.3], [80.5 & 80.9], [122.9 & 123.1], [126.0 & 126.2], [126.7 & 126.8], 127.8, [128.9 & 129.0], [129.7 & 129.8], 131.5, [132.4 & 132.5], [137.0 & 137.1], [138.9 & 139.0], 154.7, [159.6 & 159.8], [168.5 & 168.7], 169.4, [173.3 & 173.5], [173.9 & 174.3], [176.3 & 176.8]; HRMS m/z 749.3735 [(M+H)$^+$ calcd for $C_{36}H_{49}N_{10}O_8^+$ 749.3729]; HPLC purity: 96.5% (UV, rt 2.69), 98.7% (ELSD, rt 2.85); BACE-1 IC_{50} = 0.68 μM.

4.1.7.12. 3-(*R*)-(2-{[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-phenyl-propionylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid ((*R*)-15). Compound (*R*)-**15** (16 mg, 15% yield) was prepared according to the General procedures from (*R*)-**6** (50 mg, 0.14 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Phe-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound (*R*)-**15**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.73 (d, J = 6.3 Hz, 3H), 0.75 (d, J = 6.3 Hz, 3H), 1.41–1.58 (m, 3H), 1.91 (m, 1H), 2.21 (dt, J = 13.7, 5.1 Hz, 1H), 2.52 (dt, J = 12.3, 5.1 Hz, 1H), 2.80–2.93 (m, 2H), 3.19 (dd, J = 14.4, 4.9 Hz, 1H), 3.81–4.00 (m, 4H), 4.09 (m, 1H), 4.40 (m, 1H), 4.66 (q, J = 4.9 Hz, 1H), 7.10 (m, 1H), 7.16–7.29 (m, 9H), 7.38 (m, 1H), 7.76 (m, 1H), 7.86 (m, 1H), 8.28 (m, 1H); ^{13}C NMR (CD_3OD) δ 22.1, 23.1, 25.7, 30.7, 38.4, 40.2, 41.3, 41.9, 53.6, 54.3, 56.8, 79.6, 122.9, 126.0, 126.7, 126.9, 127.9, 129.4, 129.5, 129.6, 129.9, 130.2, 132.5, 138.1, 139.2, 143.0, 154.1, 159.2, 168.5, 169.5, 173.5, 174.6, 175.8; $[\alpha]_D^{23}$ = -0.6° (c 0.59, MeOH); HRMS m/z 771.3578 [(M+H)$^+$ calcd for $C_{38}H_{47}N_{10}O_8^+$ 771.3573]; HPLC purity: >99% (UV, rt 2.86), >99% (ELSD, rt 3.03); BACE-1 IC_{50} >10 μM.

4.1.7.13. 3-(*S*)-(2-{[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-phenyl-propionylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid ((*S*)-16). Compound (*S*)-**16** (16 mg, 12% yield) was prepared according to the General procedures from (*S*)-**6** (59 mg, 0.16 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Phe-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound (*S*)-**16**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.76 (d, J = 6.2 Hz, 3H), 0.79 (d, J = 6.2 Hz, 3H), 1.44–1.62 (m, 3H), 1.91 (m, 1H), 2.16 (m, 1H), 2.52 (dt J = 12.3, 5.1 Hz, 1H), 2.79–2.89 (m, 2H), 3.13 (dd, J = 14.0, 5.3 Hz, 1H), 3.54 (d, J = 13.9 Hz, 1H), 3.62 (d, J = 13.9 Hz, 1H), 3.85 (dd, J = 14.8, 5.6 Hz, 1H), 3.94 (dd, J = 14.8, 4.4 Hz, 1H), 4.08 (m, 1H), 4.39 (m, 1H), 4.64 (q, J = 5.1 Hz, 1H), 7.10 (m, 1H), 7.14–7.29 (m, 9H), 7.36–7.42 (m,1H), 7.77 (m, 1H), 7.84 (m, 1H), 8.30 (m, 1H); ^{13}C NMR (CD_3OD) δ 22.0, 23.2, 25.8, 30.7, 38.4, 40.3, 41.3, 42.0, 53.4, 54.3, 56.7, 80.0, 123.0, 126.2, 126.8, 126.9, 127.9, 129.4 (2C), 129.6, 129.9, 130.2, 132.6, 138.0, 139.1, 143.0, 154.5, 159.6, 168.4, 169.5, 173.4, 174.9, 176.3; HRMS m/z 771.3578 [(M+H)$^+$ calcd for $C_{38}H_{47}N_{10}O_8^+$ 771.3573]; $[\alpha]_D^{23}$ = -36.7° (c 0.58, MeOH); HPLC purity: 98.8% (UV, rt 2.85), >99% (ELSD, rt 3.02); BACE-1 IC_{50} >10 μM.

4.1.7.14. 3-(2-{[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-hexanoylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid (17). Compound **17** (5 mg, 3% yield) was prepared according to the General procedures from **6** (92 mg, 0.25 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Nle-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **17**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.72–0.89 (m, 9H), 1.22–1.34 (m, 4H), 1.44–1.78 (m, 6H), 1.90 (m, 1H), 2.17 (m, 1H), 2.52 (m, 1H), 2.84 (m, 1H), 3.58 (m, 1H), 3.77–3.96 (m, 2H), 4.16 (m, 1H), 4.24 (m, 1H), 4.37 (m, 1H), 7.10 (m, 1H), 7.14 (m, 3H), 7.40 (m, 1H), 7.74–7.81 (m, 2H), 7.86 (m, 1H), 8.27 (m, 1H); ^{13}C NMR (CD_3OD) δ 14.2, [21.9 & 22.0], [23.2 & 23.3], [23.4 & 23.5], [25.8 & 25.9], 29.1, 29.5, 30.7, [32.5 & 32.6], 40.3, 41.8, [53.5 & 53.6], [54.7 & 54.8], [55.7 & 55.8], [79.8 & 79.9], [123.1 & 123.2], 125.7, [126.6 & 126.7], 126.8, 129.3, 129.4, 129.7, [134.1 & 134.4], [139.1 & 139.2], [143.0 & 143.1], 153.7, 158.5, 163.7, [169.4 & 169.5], [174.0 & 174.1], [174.6 & 174.9], [176.1 & 176.5]; HRMS m/z 737.3735 [(M+H)$^+$ calcd for $C_{35}H_{49}N_{10}O_8^+$ 737.3729]; HPLC purity: 98.4% (UV, rt 5.37, MeOH), 98.7% (ELSD, rt 5.89, MeOH); BACE-1 IC_{50} >10 μM.

4.1.7.15. 3-(2-{[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-4-methyl-pentanoylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid (18). Compound **18** (8 mg, 4% yield) was prepared according to the General procedures from **6** (92 mg, 0.25 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Leu-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **18**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.69–0.79 (m, 6H), 0.86–0.93 (m, 6H), 1.42–1.70 (m, 7H), 1.91 (m, 1H), 2.19 (m, 1H), 2.52 (m, 1H), 2.85 (m, 1H), 3.58 (m, 1H), 3.81–3.97 (m, 2H), 4.20 (m, 1H), 4.33–4.41 (m, 2H), 7.09 (m, 1H), 7.14–7.23 (m, 4H), 7.40 (m, 1H), 7.76 (m, 1H), 7.85 (m, 1H), 8.30 (m, 1H); ^{13}C NMR (CD_3OD) δ 21.7, [21.9 & 22.0], [23.2 & 23.3], [23.4 & 23.5], 25.8, 25.9, 30.7, [40.2 & 40.3], [41.5 & 41.6], 41.7, [41.8 & 41.9], [53.4 & 53.6], 54.0, [54.7 & 54.8], [79.6 & 80.0], [123.0 & 123.2], [125.9 & 126.0], [127.0 & 126.8], 126.9, 129.4, 129.5, [129.7 & 129.8], [133.6 & 133.4], [139.1 & 139.2], [143.0 & 143.1], 152.7, 158.5, 163.7, [169.0 & 169.1], [174.4 & 174.5], [174.6 & 174.9], [175.9 & 176.5]; HRMS m/z 737.3735 [(M+H)$^+$ calcd for $C_{35}H_{49}N_{10}O_8^+$ 737.3729]; HPLC purity: >99% (UV, rt 5.53, MeOH), 98.8% (ELSD, rt 5.50, MeOH); BACE-1 IC_{50} >10 μM.

4.1.7.16. 3-(2-{[(*S*)-2-((*R*)-3-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-4-methyl-pentanoylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid (19). Compound **19** (15 mg, 8% yield) was prepared according to the General procedures from **6** (92 mg, 0.25 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-β-Leu-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **19**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.62–0.8 (m, 6H), 0.87–1.02 (m, 6H), 1.35–1.90 (m, 5H), 2.08–2.21 (m, 2H), 2.40–2.55 (m, 2H), 2.77 (m, 1H), [3.25 & 3.90 (d, J = 13.7 Hz, 1H), 3.50 & 3.64 (d, J = 13.7 Hz, 1H)], 3.77 (m, 1H), 3.98–4.13 (m, 3H), 4.47 (m, 1H), 7.08–7.23 (m, 5H), 7.42 (m, 1H), 7.74–7.89 (m, 2H), [8.26 & 8.36 (m, 1H)]; ^{13}C NMR (CD_3OD) δ 18.5, [19.5 & 19.6], [22.0 & 22.1], [23.1 & 23.2], [25.7 & 25.8], 29.4, [30.6 & 30.7], [33.7 & 33.8], [39.1 & 39.4], [40.1 & 40.2], [41.4 & 41.6], 53.4, 53.9. [54.5 & 54.7], [79.7 & 79.8], [122.6 & 123.0], 125.7, 126.2, [126.6 & 126.8], [126.8 & 126.9], 129.3, [129.8 & 129.9], 132.6, [139.1 & 139.3], [143.0 & 143.1], 154.0, [158.9 & 159.4], [167.3 & 167.4], 169.5, [174.0 & 174.3], [174.4 & 174.6], 176.5; HRMS m/z 737.3735 [(M+H)$^+$ calcd for $C_{35}H_{49}N_{10}O_8^+$ 737.3729]; HPLC purity: 99.0% (UV, rt 2.50), >99% (ELSD, rt 2.63); BACE-1 IC_{50} >10 μM.

4.1.7.17. 3-(2-{[(*S*)-2-((*S*)-2-{(*R*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-methyl}-2-hydroxy-4-phenyl-butyrylamino)-benzoic acid (20). Compound **20** (13 mg, 7% yield) was prepared according to the General procedures from **6** (92 mg, 0.25 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-D-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **20**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.72–0.82 (m, 6H), 0.87–0.94 (m, 6H), 1.42–1.64 (m, 3H), 1.89 (m, 1H), 2.04 (m, 1H), 2.17 (m, 1H), 2.52 (m, 1H), 2.85 (m, 1H), [3.33 & 3.83 (d, J = 14.0 Hz, 1H), 3.51 & 3.65 (d, J = 14.0 Hz, 1H)], 3.83–3.89 (m, 2H), 4.15 (m, 1H), 4.23 (m, 1H), 4.36 (m, 1H), 7.11 (m, 1H), 7.16–7.25 (m, 4H), 7.44 (m, 1H), 7.77–7.90 (m, 2H), 8.34 (m, 1H); ^{13}C NMR (CD_3OD) δ [18.6 & 18.7], [19.7 & 19.8], [21.9 & 22.0], [23.1 & 23.2], [25.6 & 25.7], 30.6, 31.9, 40.3, 41.4, [42.0 & 42.2], [53.1 & 53.2], [54.2 & 54.3], [60.7 & 60.8], [79.7 & 79.9], [123.0 & 123.1], [126.0 & 126.1], [126.7 & 126.8], 126.9, 129.2, 129.3, 129.9, [132.6 & 132.7], [139.1 & 139.2], [142.9 & 143.0], 154.2, 158.4, 168.2, [169.4 & 169.5], [173.3 & 173.4], [174.5 & 174.8], [175.7 & 176.2]; HRMS m/z 723.3578 [(M+H)$^+$ calcd for $C_{34}H_{47}N_{10}O_8^+$ 723.3573]; HPLC purity: >99% (UV, rt 2.56), 98.1% (ELSD, rt 2.73); BACE-1 IC_{50} >10 μM.

4.1.7.18. 3-[2-({(*S*)-2-[(*S*)-2-((*S*)-2,3-Diamino-propionylamino)-3-methyl-butyrylamino]-4-methyl-pentanoylamino}-methyl)-2-hydroxy-4-phenyl-butyrylamino]-benzoic acid (21). Compound **21** (10 mg, 10% yield) was prepared according to the General procedures from **6** (70 mg, 0.19 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, and finally Boc-L-DAP(Fmoc)-OH. Compound **21**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.72–0.81 (m, 6H), 0.91–1.01 (m, 6H), 1.39–1.65 (m, 4H), 2.11–2.24 (m, 2H), 2.53 (m, 1H), 2.85 (m, 1H), [3.22 & 3.91 (d, J = 13.8 Hz, 1H), 3.50 & 3.64 (d, J = 13.8 Hz, 1H)], 3.36 (m, 1H), 3.50 (m, 1H), 4.23 (m, 1H), 4.37–4.45 (m, 2H), 7.11 (m, 1H), 7.16–7.24 (m, 4H), 7.44 (m, 1H), 7.78–7.87 (m, 2H), 8.33 (m, 1H); ^{13}C NMR (CD_3OD) δ [16.7 & 16.9], [18.5 & 18.6], [20.7 & 20.8], [21.9 & 22.0], [24.5 & 24.6], 29.5, 30.2, [39.0 & 39.1], 40.0, [41.0 & 41.2], [52.0 & 52.1], [53.0 & 53.1], 60.1, [78.3 & 78.7], [121.6 & 121.7], [124.7 & 124.9], [125.5 & 125.6], 125.7, 128.2, 128.3, 128.8, 131.5, [138.0 & 138.1], [141.7 & 141.8], 168.3, [172.6 & 172.7], [173.3 & 173.7], 174.2, 174.9; HRMS m/z 623.3506 [(M+H)$^+$ calcd for $C_{32}H_{47}N_6O_7^+$ 627.3501]; HPLC purity: 97.8% (UV, rt 2.17), >99% (ELSD, rt 2.08); BACE-1 IC_{50} >10 μM.

4.1.7.19. 3-(2-Hydroxy-2-{[(*S*)-2-((*S*)-2-{(*R*)-3-hydroxy-2-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-methyl}-4-phenyl-butyrylamino)-benzoic acid (22). Compound **22** (9 mg, 5% yield) was prepared according to the General procedures from **6** (81 mg, 0.22 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Fmoc-D-Ser(^{t}Bu)-OH, and finally tetrazole **9**. Compound **22**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.77–0.80 (m, 6H), 0.92–0.95 (m, 6H), 1.44–1.60 (3H), 1.91 (m, 1H), 2.08–2.25 (m, 2H), 2.52 (m, 1H), 2.85 (m, 1H), [3.38 & 3.77 (d, J = 13.8 Hz, 1H), 3.51 & 3.67 (d, J = 13.8 Hz, 1H)], 3.86–4.01 (m, 2H), 4.15 (m, 1H), 4.33 (m, 1H), 4.80 (m, 1H), 7.10 (m, 1H), 7.17–7.22 (m, 4H), 7.42 (m, 1H), 7.77 (m, 1H), 7.87 (m, 1H), 8.33 (m, 1H); ^{13}C NMR (CD_3OD) δ [18.2 & 18.3], 19.6, [21.6 & 21.7], 23.3, [25.8 & 25.9], [30.6 & 30.7], [31.2 & 31.3], 40.1, [41.8 & 41.9], [53.3 & 53.4], [56.4 & 56.5], [60.9 & 61.0], [63.0 & 63.2], [79.8 & 80.0], [123.0 & 123.1], [126.1 & 126.2], [126.6 & 126.7], 126.9, 129.3, 129.5, [129.8 & 129.9], [132.5 & 132.6], [139.1 & 139.2], [143.0 & 143.1], 153.8, 158.8, [169.2 & 169.3], [171.3 & 171.4], [173.4 & 173.5], [174.5 & 174.6], [175.9 & 176.2]; HRMS m/z 724.3418 [(M+H)$^+$ calcd for $C_{34}H_{46}N_9O_9^+$ 724.3413]; HPLC purity: 99.0% (UV, rt 3.03), >99% (ELSD, rt 2.85); BACE-1 IC_{50} >10 μM.

4.1.7.20. 3-(2-Hydroxy-2-{[(*S*)-2-((*S*)-2-{(*S*)-3-hydroxy-2-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-methyl}-4-phenyl-butyrylamino)-benzoic acid (23). Compound **23** (7 mg, 4% yield) was prepared according to the General procedures from **6** (81 mg, 0.22 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Fmoc-L-Ser(^{t}Bu)-OH, and finally tetrazole **9**. Compound **23**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.76–0.79 (m, 6H), 0.91–0.95 (m, 6H), 1.43–1.62 (m, 3H), 1.85 (m, 1H), 2.07–2.18 (m, 2H), 2.48 (m, 1H), 2.78 (m, 1H), [3.28 & 3.75 (d, J = 13.7 Hz, 1H), 3.45 & 3.56 (d, J = 13.7 Hz, 1H)], 3.92–3.95 (m, 2H), 4.15 (m, 1H), 4.34 (m, 1H), 4.70 (m, 1H), 7.11 (m, 1H), 7.15–7.23 (m, 4H), 7.43 (m, 1H), 7.78 (m, 1H), 7.86–7.92 (m,1H), 8.35 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.5, [19.7 & 19.8], [21.6 & 21.8], [23.2 & 23.3], [25.7 & 25.8], 30.6, [31.3 & 31.4], [40.0 & 40.2], [41.6 & 41.7], [53.4 & 53.5], [57.1 & 57.2], [60.7 & 61.0], 62.8, [79.6 & 79.8], 123.0, 126.1, [126.6 & 126.7], 126.9, 129.4, 129.5, [129.8 & 129.9], [132.5 & 132.6], [139.1 & 139.2], [143.0 & 143.1], 154.1, 158.6, [169.5 & 169.6], 170.9, [172.4 & 172.6] [173.4 & 173.8], [174.6 & 174.9]; HRMS m/z 724.3418 [(M+H)$^+$ calcd for $C_{34}H_{46}N_9O_9^+$ 724.3413]; HPLC purity: 99.0% (UV, rt 2.84), 98.3% (ELSD, rt 2.97); BACE-1 IC_{50} >10 μM.

4.1.7.21. 3-{3-[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-2-hydroxy-propionylamino}-benzoic acid (28). Compound **28** (10.5 mg, 15% yield) was prepared according to the General procedures from **26** (31 mg, 0.11 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **28**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.77–1.00 (m, 12 H), 1.45–1.69 (m, 3H), 2.04–2.14 (m, 1H), 3.48–3.57 (m, 1H), 3.62–3.79 (m, 1H), 3.86–3.99 (m, 2H), 4.18–4.24 (m, 2H), 4.26–4.32 (m, 1H), 4.39–4.44 (m, 1H), 7.39–7.44 (m, 1H), 7.75–7.78 (m, 1H), 7.84–7.89 (m, 1H), 8.29–8.31 (m, 1H); ^{13}C NMR (CD_3OD) δ [18.6 & 18.7], 19.7, 22.0, [23.2 & 23.3], [25.8 & 25.9], 31.6, 41.4, [44.2 & 44.4], [53.4 & 53.5], 54.3, [61.2 & 61.3], [72.5 & 72.6], [122.7 & 122.8], [125.8 & 125.9], [126.6 & 126.7], 129.9, 132.6, [139.3 & 139.4], [154.9 & 155.0], 160.0, 168.6, 169.5, 173.2, [173.3 & 173.4], [175.4 & 175.5]; HRMS m/z 619.2961 [(M+H)$^+$ calcd for $C_{26}H_{39}N_{10}O_8^+$ 619.2952]; HPLC (10–100% MeOH) purity: 99.0% (C4, rt 1.27), >99% (C18, rt 2.84); BACE-1 IC_{50} = 0.44 μM.

4.1.7.22. 3-{3-[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-3-methyl-butyrylamino]-2-hydroxy-propionylamino}-benzoic acid (29). Compound **29** (6.0 mg, 9% yield) was prepared according to the General procedures from **26** (31 mg, 0.11 mmol) in the following order: Fmoc-L-Val-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **29**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.82–1.01 (m, 12H), 1.96–2.15 (m, 2H), 3.54–3.60 (m, 1H), 3.64–3.75 (m, 1H), 3.94–4.00 (m, 1H), 4.17–4.25 (m, 3H), 4.24–4.32 (m, 1H), 7.42 (t, J = 8.2 Hz, 1H), 7.76–7.79 (m, 1H), 7.84–7-89 (m, 1H), 8.29–8.31 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.7, [18.8 & 18.9], 19.7, 31.6, 31.9, 41.7, [44.2 & 44.3], 53.4, 60.5, [61.1 & 61.2], 72.3, [122.7 & 122.8], [125.8 & 125.9], 126.7, 139.5, 154.7, 159.9, [168.3 & 168.4], 169.5, [173.1 & 173.2], [173.4 & 173.5], 174.2; HRMS m/z 605.2805 [(M+H)$^+$ calcd for $C_{25}H_{37}N_{10}O_8^+$ 605.2796]; HPLC (10–100% MeOH) purity: >99% (C4, rt 1.49), 99.0% (C18, rt 3.03); BACE-1 IC_{50} = 3 μM.

4.1.7.23. 3-(3-{(*S*)-2-[(*S*)-2-((*S*)-2,3-Diamino-propionylamino)-3-methyl-butyrylamino]-4-methyl-pentanoylamino}-2-hydroxy-propionylamino)-benzoic acid (30). Compound **30** (9.2 mg, 13% yield) was prepared according to the General procedures from **26** (31 mg, 0.11 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, and finally Boc-L-DAP(Fmoc)-OH. The Fmoc group was then removed according to the General procedure. Compound **30**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.80–1.03 (m, 12 H), 1.46–1.70 (m, 3H), 2.13–2.22 (m, 1H), 3.34–3.36 (m, 4H), 4.22–4.30 (m, 2H), 4.36–4.45 (m, 2H), 7.44 (t, J = 8.1 Hz, 1H), 7.77–7.81 (m, 1H), 7.83–7.88 (m, 1H), 8.23–8.32 (m, 1H); ^{13}C NMR (CD_3OD) δ [18.0 & 18.1], 19.7, [21.8 & 21.9], 23.3, 25.8, 31.3, 41.2, 42.1, 44.3, 51.4, 53.4, 61.5, [72.4 & 72.6], [122.6 & 122.7], [125.8 & 125.9], 126.7, 130.0, 132.7, 139.3, 168.1, 169.5, 173.2, [175.0 & 175.1]; HRMS m/z 523.2893 [(M+H)$^+$ calcd for $C_{24}H_{39}N_6O_7^+$ 523.2880]; HPLC (10–100% MeOH) purity: 98.3% (C4, rt 0.65), 96.1% (C18, rt 2.52); BACE-1 IC_{50} >10 μM.

4.1.7.24. 3-(3-{(*S*)-2-[(*S*)-2-((*R*)-2-Amino-3-hydroxy-propionylamino)-3-methyl-butyrylamino]-4-methyl-pentanoylamino}-2-hydroxy-propionylamino)-benzoic acid (31). Compound **31** (9.2 mg, 16% yield) was prepared according to the General procedures from **26** (31 mg, 0.11 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, and finally Fmoc-D-Ser(^{t}Bu)-OH. The Fmoc group then removed according to General procedure. Compound **31**: white powder after lyophilization. ^{1}H NMR (300.0 MHz, CD_3OD) δ 0.81–0.97 (m, 12H), 1.50–1.62 (m, 3H), 2.03–2.15 (m, 1H), 3.45–3.75 (m, 2H), 3.80–3.90 (m, 1H), 3.91–3.99 (m, 1H), 4.03–4.12 (m, 1H), 4.16–4.25 (m, 1H), 4.30–4.45 (m, 2H), 7.48 (t, J = 8.1 Hz, 1H), 7.77–7.90 (m, 2H), 8.29–8.32 (m, 1H); ^{13}C NMR (75.4 MHz, CD_3OD) δ 18.6, 19.7, 21.9, 23.3, 25.8, 31.9, 42.0, [44.3 & 44.4], 53.2, 56.2, [60.6 & 60.7], 61.8, 72.5, [122.7 & 122.9], [125.8 & 126.1], [126.6 & 126.7], 129.0, 132.6, 139.3, [168.6 & 168.8], 169.5, [173.1 & 173.2], 173.4, [175.1 & 175.2]; HRMS m/z 524.2717 [(M+H)$^+$ calcd for $C_{24}H_{38}N_5O_8^+$ 524.2720]; HPLC (10–100% MeOH) purity: 98.5% (C4, rt 1.01), 97.0% (C18, rt 2.49); BACE-1 IC_{50} >10 μM.

4.1.7.25. 3-{3-[(*S*)-2-((*S*)-2-{(*R*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-2-hydroxy-propionylamino}-benzoic acid (32). Compound **32** (10.0 mg, 15% yield) was prepared according to the General procedures from **26** (31 mg, 0.11 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-D-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **32**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.80–0.99 (m, 12 H), 1.48–1.69 (m, 3H), 2.00–2.20 (m, 1H), 3.47–3.74 (m, 2H), 3.82–3.95 (m, 2H), 4.12–4.17 (m, 1H), 4.23–4.32 (m, 2H), 4.35–4.41 (m, 1H), 7.43 (t, J = 8.2 Hz, 1H), 7.76–7.80 (m, 1H), 7.82–7.89 (m, 1H), 8.29–8.32 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.7, 19.7, 21.9, [23.2 & 23.4], [25.7 & 25.8], 31.8, 41.4, [44.2 & 44.3], 53.2, [54.2 & 54.3], [61.0 & 61.2], [72.4 & 72.5], [122.7 & 122.8], [125.8 & 125.9], 126.7, 129.9, 132.6, 139.3, 154.4, 159.3, [168.4 & 168.6], 169.5, 173.2, 173.4, 175.2; HRMS m/z 619.2943 [(M+H)$^+$ calcd for $C_{26}H_{39}N_{10}O_8^+$ 619.2952]; HPLC (10–100% MeOH) purity: 99.0% (C4, rt 1.60), >99% (C18, rt 3.28); BACE-1 IC_{50} >10 μM.

4.1.7.26. 3-{2-Hydroxy-3-[(*S*)-2-((*S*)-2-{(*R*)-3-hydroxy-2-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-3-methyl-butyrylamino]-propionylamino}-benzoic acid (33). Compound **33** (6.0 mg, 9% yield) was prepared according to the General procedures from **26** (31 mg, 0.11 mmol) in the following order: Fmoc-L-Val-OH, Fmoc-L-Val-OH, Fmoc-D-Ser(^{t}Bu)-OH, and finally tetrazole **9**. Compound **33**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.86–0.97 (m, 12H), 1.99–2.17 (m, 2H), 3.47–3.65 (m, 2H), 3.90–3.97 (m, 2H), 4.10–4.15 (m, 1H), 4.22–4.27 (m, 2H), 4.67–4.72 (m, 1H), 7.42 (t, J = 7.9 Hz, 1H), 7.76–7.79 (m, 1H), 7.84–7.90 (m, 1H), 8.29–8.32 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.6, [18.8 & 18.9], 19.8, [31.6 & 31.7], 44.2, 57.0, [60.5 & 60.6], 60.8, 62.9, 72.6, 122.8, 125.9, 126.7, 129.9, 132.6, 139.3, 154.5, 158.2, 169.5, 172.1, 173.1, [173.5 & 173.6], 174.2; HRMS m/z 606.2639 [(M+H)$^+$ calcd for $C_{25}H_{36}N_9O_9^+$ 606.2636]; HPLC (10–100% MeOH) purity: 98.5% (C4, rt 1.05), 97.4% (C18, rt 3.07); BACE-1 IC_{50} >10 μM.

4.1.7.27. 3-{3-[(*S*)-2-((*S*)-2-{(*S*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-propionylamino}-benzoic acid (35). Compound **35** (57 mg, 32% yield) was prepared according to the General procedures from Fmoc-3-Abz-OH (108 mg, 0.30 mmol) in the following order: Fmoc-β-Ala-OH, Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **35**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.81–0.89 (m, 6H), 0.93–0.97 (m, 6H), 1.50–1.68 (m, 3H), 2.11 (m, 1H), 2.57–2.63 (m, 2H), 3.46–3.62 (m, 2H), 3.86–3.97 (m, 2H), 4.20–4.27 (m, 2H), 4.38 (m, 1H), 7.38 (m, 1H), 7.72 (m, 1H), 7.9 (m, 1H), 8.22 (m, 1H); ^{13}C NMR (CD_3OD) δ 17.4, 18.5, 20.9, 22.1, 24.7, 30.4, 35.6, 36.2, 40.2, 40.6, 52.3, 53.1, 60.0, 121.1, 124.2, 125.0, 128.7, 131.3, 139.0, 153.3, 158.3, 167.5, 168.3, 171.1, 172.1, 173.4; HRMS m/z 603.3003 [(M+H)$^+$ calcd for $C_{26}H_{39}N_{10}O_7^+$ 603.2998]; HPLC purity: 95.9% (UV, rt 1.32), 96.7% (ELSD, rt 1.31); BACE-1 IC_{50} = 0.87 μM.

4.1.7.28. 3-{3-[(*S*)-2-((*S*)-2-{(*R*)-2-Amino-3-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-propionylamino}-benzoic acid (36). Compound **36** (45 mg, 25% yield) was prepared according to the General procedures from Fmoc-3-Abz-OH (108 mg, 0.30 mmol) in the following order: Fmoc-β-Ala-OH, Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-D-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **36**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.86–0.97 (m, 12H), 1.53–1.66 (m, 3H), 2.08 (m, 1H), 2.60–2.64 (m, 2H), 3.47–3.58 (m, 2H), 3.86–3.89 (m, 2H), 4.17 (m, 1H), 4.28–4.38 (m, 2H), 7.40 (m, 1H), 7.71–7.81 (m, 2H), 8.26 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.7, 19.7, 22.0, 23.3, 25.8, 31.8, 36.7, 37.2, 41.4, 41.8, 53.4, 54.4, 61.0, 122.4, 125.5, 126.3, 129.9, 132.5, 140.1, 153.0, 160.8, 168.6, 169.6, 172.3, 173.3, 174.6; HRMS m/z 603.3003 [(M+H)$^+$ calcd for $C_{26}H_{39}N_{10}O_7^+$ 603.2998]; HPLC purity: 96.2% (UV, rt 3.25, MeOH), 96.4% (ELSD, rt 3.14, MeOH); BACE-1 IC_{50} >10 μM.

4.1.7.29. 3-{3-[(*S*)-2-((*S*)-2-{(*R*)-3-Hydroxy-2-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-propionylamino}-benzoic acid (37). Compound **37** (26 mg, 14% yield) was prepared according to the General procedures from Fmoc-3-Abz-OH (108 mg,

0.30 mmol) in the following order: Fmoc-β-Ala-OH, Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Fmoc-D-Ser(^{t}Bu)-OH, and finally tetrazole **9**. Compound **37**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.84–0.90 (m, 6H), 0.94–0.98 (m, 6H), 1.53–1.64 (m, 3H), 2.16 (m, 1H), 2.60–2.64 (m, 2H), 3.51–3.56 (m, 2H), 3.87–3.98 (m, 2H), 4.20 (m, 1H), 4.32 (m, 1H), 4.79 (m, 1H), 7.41 (m, 1H), 7.72 (m, 1H), 7.80 (m, 1H), 8.23 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.3, 19.6, 21.7, 23.4, 25.9, 31.3, 36.9, 37.3, 41.6, 53.4, 56.3, 61.0, 63.1, 122.3, 125.5, 126.2, 129.9, 132.5, 140.1, 154.1, 157.6, 169.5, 172.2, 172.6, 173.5, 174.8; HRMS *m/z* 604.2843 [$(M+H)^+$ calcd for $C_{26}H_{38}N_9O_8^+$ 604.2838]; HPLC purity: 95.1% (UV, rt 1.67), 98.1% (ELSD, rt 1.66); BACE-1 IC_{50} >10 μM.

4.1.7.30. 3-{3-[(*S*)-2-((*S*)-2-{(*S*)-3-Hydroxy-2-[(2*H*-tetrazole-5-carbonyl)-amino]-propionylamino}-3-methyl-butyrylamino)-4-methyl-pentanoylamino]-propionylamino}-benzoic acid (38). Compound **38** (22 mg, 12% yield) was prepared according to the General procedures from Fmoc-3-Abz-OH (108 mg, 0.30 mmol) in the following order: Fmoc-β-Ala-OH, Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Fmoc-L-Ser(^{t}Bu)-OH, and finally tetrazole **9**. Compound **38**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.83–0.89 (m, 6H), 0.93–0.97 (m, 6H), 1.53–1.66 (m, 3H), 2.14 (m, 1H), 2.54–2.59 (m, 2H), 3.42–3.59 (m, 2H), 3.92–3.96 (m, 2H), 4.17 (m, 1H), 4.34 (m, 1H),4.69–4.72 (m,1H), 7.41 (m, 1H), 7.72 (m, 1H), 7.80 (m, 1H), 8.23 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.5, 19.7, 21.7, 23.3, 25.8, 31.3, 36.9, 37.4, 41.6, 53.4, 57.0, 60.9, 62.9, 122.3, 125.4, 126.2, 129.8, 132.5, 140.1, 154.7, 158.0, 169.5, 172.1, 172.5, 173.5, 174.7; HRMS *m/z* 604.2843 $[(M+H)^+$ calcd for $C_{26}H_{38}N_9O_8^+$ 604.2838]; HPLC purity: 95.5% (UV, rt 1.64), 95.6% (ELSD, rt 1.63); BACE-1 IC_{50} >10 μM.

4.1.7.31. (4*S*,7*S*,10*S*)-4-Amino-14-hydroxy-10-isobutyl-7-isopropyl-1,5,8,11-tetraoxo-1-(2*H*-tetrazol-5-yl)-2,6,9,12-tetraazapentadecan-15-oic acid (39). Compound **39** (6 mg, 12% yield) was prepared according to the General procedures from Fmoc-D,L-Isoserine-OH (36.0 mg, 0.11 mmol) in the following order: Fmoc-L-Leu-OH, Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **39**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ0.88–0.91 (m, 3H), 0.93–0.96 (m, 3H), 0.98–1.03 (m, 6H), 1.54–1.73 (m, 3H), 2.09–2.17 (m, 1H), 3.01–3.08 (m, 2H), 3.49–3.74 (m, 1H), 3.89–4.00 (m, 2H), 4.20–4.25 (m, 2H), 4.42–4.48 (m, 1H); ^{13}C NMR (CD_3OD) δ 18.7, 19.8, [22.0 & 22.1], 23.4, 25.6, 31.7, 41.5, 41.8, 44.1, [53.4 & 53.5], 54.5, 61.2, [70.6 & 70.7], 165.5, 161.7, 168.7, 173.3, 175.1, 175.7; HRMS *m/z* 500.2577 $[(M+H)^+$ calcd for $C_{19}H_{34}N_9O_7^+$ 500.2581]; HPLC purity: >99.0% (C4, rt 0.69), 97.9% (C18, rt 0.91); BACE-1 IC_{50} = 6.1 μM.

4.1.7.32. (*S*)-2-((*S*)-2-((*S*)-2-Amino-3-(2*H*-tetrazole-5-carboxamido)propanamido)-3-methylbutanamido)-4-methylpentanoic acid (40). Compound **40** (11 mg, 24% yield) was prepared according to the General procedures from Fmoc-L-Leu-OH (38.9 mg, 0.11 mmol) in the following order: Fmoc-L-Val-OH, Boc-L-DAP(Fmoc)-OH, and finally tetrazole **9**. Compound **40**: white powder after lyophilization. ^{1}H NMR (CD_3OD) δ 0.87–0.90 (m, 3H), 0.92–0.95 (m, 3H), 0.98–1.04 (m, 6H), 1.62–1.75 (m, 3H), 2.08–2.18 (m, 1H), 3.72–3.83 (m, 1H), 3.89–3.95 (m, 1H), 3.21–3.34 (m, 2H), 4.41–4.48 (m, 1H); ^{13}C NMR (CD_3OD) δ 17.7, 19.7, 21.9, 23.4, 26.0, 31.8, 41.6, 41.8, 52.4, 54.8, 60.9, 158.4, 163.7, 168.5, 173.2, 176.2; HRMS *m/z* 413.2256 $[(M+H)^+$ calcd for $C_{16}H_{29}N_8O_5^+$ 413.2261]; HPLC purity: >99.0% (C4, rt 0.78), 97.6% (C18, rt 1.33); BACE-1 IC_{50} = 4.4 μM.

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