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Design, synthesis and SAR of potent statine-based BACE-1 inhibitors: Exploration of P1 phenoxy and benzyloxy residues

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ABSTRACT

Several BACE-1 inhibitors with low nanomolar level activities, encompassing a statine-based core structure with phenyloxymethyl- and benzyloxymethyl residues in the P1 position, are presented. The novel P1 modification introduced to allow the facile exploration of the S1 binding pocket of BACE-1, delivered highly promising inhibitors.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by loss of memory and cognition. It is the most common form of dementia among elderly afflicting up to 30 million people worldwide,[1] with the suffering amount expected to grow due to an aging population.[2] The current therapy, mainly consisting of cholinesterase inhibitors for mild to moderate AD, has only modest symptomatic effects. Other pharmaceutical treatments address mood disorders, agitation and psychosis in the later stages of the disease.[3] In all, these drugs are safe but cannot offer disease modifying treatment regimens to the AD patient population.[4]

The disease is associated with accumulation and aggregation of amyloid plaques, consisting of Aβ40 and Aβ42 peptides along with neurofibrillary tangles, in the brain. This overproduction of Aβ40,42 within the neurons is believed to be central for the development of the disease.[5–8] The peptides result from cleavage of the amyloid precursor protein (APP) by β-secretase, or beta-site APP cleaving enzyme (BACE-1), and γ-secretase, respectively.[9]

BACE-1 knockout mice have been shown to display greatly diminished Aβ40,42 production and the phenotype does not give rise to any major adverse effects.[10–12] Moreover, BACE-1 appears to have a limited number of substrates[13] whereas γ-secretase is involved in numerous transmembrane cleavages.[14,15] Taken together, these studies portray BACE-1 as a promising target for the treatment of AD.[16]

Previously BACE-1 inhibitors encompassing the central cores **I** (Fig. 1), characterized by a methylphenyl moiety in the P1 position, have been presented.[17,18] Herein we disclose inhibitors comprising

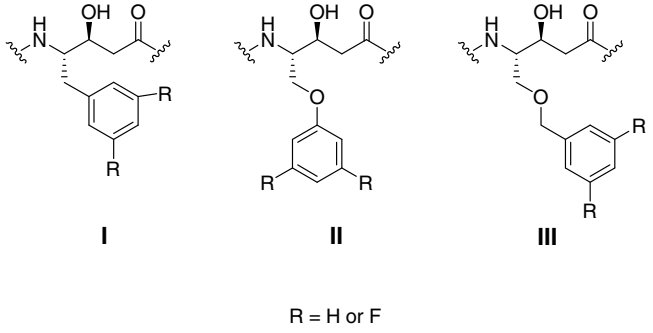

Figure 1. A comparison of three statine-based central cores incorporating different P1 residues. Central core **I** has been used recently in inhibitors targeting BACE-1 and the modified central cores **II** and **III** are described in this report.

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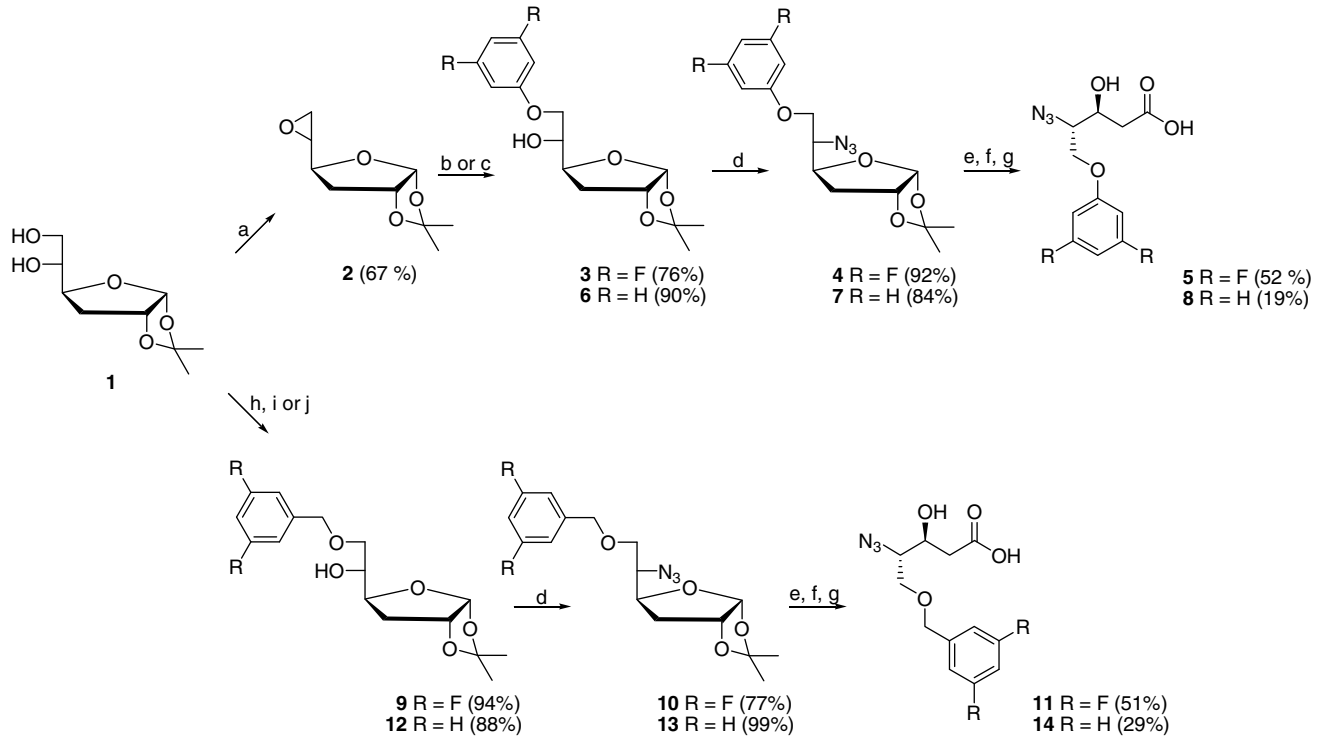


Scheme 1. Reagents and conditions: (a) PPh_3, DIAD, $CHCl_3$; (b) 3,5-difluorophenol, K_2CO_3, DMF, 100 °C; (c) phenol, K_2CO_3, DMF, 100 °C; (d) PPh_3, DIAD, DPPA, THF (dry); (e) HOAc/H_2O 1:1, 110 °C; (f) $NaIO_4$, $KMnO_4$, *t*-BuOH/H_2O 2:3; (g) 1 M NaOH; (h) Bu_2SnO, toluene, reflux; (i) tetrabutylammonium bromide (TBAB), 3,5-difluoroBnBr, toluene, 90 °C; (j) TBAB, BnBr, toluene, 90 °C.

the central cores **II** with a phenyloxymethyl P1 group for which the norstatine thio analog was recently reported.[19] In addition we present inhibitors encompassing the extended central core **III**[20,21] with a benzyloxymethyl residue in the P1 position.

Flexible synthesis provided compounds exhibiting IC_{50} values in the low nanomolar range.

2. Results and discussion

Previous reports from our laboratories have shown that insertion of oxygen in the appropriate position of peptidomimetic structures, not only provides new centers for diversification, but also simplified synthesis and may when appropriately positioned provide increased potency against the desired proteases.[20–23]

The syntheses of the central core building blocks, comprising the different phenyloxy-, and bensyloxy P1 residues, were performed as presented in scheme 1. The couplings of various amines and carboxylic acids to these building blocks furnished the target compounds. In Figure 2 the general structure of the inhibitors are presented.

2.1. Chemistry

Amines **C** and **D** are commercially available whereas substituents **A**–**B**, **E**–**G**, **L**, **N**, and **P** were synthesized from suitably protected commercially available precursors. Sulfonamides **M**, **O**, and **Q** were obtained as described by Stachel et al.[24]

The synthesis of the statine-based templates is outlined in Scheme 1. 3-Deoxy-1,2-*O*-isopropylidene-α-D-glucose (**1**) was synthesized in three steps according to literature procedures.[25–27] In order two obtain the phenyloxymethyl templates **5** and **8** and the benzyloxymethyl templates **11** and **14**[20] two slightly different routes were employed.

For the synthesis of templates **5** and **8** diol **1** was first converted into the corresponding epoxide[28] using triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate (DIAD) in refluxing chloroform,[28] furnishing **2** in 67% yield. Subsequent reaction of epoxide **2** at 100 °C in the presence of 3,5-difluorophenol or phenol and potassium carbonate in DMF[29] delivered compounds **3** and **6** in 76% and 90% yields, respectively. Phenoxy ethers **3** and **6** were then subjected to PPh_3, DIAD and diphenyl phosphoryl azide (DPPA) in dry THF[29] affording azides **4** and **7**, with inversion of configuration, in 92% and 84% yields, respectively. The statine-based templates **5** and **8** were then delivered in a three-step reaction sequence starting with hydrolysis of the isopropylidene groups of **4** and **7** using 50% acetic acid at 110 °C, producing the corresponding diols, followed by oxidative cleavage of the 1,2-diols using sodium periodate and potassium permanganate, rendering a mixture of carboxylic acids **5** and **8** along with their corresponding formic esters. Treating the mixtures with 1 M aqueous sodium hydroxide provided compounds **5** and **8** in 52% and 19% yields, respectively, over three steps.[20,30]

For the synthesis of compounds **11** and **14**, diol **1** was first transformed into its corresponding tin acetal by reaction with dibutyltin oxide in refluxing toluene followed by treatment with tetrabutylammonium bromide and 3,5-difluorobenzyl bromide or benzyl bromide to furnish the selectively alkylated[31] benzyl ethers **9** and **12** in 94% and 88% yields, respectively. Azides **10** and **13** were obtained in 77% and 99% yields, respectively, using the same procedure as employed for the synthesis of compounds **4** and **7**, and templates **11** and **14** were delivered in 51% and 29% yields, respectively, according to the same three-step procedure used when synthesizing templates **5** and **8**.

The synthesis of target compounds **16** and **17** (Table 1) was performed as outlined in Scheme 2. Template **5** was coupled to amine **A** (Fig. 2) employing *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and *N*,*N*-diisopropylethylamine (DIPEA) in DMF to give compound **15** in 76% yield. Reduction of the azide group was achieved using PPh_3 and methanol containing a few drops of water. Subsequent coupling of the

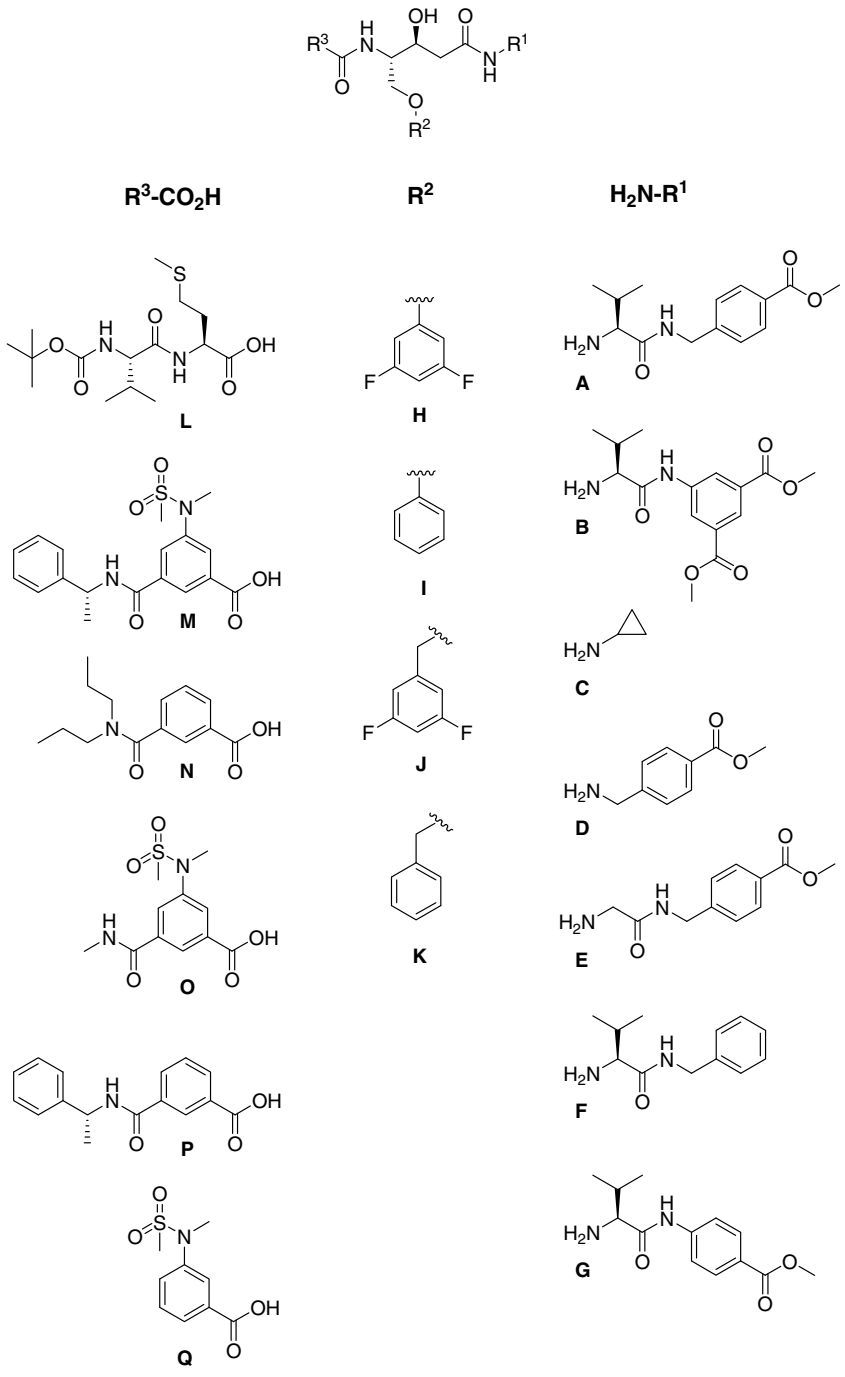
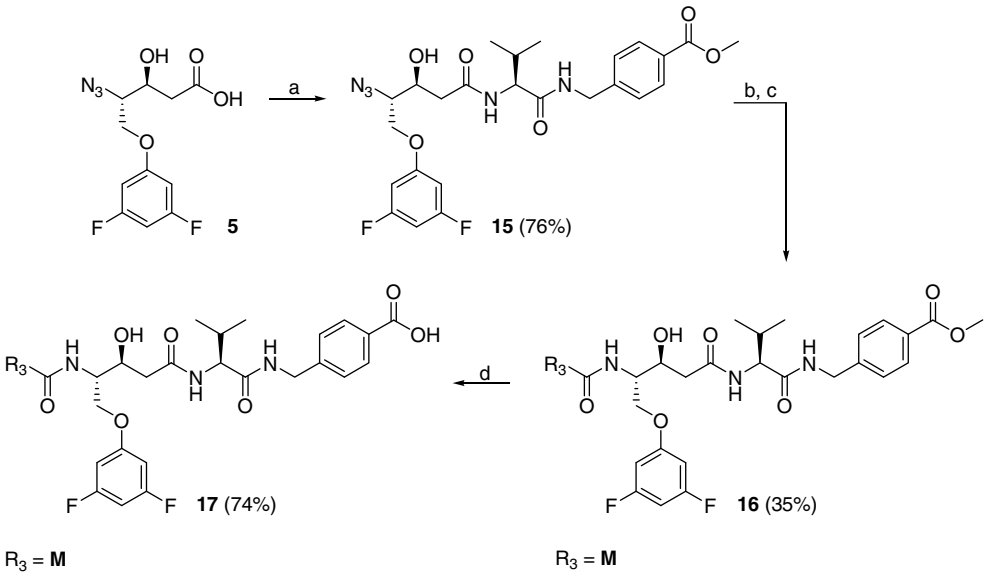


Figure 2. General picture of the different R-moieties connected or coupled to the central core.

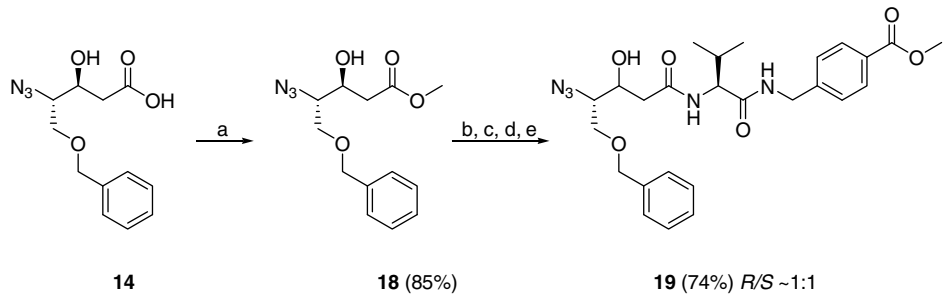
corresponding amine to carboxylic acid **M** (Fig. 2) using HATU and DIPEA in DMF furnished target compound **16** in 35% yield over the two steps. Finally, methyl ester hydrolysis using lithium hydroxide in THF/MeOH/H_2O 2:1:1 provided target compound **17** in 74% yield.

Scheme 3 depicts the synthesis of building block **19** having the alcohol of the central core with inverted configuration. The carboxylic acid **14** was treated with thionyl chloride in MeOH to afford methyl ester **18** in 85% yield. Oxidation of the hydroxyl group using Dess–Martin periodinane furnished the corresponding ketone, which after workup was subsequently reduced using $NaBH_4$ in MeOH. Workup followed by ester hydrolysis using LiOH provided the corresponding acid, which was subsequently coupled to amine **A** to give a diastereomeric mixture (**19**) (*R*/*S* ~ 1:1) in 74% yield over four steps. The diastereomers were separated on HPLC and the desired *R*-isomer was confirmed by HPLC retention times- and NMR analysis. Target compound **25** (Table 1) with *R*-configuration of the hydroxyl was synthesized in three steps according to Scheme 2, from the *R*-isomer of compound **19**.

All target compounds were synthesized according to Scheme 2, starting from templates **5**, **8**, **11** or **14**, employing the appropriate amines and carboxylic acids from Figure 2. In Tables 1–3 are given the structures of all target compounds synthesized and enzyme inhibition data.



Scheme 2. Reagents and conditions: (a) **A**, DIPEA, HATU, DMF; (b) PPh_3, MeOH, H_2O; (c) **M**, DIPEA, HATU, DMF; (d) 1 M LiOH, THF/MeOH/H_2O 2:1:1.



Scheme 3. Reagents and conditions: (a) $SOCl_2$, MeOH; (b) Dess–Martin periodinane, DCM; (c) $NaBH_4$, MeOH, −15 °C; (d) LiOH, dioxane/H_2O 1:1; (e) **A**, DIPEA, HATU, DMF.

2.2. Biological data and structure–activity relationships

All target compounds were screened against BACE-1 and the IC_{50} values are shown in Tables 1–3 along with K_i values for the inhibition of human cathepsin D.

Potent BACE-1 inhibitors incorporating methionine and valine in P2 and P3 positions, respectively, have previously been reported.[32] Moreover, statine-based inhibitors carrying carboxylic acid moieties on the P′ side have resulted in highly potent inhibitors.[18] These results prompted the synthesis of compounds **20** and **21** (Table 1) with the 3,5-difluorophenyloxymethyl residue in the P1 position where both methyl ester **20** and carboxylic acid **21** turned out to be inactive with IC_{50} values of >10 μM.

Replacing the P2–P3 groups with isophthalamide **M**, reported by Vacca and co-workers,[24] (Fig. 2) furnished methyl ester **16** and carboxylic acid **17** with promising IC_{50} values of 0.2 μM and 0.013 μM, respectively.

Exploration of the SAR of the P1 residue resulted in compounds **22**–**24**, where compound **22**, incorporating a phenyloxymethyl P1 residue, is equipotent with the corresponding 3,5-difluorophenyloxymethyl analog **17** displaying an IC_{50} value of 0.014 μM. Compound **23** with a P1 3,5-difluorobenzyloxymethyl residue is about nine times less active, whereas compound **24** incorporating a benzyloxymethyl residue is only slightly less active than compound **17**, with IC_{50} values of 0.12 μM and 0.024 μM for **23** and **24**, respectively (Table 1). This indicates that there is limited size of the S1 pocket, as it is accessed and utilized by the statine-based inhibitor cores.

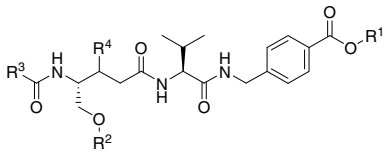
Studies on HIV protease inhibitors have shown that the (*R*)-hydroxyl stereochemistry are preferred for rather small HEA motif inhibitors,[33] whereas the opposite *S* stereochemistry is preferred for hydroxyethylene and statine-based isosteres. In order to confirm the preference for *S*-configuration of the hydroxyl group when employing these statine cores, we prepared compound **25** having the *R*-configuration, and as expected this resulted in a large drop in activity delivering an IC_{50} value of 1.2 μM (Table 1).

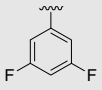
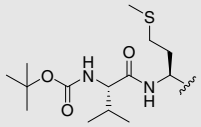
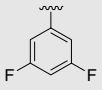
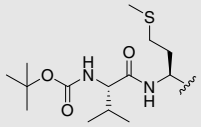
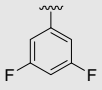
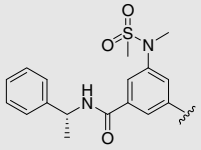
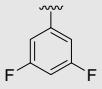
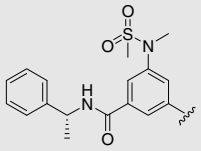
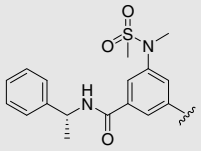
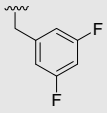
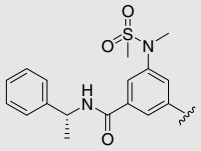
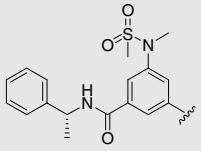
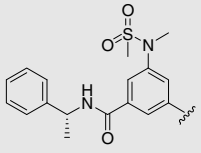
Further explorations into interactions with the S3′ pocket resulted in Kiso[34] analog inhibitors **26**–**32**, carrying an isophthalic-diacid group (Table 2). The diester **26** was found to have an activity of 0.22 μM while the target diacid **27** displays an IC_{50} value of 0.037 μM; thus making it slightly less active than monoacid analogue **17** (IC_{50} value of 0.013 μM). When extending the P1 moiety from 3,5-difluorophenyloxymethyl to 3,5-difluorobenzyloxymethyl as in compound **28**, no change in activity was observed compared to **27**, whereas enhanced activity results from the non-fluorinated extended P1 group as seen for compound **29**, delivering an IC_{50} value of 0.012 μM.

Replacing the **M** P2–P3 group in this series with simplified isophathalamides furnished inhibitors **30**–**32** displaying considerable lower potency (Table 2).

In Table 3 are depicted inhibitors featuring the central core of the potent inhibitor **17**. Truncation of the P2′–P3′ portion and replacing it with the small cyclopropyl residue or *p*-carboxybenzyl residue or removing the P2' side chain, in compound **17**, (compounds **33**–**35**) renders essentially inactive inhibitors of BACE-1. Notably, compound **36**, which lacks the carboxylate functionality

Table 1
Target compounds and inhibition data



Compound	R^1	R^2	R^3	R^4	IC_{50} (μM) BACE-1	K_i (μM) Cat D
20	Me			(*S*)-OH	>10	0.015
21	H			(*S*)-OH	>10	0.024
16	Me			(*S*)-OH	0.20	0.73
17	H			(*S*)-OH	0.013	0.51
22	H			(*S*)-OH	0.014	0.36
23	H			(*S*)-OH	0.12	0.43
24	H			(*S*)-OH	0.024	0.18
25	H			(*R*)-OH	1.2	>5

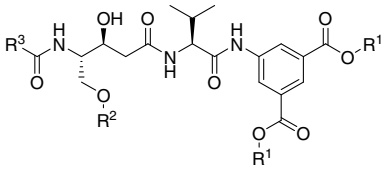
of **17**, is almost equipotent to the methyl ester analog **16**, with an IC_{50} value of 0.21 μM indicating the importance of the acidic function in this position of the S3′ pocket. A slightly less extended P3′ group as in compound **37** leads to a 20-fold drop in activity compared to **17**, that is, 0.26 μM versus 0.013 μM. An exploration in truncation of the P2–P3 **M** group resulted in inactive inhibitors, that is, compounds **38** and **39**, confirming the importance of both the S2 and S3 groups for these BACE-1 inhibitors (Table 3).

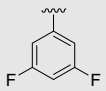
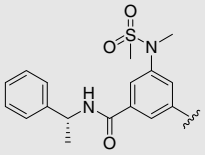
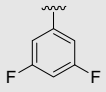
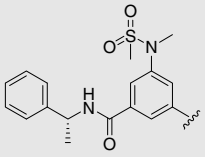
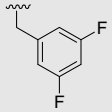
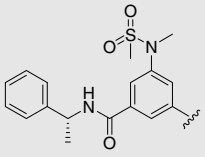
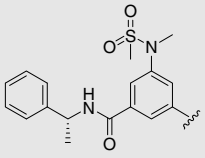
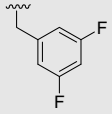
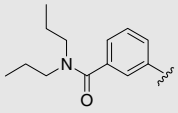
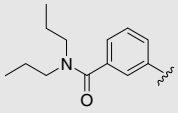
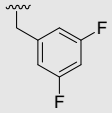
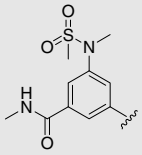
For all inhibitors the BACE cellular activities were determined measuring production of secreted soluble Aβ40 in cultured HEK-293 cells. Whereas many compounds showed good inhibition of BACE-1 in cell-free systems, they lost all activity in the cell-based assay resulting in IC_{50} values above 10 μM.

2.2.1. X-ray crystal structure results of inhibitor 27 (Fig. 3)

Compound **27** was co-crystallized with BACE-1 (PDB code, 3dm6) and the key binding interactions, obtained from the X-ray crystal structure, are described below. The hydrogen bonds from the nonprime side inhibitor backbone are made up by the P3 NH to the carbonyl of Gly230, the two P2 carbonyls that bind to the

Table 2
Target compounds and inhibition data

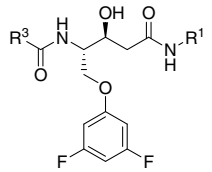


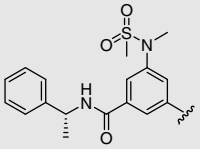
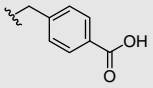
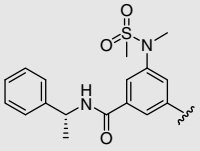
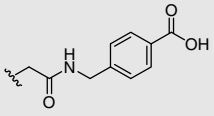
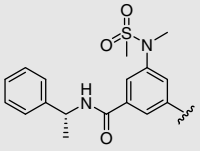
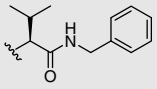
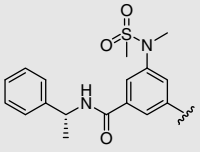
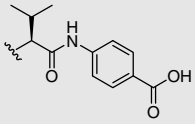
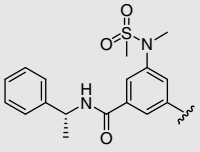
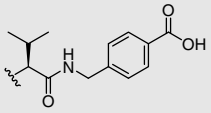
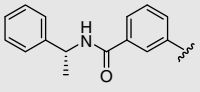
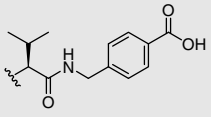
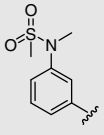
Compound	R^1	R^2	R^3	IC_{50} (μM) BACE-1	K_i (μM) Cat D
26	Me			0.22	1.95
27	H			0.037	0.78
28	H			0.040	0.44
29	H			0.012	0.63
30	H			2.23	0.93
31	H			1.89	0.35
32	H			1.6	>5

side chain of Thr232 and to the back bone of the flap residue Gln73, respectively, and the P1 NH that (similar to the P3 NH) also interacts with the carbonyl of Gly230. The hydrogen bond interactions of the backbone prime side are composed of the P1′ carbonyl to the back bone NH of the flap residue Thr72, the P2′ NH to the carbonyl of Gly34, the P2′ carbonyl to the side chain hydroxyl of Tyr198, and the P3′ NH to the carbonyl of Pro70. The hydroxyl group of the statine-based transition-state isostere is positioned between the two catalytic residues Asp32 and Asp228, forming a hydrogen bond network.

The P3 capping phenyl makes close interactions with several residues in the S3 subpocket. It is stacked between Thr232 and Gly13 from top and bottom and has edge on close contact interactions with Gly11, Tyr14, Ser229, Gly230, and Arg307. The P3 methyl group has close contact interactions with Gln12, Gly13, Leu30, and Ile110 in the S3 pocket. The P2 aromatic ring is stacked between Thr231 and the side chain of the flap residue Gln73. The sulfonamide makes hydrogen bond interactions with the back bone NH of Thr232 and Asn233 and with the side chains of Ser325 and Arg235. The P1 side chain is contributing considerably to the activity of inhibitor **27** with aromatic stacking interactions with the side chains of Tyr71, Phe108 and Trp115, and close contact interactions with Gln73, Gly74, Lys107, and Ile110 in the S1–S3 pocket. The P2′ valine side chain interacts mainly with Ser35, Val69, Ile126, and Arg128 in the S2′ pocket. The P3′ isophthalic acid group interacts weakly with the solvent exposed S3′ pocket and appears less hindered to rotate then any of the other side groups. However, in one out of three observations in the asymmetric unit, the isophthalic acid group is locked in position by two hydrogen bonds formed between the carboxylates and

Table 3
Target compounds and inhibition data



Compound	R^1	R^3	IC_{50} (μM) BACE-1	K_i (μM) Cat D
33			>10	>5
34			4.0	>5
35			2.3	>5
36			0.21	0.57
37			0.26	1.5
38			>10	>5
39			>10	>5

the side chains of Thr72 and Arg128. This gain in interactions with the S3′ pocket may relate to the observed increase in efficacy of this class of inhibitors upon addition of carboxylate functionalities in P3′.

2.2.2. Modeling of selected compounds in BACE-1 and cathepsin D

Modeling of compounds **17**, **36**, and **37**, using the structural information obtained from the crystal structure of **27**, revealed information that can rationalize some of the inconsistency found in potency. Especially modeling of compound **17** showed a possibility of an additional strong interaction between the P3′ *p*-carboxyl group and the side chain of Lys75. This explains the increased activity of compound **17** over compound **36**, lacking the carboxyl. Moreover, according to modeling results, none of the carboxyl groups of compounds **27** and **37** interact with Lys75.

Modeling of compounds **23** and **24** could not account for the differences in potency seen from the change in substitution of the P1 substituent when going from difluorobenzyloxymethyl analog **23** to the corresponding benzyloxymethyl analog **24**.

All the described inhibitors were also tested for inhibition of human cathepsin D. Generally, the explored variations of the P1 and P2′–P3′ side chains in this series of inhibitors are having minor effects on the cathepsin D selectivity. The variations of P2–P3 side chains are on the other hand of great importance for the cathepsin D activity as can be seen for compounds **20**, **21**, **30**, and **31** displaying cathepsin D IC_{50} values of 0.015, 0.024, 0.93, and 0.35 μM, respectively. Compounds **20** and **21** are completely inactive on

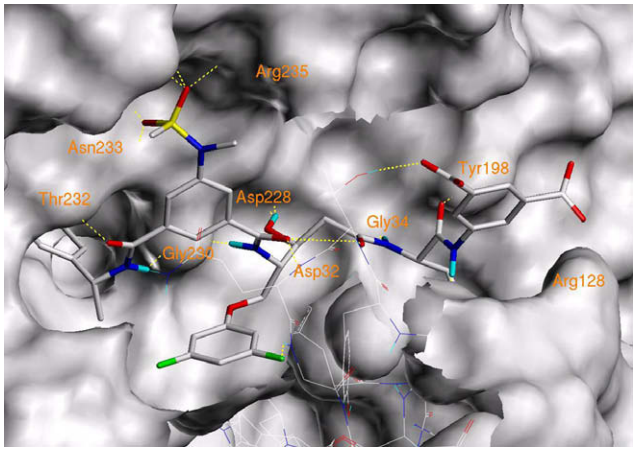


Figure 3. X-ray crystal structure of inhibitor **27** and BACE-1. Depicted are the key interactions between the inhibitor and the active site.

BACE-1 while compounds **30** and **31** are weak BACE-1 inhibitors with IC_{50} values of 2.2 and 1.9 μM, respectively.

The excellent potency of compound **20** for cathepsin D can be explained from docking of compound **20** with the crystal structure of cathepsin D (1lyb).[35] Accordingly, compound **20** shows an extensive hydrogen bonding network to the active site in cathepsin D including four strong hydrogen bonds to the flap. The methionine side chain in the P2 position of the inhibitor is interacting closely with the side chains of methionine 307 and 309 in the S2 pocket. The difluorophenyloxy P1 group also fits well having favorable aromatic stacking interactions in the S1 pocket.

A model of compound **20** in BACE-1 on the other hand reveals that the large hydrophobic P2 methionine does not accommodate well to the polar environment of the BACE-1 S2 pocket. The lack of BACE-1 activity of compound **20** may also be a result of the bulky and unflexible *tert*-butoxycarbonyl group that does not fit well in the narrow path between the flap of Gln73 and the S3 and S4 pockets of BACE-1.

3. Conclusion

Several potent BACE-1 inhibitors have been synthesized comprising a statine-based central core containing novel phenyloxymethyl- and benzyloxymethyl residues in the P1 position. These novel templates were obtained by employing an efficient synthesis route starting from 3-deoxy-1,2-*O*-isopropylidene-α-D-glucose (**1**).

Different substituents were evaluated in order to study the SAR for this inhibitor class. Carboxylate functionalities in P3′ position were found to be of importance in order to obtain inhibitors of high potency, for example, **17** (13 nM), **22** (14 nM), **27** (37 nM), and **29** (12 nM). X-ray crystal data revealed the binding mode of inhibitor **27**.

4. Experimental

4.1. Production of soluble BACE-1

Construction of soluble BACE-1. The cDNA for human BACE-1 was isolated from human brain. Two different expression constructs were made; one containing residues 42–446 and one containing residues 42–454. Both were cloned into the expression vector pET11a. In order to improve protein expression the first 205 bp of the expression constructs were changed into more common *Escherichia coli* codons.

4.1.1. Growth of cells and collection of inclusion bodies

Escherichia coli cells (BL21(DE3)) expressing human BACE-1 were grown in Terrific Broth with 50 μg/mL carbenicillin added. When cell density reached $OD_{600} \approx 1.3$, 1 mM IPTG was added and the growth was continued for 3 h. The cells were harvested by centrifugation at 6000*g* for 10 min at 4 °C. Cell pellets were stored at −20 °C. The cells were solubilized in a glass homogenizer in lysis buffer (50 mM Tris–HCl, pH 7.6, 10 mM EDTA, 50 mM $MgCl_2$, and 0.1% (w/v) TX-100) with DNAse and Complete protease inhibitor cocktail (Merck) added. The cells were disrupted in a cell disrupter (Constant cell system) at 1.7 kbar. The inclusion bodies were collected from the lysate by centrifugation at 20,000*g* for 15 min at 4 °C and washed with wash buffer (50 mM Tris–HCl, pH 7.6, and 100 mM NaCl) with 0.5% Triton X-100 added twice and twice with wash buffer without the addition of Triton X-100. Washed inclusion bodies were stored at −70 °C.

4.1.2. Denaturing and refolding of BACE-1

Inclusion bodies were dissolved in denaturing buffer (50 mM Tris–HCl, pH 9, 8 M urea, and 10 mM β-mercaptoethanol) 35 mL buffer/g cell pellet). The sample was further diluted 1:20 in refolding buffer (20 mM Tris–HCl, pH 9, 0.5 mM oxidized glutathione, and 1.25 mM reduced glutathione) at room temperature. It was important that the solutions were thoroughly degassed before the reducing agents were added. The sample was stirred at 4 °C for 4 h, the pH was adjusted to 8.0 and the solutions were stirred for 48–72. The pH was further adjusted to 6.8 and the solutions were stirred for an additional 48–72 h.

4.1.3. Chromatography of BACE-1

Precipitate was removed by centrifugation at 18,500*g* for 1 h followed by filtration through a 0.8/02 μm filter. The sample was loaded onto an equilibrated 5 mL HiTrap Q-sepharose (GE Health Care) overnight. The column was washed with A-buffer (20 mM Tris–HCl, pH 7.6, 0.4 M urea) until the absorbance was stabilized. BACE-1 was eluted by A-buffer with 0.25 M NaCl added and the peak was collected and concentrated in a 20 mL Viva Science spin concentrator (MWCO 10 000). The concentrated sample was applied onto a HiLoad Superdex 200 26/60 (GE Health Care) previously equilibrated with 20 mM Tris–HCl, pH 7.6. The eluted monomeric form of BACE-1 could easily be distinguished from multi-meric forms by running native PAGE. Fractions containing monomer BACE-1 were pooled and applied to a 1 mL HiTrap Q-sepharose equilibrated with 20 mM Tris–HCl buffer, pH 7.6. Pure BACE-1 was eluted by a gradient of 0–0.25 M NaCl for 20 CV. The total yield of pure BACE-1 from 1 L of bacterial cell culture was generally 1–2 mg.

4.2. BACE-1 assay

The inhibition of BACE-1 was determined in a homogeneous time resolved fluorescence (TRF) assay (True Point kit, Perkin-Elmer). The assay buffer contained sodium acetate, CHAPS, Triton-X 100, and EDTA, pH 4.5. The substrate used was the Swedish mutant sequence Eu-EVNLDAEFK-Quencher.

Inhibitor dilutions were made in DMSO at 30× final assay concentration. 1 μl per well of inhibitor dilutions were added to 15 μl per well of 20 nM BACE-1 in assay buffer on a black half area 96-well plate. The plate was covered and incubated at room temperature for 30 min. The assay was initiated by the addition of 15 μl per well of 400 nM substrate in assay buffer (final concentration in assay 200 nM) and the plate was read for 60 min at room temperature in a Victor-2 (Wallac). The excitation wavelength was 340 nm and the emission was monitored at 615 nm. The percent inhibition was calculated from initial velocities of the inhibited reactions relative to the uninhibited control. IC_{50} values were

determined using the following relationship: % inhibition = 100[I]/([I] + IC_{50}).

4.3. Cathepsin D assay

Cathepsin D, purified from human liver, was purchased from Sigma. The assay buffer was 125 mM sodium acetate, 12.5% glycerol, and 0.0125% Tween 20, pH 3.5. The substrate used was DABCYL-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-EDANS, purchased from Anaspec. Inhibitor dilutions were made in DMSO at 100× final assay concentration. One microliter per well of inhibitor dilutions were added to 95 μl per well of 175 ng/mL cathepsin D in assay buffer on a 96-well plate. The plate was covered and incubated at 37 °C for 20 min. The assay was initiated by the addition of 5 μl per well of 40 μM substrate in assay buffer (final concentration in assay 2 μM) and the plate was read for 20 min at 37 °C in a Fluoroskan Ascent (Thermo Labsystems). The excitation wavelength was 355 nm and the emission was monitored at 500 nm. Data were fit to the competitive inhibition equation by non-linear regression with the substrate concentration and K_M fixed at 2 μM and 2.7 μM, respectively.

4.4. Crystallography

The crystals were grown using the hanging drop vapor diffusion method. The protein was concentrated to 5 mg/mL in 20 mM Tris, pH 7.6, 200 mM sodium chloride. Inhibitory compounds (dissolved at 10 mM in 16% DMSO and 33% isopropanol) were added to the concentrated protein in twofold molar excess and allowed for complex formation over 30 min at 4 °C. Hanging drops were formed by the addition of 1 μL reservoir solution (100 mM sodium citrate, pH 5.0, 3–15% PEG8000, and 300 mM lithium sulphate) to 1 μL of protein–compound complex. Typically, single crystals appeared after one day and grew to dimensions of 200 μm × 150 μm × 80 μm over a period of two weeks. Prior to diffraction experiments, single crystals were transferred to a cryo-protecting solution that contained the reservoir solution plus 25% glycerol, soaked briefly and frozen in liquid nitrogen. Crystals diffracted to 2.2–2.8 Å and belonged to the monoclinic space group *P*21 with cell dimensions of $\alpha = 83.435°$, $\beta = 103.017°$, $\gamma = 103.110°$, and $\delta = 103.50°$.

4.5. Experimental modeling

Inhouse cocrystal structure of compound **27** in BACE-1 (PDB code, 3dm6) was used for the analysis of the binding interactions. Inhibitor **20** was docked and minimized in the crystal structure of cathepsin D (1lyb).[35] All modeling experiments were performed using SYBYL 7.3 (Tripos Inc. 1699 South Hanley Road, St. Louis, Missouri, 63144, USA).

4.6. General methods

NMR spectra were recorded on a Varian 300 MHz instrument using $CDCl_3$, CD_3OD and $(CD_3)_2SO$ as solvent. TLC was carried out on Merck precoated 60 F_{254} plates using UV-light and charring with ethanol/sulfuric acid/*p*-anisaldehyde/acetic acid 90:3:2:1, and a solution of 0.5% ninhydrin in ethanol for visualization. Flash column (FC) chromatography was performed using silica gel 60 (0.040–0.063 mm, Merck). Organic phases were dried over anhydrous magnesium sulfate. Concentrations were performed under diminished pressure (1–2 KPa) at a bath temperature of 40 °C. Optical rotations were measured using a Perkin-Elmer 141 polarimeter. Isocratic HPLC was performed on a preparative C-18 column.

Gradient HPLC-MS (used mainly for mass detection and purity measurements) was performed on a Gilson system (Column: Phenomenex C-18 250 × 15 mm and Phenomenex C-18 150 × 4.6 mm for preparative and analytical runs, respectively; Pump: Gilson gradient pump 322; UV/vis-detector: Gilson 155; MS detector: Thermo Finnigan Surveyor MSQ; Gilson Fraction Collector FC204) using methanol with 0.1% formic acid and deionized water with 0.1% formic acid as mobile phase.

4.7. LC–MS purity measurements

4.7.1. Chromatography system A

Column: Phenomenex C-18 150 × 4.6 mm; Pump: Gilson gradient pump 322; UV/vis-detector: Gilson 155; MS detector: Thermo Finnigan Surveyor MSQ; Software: Gilson UniPoint 4.0 and Xcalibur 1.3. Gradient: methanol 40–100% over 10 min at 1 mL/min followed by 100% for 5 min at 1 mL/min. To all solvents formic acid (0.1% v/v) was added. Peaks were detected at 254 nm.

4.7.2. Chromatography system B

As system A except: Gradient: acetonitrile 60–100% over 10 min at 1 mL/min followed by 100% for 5 min at 1 mL/min.

4.8. General procedures (used in the synthesis and deprotections of building blocks A–B, E–G, and L)

4.8.1. Peptide coupling

To a cooled (0 °C) solution of the acid (4.95 mmol), the amine (5.44 mmol), and DIPEA (14.84 mmol) in DMF (20 mL) was added HATU (5.94 mmol). The solution was stirred for 0.5 h and for an additional 2 h at room temperature. EtOAc and brine were added and the organic phase was separated and washed once more with brine, dried and concentrated. The crude peptide was purified by flash column (FC) chromatography or HPLC.

4.8.2. Boc deprotection

To a solution of the protected compound (0.167 mmol) and triethylsilane (0.419 mmol) in DCM (3 mL) was added TFA (1 mL). The solution was stirred for 2 h at room temperature and then concentrated and co-concentrated with toluene.

4.8.3. Methyl ester deprotection

To a cooled (0 °C) solution of the ester (0.021 mmol) in dioxane/water 1:1 (1 mL) was added 1 M LiOH (0.031 mmol) and the mixture was stirred for 0.5 h and for an additional 0.5 h in room temperature. The solution was neutralized with 1 M HCl and concentrated and co-concentrated with toluene.

4.9. Synthetic procedures

4.9.1. 5-(Methanesulfonyl-methyl-amino)-*N*-((*R*)-1-phenyl-ethyl)-isophthalamic acid (M)

Synthesized according to Ref. 24.

4.9.2. *N*,*N*-Dipropyl-isophthalamic acid (N)

Synthesized in 50% yield over three steps from commercially available isophthalic acid performing in sequential order: (1) monoester hydrolysis according to the monoester hydrolysis step in Ref. 24; (2) peptide coupling using dipropyl amine according to General procedure 1 (Section 4.8.1); (3) ester hydrolysis according to General procedure 3 (Section 4.8.3).

4.9.3. 5-(Methanesulfonyl-methyl-amino)-*N*-methyl-isophthalamic acid (O)

Synthesized according to Ref. 24 except for the amide formation step where methyl amine was coupled instead of *R*-(+)-α-methylbenzylamine.

4.9.4. *N*-((R)-1-Phenyl-ethyl)-isophthalamic acid (P)

Synthesized in 50% total yield over three steps from commercially available isophthalic acid performing in sequential order: (1) monoester hydrolysis according to the monoester hydrolysis step in Ref. 24; (2) coupling of (*R*)-(−)-1-phenylethylamine using General Synthetic Procedure 1 (Peptide coupling); (3) ester hydrolysis using General Synthetic Procedure 3 (Section 4.8.3).

4.9.5. 3-(Methanesulfonyl-methyl-amino)-benzoic acid (Q)

Commercially available 3-amino-benzoic acid methyl ester was used as starting material. Mesylation and methylation were performed according to Ref. 24 and the final hydrolysis step was performed according to General procedure 3 (Section 4.8.3).

4.9.6. 3-Deoxy-1,2-*O*-isopropylidene-α-D-glucose (1)

The diol **1** was synthesized from 1,2,5,6-diisopropylidene-D-glucose in 60% yield over three steps according to Refs. 25–27.

4.9.7. 5,6-Anhydro-3-deoxy-1,2-*O*-isopropylidene-α-D-glucose (2)

To a mixture of diol 1 (1.49 g, 7.27 mmol) and triphenylphosphine (2.29 g, 8.73 mmol) in chloroform (120 mL) were added diisopropyl azodicarboxylate (DIAD) (1.72 mL, 8.28 mmol) and the mixture was stirred at reflux overnight. Concentration and flash column (FC) chromatography gradient (toluene/EtOAc 15:1 and 9:1) gave 2 (0.91 g, 67%) as a colorless oil. Analytical data in accordance with Ref. 28.

4.9.8. 6-(3,5-Difluorophenoxy)-3-deoxy-1,2-*O*-isopropylidene-α-D-glucose (3)

To epoxide **2** (0.893 g, 4.80 mmol), and 3,5-difluorophenol (0.690 g, 5.30 mmol) in DMF (20 mL) was added K_2CO_3 (0.332 g, 2.40 mmol). The mixture was heated to 100 °C and stirred overnight. After cooling the solution aqueous NH_4Cl (20 mL) was added and the reaction mixture was extracted with diethyl ether. The organic phase was washed twice with water, dried and concentrated. FC gradient (toluene/EtOAc 15:1, 9:1 and 6:1) gave **3** (1.16 g, 76%) as a colorless oil. $[\alpha]_D^{22}$ −17.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 3H), 1.52 (m, 3H), 1.81–1.96 (m, 1H), 2.16 (dd, J = 4.2, 13.5 Hz, 1H), 2.62 (d, J = 4.2 Hz, 1H), 3.94 (dd, J = 6.6, 9.6 Hz, 1H), 4.05 (dd, J = 3.9, 9.6 Hz, 1H), 4.09–4.17 (m, 1H), 4.26–4.35 (m, 1H), 4.74–4.79 (m, 1H), 5.82 (d, J = 3.9 Hz, 1H), 6.38–6.49 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.1, 26.8, 34.1, 69.9, 70.1, 78.1, 80.6, 96.9 (t, J_{CF} = 25.9 Hz), 98.5 (d, J_{CF} = 27.8 Hz, 2C), 105.5, 111.5, 160.4, 163.6 (d, J_{CF} = 246.6 Hz), 163.8 (d, J_{CF} = 246.6 Hz).

4.9.9. 5-Azido-6-(3,5-difluorophenoxy)-3,5 dideoxy-1,2-*O*-isopropylidene-α-L-idose (4)

Compound **3** (1.16 g, 3.65 mmol) and triphenylphosphine (1.44 g, 5.64 mmol) were dissolved in dry THF (19 mL). The mixture was cooled to −15 °C and diisopropyl azodicarboxylate (DIAD) (1.80 mL, 9.13 mmol) was added. After stirring the solution for 10 min at −15 °C the temperature was raised to 0 °C and diphenylphosphoryl azide (DPPA) (1.23 mL, 5.48 mmol) was added. The solution was stirred for 30 min at 0 °C and then overnight at room temperature. Concentration and FC gradient (toluene, toluene/EtOAc 24:1, 15:1, 9:1) gave **4** (1.15 g, 92%) as a colorless oil. $[\alpha]_D^{22}$ −21.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 3H), 1.51 (s, 3H), 1.92–2.03 (m, 1H), 2.13 (dd, J = 4.7, 13.4 Hz, 1H), 3.70–3.78 (m, 1H), 4.17–4.22 (m, 2H), 4.36–4.44 (m, 1H), 4.74–4.80 (m, 1H), 5.84 (d, J = 3.6 Hz, 1H), 6.41–6.51 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.2, 26.8, 35.3, 61.6, 69.2, 77.1, 80.2, 97.2 (t, J_{CF} = 25.8 Hz), 98.6 (d, J_{CF} = 28.9 Hz, 2C), 105.6, 111.7, 160.0, 163.6, (d, J_{CF} = 247.1 Hz) 163.8 (d, J_{CF} = 246.8 Hz).

4.9.10. (3*S*,4*S*)-4-Azido-5-(3,5-difluorophenoxy)-3-hydroxy-pentanoic acid (5)

Compound **4** (185 mg, 0.542 mmol) was refluxed (115 °C) in acetic acid/water (1:1, 4 mL) for 1 h. The mixture was concentrated and the residual was dissolved in *tert*-butyl alcohol/water (2:3, 4 mL) and sodium periodate ($NaIO_4$) (581 mg, 2.72 mmol) was added. After stirring for 20 min potassium permanganate ($KMnO_4$) (12.8 mg, 0.081 mmol) was added and the reaction mixture was allowed to stir for an additional 1.5 h. The mixture was extracted three times with $CHCl_3$ and the combined organic extracts were dried and concentrated.

The crude residual was dissolved in water (5 mL) and the mixture was cooled to 0 °C. One molar NaOH (2.2 mL) was added drop wise and the solution was stirred at 0 °C for 30 min and for an additional 1 h at room temperature. The reaction mixture was washed twice with diethyl ether and was then acidified with 6 M HCl to pH 1–2. The acidified water phase was extracted three times with $CHCl_3$ and the combined organic extracts were dried and concentrated. FC gradient (toluene/EtOAc 6:1 + 1% HOAc and toluene/EtOAc 2:1 + 1% HOAc) gave **5** (52%) as white crystals. $[\alpha]_D^{22}$ −14.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 2.58 (dd, J = 7.7, 15.9 Hz, 1H), 2.65 (dd, J = 5.2, 15.9 Hz, 1H), 3.81–3.88 (m, 1H), 4.14–4.31 (m, 3H), 4.94 (br s, 1H), 6.47–6.64 (m, 3H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 39.7, 65.4, 68.7, 70.1, 97.5 (t, J_{CF} = 26.5 Hz), 99.5 (d, J_{CF} = 29.2 Hz, 2C), 161.9, 165.0 (d, J_{CF} = 245.4 Hz), 165.2 (d, J_{CF} = 245.4 Hz), 174.7.

4.9.11. 6-Phenoxy-3-deoxy-1,2-*O*-isopropylidene-α-D-glucose (6)

Compound **6** was synthesized in 90% yield (colorless oil) according to the synthesis method of compound **3** using phenol instead of 3,5-difluorophenol. $[\alpha]_D^{22}$ −26.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 3H), 1.49 (s, 3H), 1.81–1.93 (m, 1H), 2.12 (dd, J = 4.5, 13.5 Hz, 1H), 3.45 (d, J = 4.2 Hz, 1H), 3.92 (dd, J = 6.6, 9.9 Hz, 1H), 4.01 (dd, J = 3.9, 9.9 Hz, 1H), 4.07–4.16 (m, 1H), 4.28–4.37 (m, 1H), 4.64 (app. t, J = 4.1 Hz, 1H), 5.76 (d, J = 3.6 Hz, 1H), 6.84–6.94 (m, 3H), 7.06–7.14 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 25.7, 26.3, 33.4, 68.9, 70.1, 78.1, 80.2, 105.0, 110.8, 114.2, 120.6, 129.0, 158.2.

4.9.12. 5-Azido-6-phenoxy-3,5 dideoxy-1,2-*O*-isopropylidene-α-L-idose (7)

Compound **7** was synthesized in 84% yield (colorless oil) from **6** according to the synthesis method of compound **4**. $[\alpha]_D^{22}$ −44.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 3H), 1.49 (m, 3H), 1.82–1.96 (m, 1H), 2.07 (dd, J = 4.7, 13.4 Hz, 1H), 3.63–3.73 (m, 1H), 4.10–4.22 (m, 2H), 4.32–4.41 (m, 1H), 4.65–4.72 (m, 1H), 5.79 (d, J = 3.6 Hz, 1H), 6.87–6.98 (m, 3H), 7.08–7.16 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.4, 27.1, 35.6, 62.3, 68.7, 77.6, 80.5, 105.8, 111.8, 114.9, 120.5, 129.8, 158.4.

4.9.13. (3*S*,4*S*)-4-Azido-3-hydroxy-5-phenoxy-pentanoic acid (8)

Compound **8** was synthesized in 19% yield (white crystals) from **7** according to the synthesis method of compound **5**. $[\alpha]_D^{22}$ −15.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.65 (dd, J = 4.2, 16.6 Hz, 1H), 2.77 (dd, J = 8.6, 16.6 Hz, 1H), 3.78 (ddd, J = 3.5, 5.0, 6.9 Hz, 1H), 4.22 (dd, J = 6.9, 9.9 Hz, 1H), 4.27 (dd, J = 5.0, 9.9 Hz, 1H), 4.27–4.33 (m, 1H), 6.90–7.02 (m, 3H), 7.26–7.33 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 38.3, 63.5, 67.7, 67.9, 114.6, 121.6, 129.6, 158.0, 177.0.

4.9.14. 6-(3,5-Difluorobenzyloxy)-3-deoxy-1,2-*O*-isopropylidene-α-D-glucose (9)

Compound **1** (1.50 g, 7.35 mmol) and Bu_2SnO (2.11 g, 8.48 mmol) were refluxed in toluene (46 mL) for 5 h. (The water

produced from the acetal formation was removed using a Dean-Stark trap.) The mixture was allowed to cool down to 90 °C and tetrabutylammonium bromide and 3,5-difluorobenzyl bromide were added. The solution was stirred at 90 °C overnight and concentrated. FC (toluene/EtOAc 2:1) gave **9** (2.28 g, 94%) as a colorless oil. $[\alpha]_D^{22}$ −24.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 3H), 1.49 (s, 3H), 1.78–1.90 (m, 1H), 2.07 (dd, *J* = 4.6, 13.8 Hz, 1H), 2.52 (br s, 1H), 3.49 (dd, *J* = 6.8, 9.9 Hz, 1H), 3.59 (dd, *J* = 3.9, 9.9 Hz, 1H), 3.94–4.02 (m, 1H), 4.17–4.26 (m, 1H), 4.51 (s, 2H), 4.73 (app. t, *J* = 4.2 Hz, 1H), 5.78 (d, *J* = 3.9 Hz, 1H), 6.64–6.74 (m, 1H), 6.79–6.89 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.1, 26.7, 33.9, 70.9, 71.7, 72.1, 78.2, 80.5, 102.9 (t, J_{CF} = 25.3 Hz), 105.3, 109.9 (d, J_{CF} = 25.2 Hz, 2C), 111.3, 142.0, 163.0 (d, J_{CF} = 248.5 Hz), 163.1 (d, J_{CF} = 248.5 Hz).

4.9.15. 5-Azido-6-(3,5-difluorobenzyloxy)-3,5 dideoxy-1,2-*O*-isopropylidene-α-L-idose (10)

Compound **10** was synthesized in 77% yield (colorless oil) from **9** according to the synthesis method of compound **4**. $[\alpha]_D^{22}$ −35.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 3H), 1.49 (s, 3H), 1.84–1.96 (m, 1H), 2.07 dd, *J* = 4.5, 13.2 Hz, 1H), 3.52–3.60 (m, 1H), 3.71–3.78 (m, 2H), 4.32 (app. dt, *J* = 4.2 Hz, 1H), 4.55 (s, 2H), 4.71–4.77 (m, 1H), 5.81 (d, *J* = 3.6 Hz, 1H), 6.67–6.77 (m, 1H), 6.83–6.91 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.2, 26.8, 35.9, 62.5, 71.0, 72.3, 77.3, 80.3, 103.1 (t, J_{CF} = 25.3 Hz), 105.5, 109.9 (d, J_{CF} = 25.2 Hz, 2C), 111.6, 141.9, 163.1 (d, J_{CF} = 248.8 Hz), 163.3 (d, J_{CF} = 248.8 Hz).

4.9.16. (3*S*,4*S*)-4-Azido-5-(3,5-difluorobenzyloxy)-3-hydroxy-pentanoic acid (11)

Compound **11** was synthesized in 51% yield (white crystals) from **10** according to the synthesis method of compound **5**. $[\alpha]_D^{22}$ −16.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 2.53 (dd, *J* = 8.1, 15.8 Hz, 1H), 2.60 (dd, *J* = 5.1, 15.8 Hz, 1H), 3.62–3.69 (m, 1H), 3.70–3.83 (m, 2H), 4.16 (ddd, *J* = 3.6, 5.1, 8.1 Hz, 1H), 4.57 (s, 2H), 6.77–6.87 (m, 1H), 6.91–7.01 (m, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 39.8, 66.0, 68.8 71.8, 72.7, 103.5 (t, J_{CF} = 25.8 Hz), 110.8 (d, J_{CF} = 25.2 Hz, 2C), 144.2, 164.4 (d, J_{CF} = 247.1 Hz), 164.5 (d, J_{CF} = 247.1 Hz), 174.8.

4.9.17. 6-Benzyloxy-3-deoxy-1,2-*O*-isopropylidene-α-D-glucose (12)

Compound **12** was synthesized in 88% yield (colorless oil) according to the synthesis method of compound **9** using benzyl bromide. Analytical data in accordance with Ref. 20.

4.9.18. 5-Azido-6-benzyloxy-3,5-dideoxy-1,2-*O*-isopropylidene-α-L-idose (13)

Compound **13** was synthesized in 99% yield (colorless oil) from **12** according to the synthesis method of compound **4**. Analytical data in accordance with Ref. 20.

4.9.19. (3*S*,4*S*)-4-Azido-5-benzyloxy-3-hydroxy-pentanoic acid (14)

Compound **14** was synthesized in 29% yield (white crystals) from **13** according to the synthesis method of compound **5**. Analytical data in accordance with Ref. 20.

4.10. Target compounds

4.10.1. Synthetic protocols for target compounds

4.10.1.1. Synthetic protocol A. Peptide coupling. 4-({(S)-2-[(3S,4S)-4-Azido-5-(3,5-difluoro-phenoxy)-3-hydroxy-pentanoylamino]-3-methyl-butyrylamino}-methyl)-benzoic acid methyl ester (15). To a cooled (0 °C) solution of acid **5** (121 mg, 0.421 mmol), amine **A** (175 mg, 0.480 mmol) and DIPEA (220 μL, 1.263 mmol) in DMF (4 mL) was added HATU (193 mg, 0.508 mmol) and the mixture was stirred for 0.5 h and for an additional 2 h at room temperature. EtOAc and brine were added and the organic phase was separated and washed once more with brine, dried and concentrated. Purification by HPLC ($MeOH/H_2O$ 85:15 + 0.2% TFA) gave **15** (170 mg, 76%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 1:1) δ 0.93 (d, *J* = 6.9 Hz, 6H), 2.00–2.15 (m, 1H), 2.50 (dd, *J* = 7.7, 14.6 Hz, 1H), 2.57 (dd, *J* = 5.1, 14.6 Hz, 1H), 3.71–3.79 (m, 1H), 3.87 (s, 3H), 4.09–4.25 (m, 4H), 4.43 (d, *J* = 6.0 Hz, 2H), 6.38–6.54 (m, 3H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.72 (b, 1H), 7.87 (d, *J* = 8.1 Hz, 2H), 8.32 (b, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3/CD_3OD$ 1:1) δ 18.4, 19.5, 40.6, 42.1, 43.5, 52.4, 63.6, 64.8, 68.4, 69.3, 97.2 (t, J_{CF} = 26.2 Hz), 99.0 (d, J_{CF} = 27.8 Hz, 2C), 128.0, 129.5, 130.3, 144.4, 160.9, 164.2 (d, J_{CF} = 246.5 Hz), 164.4 (d, J_{CF} = 246.5 Hz), 167.9, 172.6, 172.9.

4.10.1.2. Synthetic protocol B. Azide reduction and amide bond formation. 4-[((S)-2-{(3S,4S)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((R)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-methyl]-benzoic acid methyl ester (16). Azide **15** (160 mg, 0.300 mmol) and PPh_3 (118 mg, 0.450 mmol) were dissolved in MeOH (15 mL). A few drops of water were added and the mixture was stirred overnight. The solvent was evaporated and the residual was purified by HPLC ($MeOH/H_2O$ 80:20 + 0.2% TEA).

The amine was subsequently coupled to acid **M** (102 mg, 0.271 mmol) according to synthetic protocol A. Purification by HPLC ($MeOH/H_2O$ 85:15 + 0.2% TEA) afforded **16** (colorless solid) in 35% yield over two steps. $[\alpha]_D^{22}$ −6.4 (*c* 0.14, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (app. t, *J* = 6.9 Hz, 6H), 1.52 (d, *J* = 6.9 Hz, 3H), 1.95–2.10 (m, 1H), 2.24–2.34 (m, 2H), 2.35–2.49 (m, 1H), 2.79 (s, 3H), 3.23 (s, 3H), 3.84 (s, 3H), 4.06–4.17 (m, 2H), 4.21–4.49 (m, 5H), 4.82 (br s, 1H), 5.15–5.28 (m, 1H), 6.30–6.45 (m, 3H), 7.14–7.29 (m, 5H), 7.30–7.36 (m, 2H), 7.43–7.51 (m, 1H), 7.53–7.64 (m, 2H), 7.85 (d, *J* = 8.1 Hz, 2H), 7.92 (d, *J* = 7.8 Hz, 2H), 8.24 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.3, 19.3, 21.7, 30.8, 35.7, 37.9, 40.2, 43.1, 49.9, 52.1, 53.3, 59.2, 67.3, 67.7, 96.5 (t, J_{CF} = 26.2 Hz), 98.5 (d, J_{CF} = 28.3 Hz, 2C), 124.7, 126.2, 127.4, 127.5, 127.9, 128.2, 128.7, 129.2, 129.8, 135.2, 136.1, 142.1, 143.1, 143.2, 160.2, 163.5 (d, J_{CF} = 246.5 Hz), 163.7 (d, J_{CF} = 246.8 Hz), 165.0, 166.5, 166.8, 171.8, 172.2. HRMS calcd $(M+H)^+$: 866.3246; found 866.3271. LC–MS Purity System A: t_R = 9.20 min, 98%; System B: t_R = 8.96 min, 97%.

4.10.1.3. Synthetic protocol C. Hydrolysis of ester. 4-[((S)-2-{(3S,4S)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((R)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-methyl]-benzoic acid (17). To a cooled (0 °C) solution of ester **16** (22 mg, 0.025 mmol) in THF/MeOH/H_2O 2:1:1 (2 mL) was added 1 M LiOH (100 μL, 0.100 mmol). The solution was stirred overnight and acidified to approximately, pH 3–4, with 1 M HCl. Concentration and purification by HPLC twice using $MeOH/H_2O$ 80:20 + 0.2% TEA as the first eluent and $MeOH/H_2O$ 80:20 + 0.2% TFA as the second eluent gave **17** (16 mg, 74%) as a colorless solid. $[\alpha]_D^{22}$ −11 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.94 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 1.57 (d, *J* = 6.9 Hz, 3H), 2.09–2.24 (m, 1H), 2.48–2.65 (m, 2H), 2.96 (s, 3H), 3.35 (s, 3H), 4.11–4.28 (m, 3H), 4.39–4.55 (m, 4H), 5.19–5.31 (m, 1H), 6.44–6.59 (m, 3H), 7.18–7.35 (m, 4H), 7.36–7.44 (m, 3H), 7.90 (d, *J* = 8.4 Hz, 2H), 8.02–8.09 (m, 2H), 8.28 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 17.2, 18.6, 20.9, 30.2, 34.8, 37.2, 40.1, 42.7, 49.8, 52.9, 59.6, 67.4, 67.7, 96.0 (t, J_{CF} = 26.2 Hz), 98.4 (d, J_{CF} = 28.1 Hz, 2C), 125.0, 126.1, 127.0, 127.3, 128.1, 128.4, 129.5, 129.7, 130.5, 135.8, 136.2, 142.5, 143.8, 144.2, 161.0, 163.9 (d,

J_{CF} = 245.6 Hz), 164.1 (d, J_{CF} = 245.6 Hz), 167.8, 168.4, 172.6,172.7, 172.9. HRMS calcd (M+H)$^+$: 852.3090; found 852.3109. LC–MS Purity System A: t_R = 8.33 min, 100%; System B: t_R = 8.17 min, 100%.

4.10.1.4. Synthetic protocol D. Hydrolysis of diester. Diesters were hydrolyzed according to General protocol C using eight equivalents of LiOH instead of four.

4.10.2. 4-({(*S*)-2-[(3*S*,4*S*)-4-[(*S*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-methyl-butyrylamino)-4-methylsulfanyl-butyrylamino]-5-(3,5-difluoro-phenoxy)-3-hydroxy-pentanoylamino]-3-methyl-butyrylamino}-methyl)-benzoic acid methyl ester (20)

Compound **20** (colorless solid) was synthesized in three steps from central core **5** according to synthetic protocols A and B using amine **A** and acid **L**. $[\alpha]_D^{22}$ −30 (*c* 0.05, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–1.02 (m, 12H), 1.40 (s, 9H), 1.88–2.10 (m, 3H), 2.02 (s, overlapped, 3H), 2.11–2.27 (m, 1H), 2.39–2.56 (m, 4H), 3.79–3.88 (m, 1H), 3.82 (s, overlapped, 3H), 3.89–3.96 (m, 2H), 3.97–4.11 (m, 2H), 4.16 (d, *J* = 6.0 Hz, 1H), 4.22–4.38 (m, 3H), 6.30–6.48 (m, 3H), 7.35 (d, *J* = 8.6 Hz, 2H), 7.88 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 15.3, 18.1, 18.3, 19.4, 19.6, 28.5, 30.4, 30.6, 30.9, 38.8, 40.6, 43.2, 52.3, 54.5, 60.3, 61.4, 67.7, 68.0, 68.7, 81.1, 96.8 (t, J_{CF} = 25.9 Hz), 98.7 (d, J_{CF} = 28.1 Hz, 2C), 127.8, 129.2, 130.1, 144.7, 151.9, 160.9, 164.1 (d, J_{CF} = 244.8 Hz), 164.3 (d, J_{CF} = 244.8 Hz), 167.8, 172.3, 173.0, 173.5, 174.0. HRMS calcd (M+H)$^+$: 838.3872; found 838.3906. LC–MS Purity System A: t_R = 10.25 min, 98%; System B: t_R = 3.41 min, 99%.

4.10.3. 4-({(*S*)-2-[(3*S*,4*S*)-4-[(*S*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-methyl-butyrylamino)-4-methylsulfanyl-butyrylamino]-5-(3,5-difluoro-phenoxy)-3-hydroxy-pentanoylamino]-3-methyl-butyrylamino}-methyl)-benzoic acid (21)

Compound **21** (colorless solid) was synthesized in four steps from central core **5** according to synthetic protocols A, B, and C using amine **A** and acid **L**. $[\alpha]_D^{22}$ −20 (*c* 0.05, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.90–1.06 (m, 12H), 1.43 (s, 9H), 1.91–2.12 (m, 3H), 2.06 (s, overlapped, 3H), 2.14–2.24 (m, 1H), 2.45–2.57 (m, 3H), 2.58–2.66 (m, 1H), 3.85 (d, *J* = 6.6 Hz, 1H), 3.92–4.10 (m, 2H), 4.12–4.26 (m, 2H), 4.28–4.38 (m, 1H), 4.44–4.55 (m, 3H), 6.45–6.57 (m, 3H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.92 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 15.3, 18.4, 18.7, 18.8, 19.7, 19.8, 28.7, 31.1, 31.5, 31.5, 32.1, 41.2, 43.7, 53.3, 54.6, 60.9, 62.1, 68.2, 68.8, 80.9, 97.2 (t, J_{CF} = 27.3 Hz), 99.5 (d, J_{CF} = 28.1 Hz, 2C), 128.4, 130.9, 145.0, 154.5, 158.4, 165.0 (d, J_{CF} = 245.5 Hz), 165.2 (d, J_{CF} = 245.5 Hz), 170.4, 170.9, 173.6, 174.0, 175.1. HRMS calcd (M+H)$^+$: 824.3716; found 824.3741. LC–MS Purity System A: t_R = 9.22 min, 100%; System B: t_R = 7.01 min, 97%.

4.10.4. 4-[((*S*)-2-{(3*S*,4*S*)-3-Hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoyl- amino]-5-phenoxy-pentanoylamino}-3-methyl-butyrylamino)-methyl]-benzoic acid (22)

Compound **22** (colorless solid) was synthesized in four steps from central core **8** according to synthetic protocols A, B, and C using amine **A** and acid **M**. $[\alpha]_D^{22}$ −7.1 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.94 (d, *J* = 6.9 Hz, 3H), 0.96 (d, *J* = 6.9 Hz, 3H), 1.57 (d, *J* = 7.1 Hz, 3H), 2.10–2.23 (m, 1H), 2.54 (dd, *J* = 7.3, 14.6 Hz, 1H), 2.62 (dd, *J* = 6.7, 14.6 Hz, 1H), 2.96 (s, 3H), 3.35 (s, 3H), 4.15–4.27 (m, 3H), 4.42–4.53 (m, 4H), 5.25 (q, *J* = 7.1 Hz, 1H), 6.86–6.98 (m, 3H), 7.17–7.43 (m, 8H), 7.92 (d, *J* = 8.4 Hz, 2H), 8.01–8.06 (m, 3H), 8.26 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.4, 19.7, 22.1, 31.4, 36.0, 38.4, 41.3, 43.8, 51.0, 54.4, 60.7, 68.2, 68.8, 115.8, 122.1, 126.1, 127.3, 128.2, 128.5, 129.2, 129.6, 130.5, 130.9, 137.1, 137.4, 143.7, 145.0, 145.4, 160.1, 167.7, 169.0, 173.9, 174.0. HRMS calcd (M+H)$^+$: 816.3278; found 816.3309. LC–MS Purity System A: t_R = 7.67 min, 100%; System B: t_R = 7.87 min, 99%.

4.10.5. 4-[((*S*)-2-{(3*S*,4*S*)-5-(3,5-Difluoro-benzyloxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-methyl]-benzoic acid (23)

Compound **23** (colorless solid) was synthesized in four steps from central core **11** according to synthetic protocols A, B, and C using amine **A** and acid **M**. $[\alpha]_D^{22}$ −17 (*c* 0.15, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.94 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 1.58 (d, *J* = 7.2 Hz, 3H), 2.10–2.24 (m, 1H), 2.51 (dd, *J* = 6.9, 14.6 Hz, 1H), 2.60 (dd, *J* = 7.1, 14.6 Hz, 1H), 2.95 (s, 3H), 3.48 (s, 3H), 3.64–3.78 (m, 2H), 4.24 (d, *J* = 6.0 Hz, 1H), 4.33–4.43 (m, 2H), 4.95 (s, 4H), 5.19–5.31 (m, 1H), 6.72–6.82 (m, 1H), 6.84–6.94 (m, 2H), 7.19–7.26 (m, 1H), 7.28–7.36 (m, 2H), 7.37–7.44 (m, 4H), 7.92 (d, *J* = 8.4 Hz, 2H), 8.01–8.05 (m, 2H), 8.26 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.4, 19.7, 22.1, 31.4, 36.0, 38.4, 41.3, 43.7, 51.0, 54.2, 60.7, 68.8, 71.0, 72.5, 103.4 (t, J_{CF} = 25.8 Hz), 111.0 (d, J_{CF} = 25.5 Hz, 2C), 126.1, 127.3, 128.2, 128.5, 129.2, 129.5, 129.6, 130.7, 130.9, 137.2, 137.4, 143.7, 144.4, 145.0, 145.5, 164.4 4 (d, J_{CF} = 246.8 Hz), 164.6 4 (d, J_{CF} = 247.4 Hz), 167.8, 169.0, 169.6, 173.9, 174.0. HRMS calcd (M+H)$^+$: 866.3246; found 866.3276. LC–MS Purity System A: t_R = 8.19 min, 99%; System B: t_R = 8.12 min, 100%.

4.10.6. 4-[((*S*)-2-{(3*S*,4*S*)-5-Benzyloxy-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-methyl]-benzoic acid (24)

Compound **24** (colorless solid) was synthesized in four steps from central core **14** according to synthetic protocols A, B, and C using amine **A** and acid **M**. $[\alpha]_D^{22}$ −20 (*c* 0.14, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.92 (d, *J* = 6.9 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H) 1.57 (d, *J* = 7.1 Hz, 3H), 2.09–2.23 (m, 1H), 2.48 (dd, *J* = 7.1, 14.6 Hz, 1H), 2.57 (dd, *J* = 6.7, 14.6 Hz, 1H), 2.94 (s, 3H), 3.34 (s, 3H), 3.65–3.72 (m, 2H), 4.23 (d, *J* = 6.2 Hz, 1H), 4.32–4.40 (m, 2H), 4.46 (s, 2H), 4.49 (d, *J* = 11.8 Hz, 2H), 5.24 (q, *J* = 7.1 Hz, 1H), 7.17–7.36 (m, 7H), 7.36–7.42 (m, 5H), 7.92 (d, *J* = 8.5 Hz, 2H), 8.00–8.02 (m, 2H), 8.25 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.4, 19.7, 22.1, 31.4, 36.0, 38.4, 41.4, 43.8, 51.0, 54.3, 60.7, 69.0, 70.7, 74.0, 126.1, 127.3, 128.2, 128.4, 128.5, 128.7, 128.9, 129.1, 129.1, 129.2, 129.3, 129.4, 129.6, 130.6, 130.9, 137.2, 137.3, 139.4, 143.7, 145.0, 145.4, 167.8, 168.9, 169.6, 173.9, 174.0. HRMS calcd (M+H)$^+$: 830.3435; found 830.3445. LC–MS Purity System A: t_R = 7.66 min, 100%; System B: t_R = 7.88 min, 100%.

4.10.7. 4-[((*S*)-2-{(3R,4*S*)-5-Benzyloxy-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-methyl]-benzoic acid (25)

Compound **25** (colorless solid) was synthesized in three steps from compound (*R*)-**19** (below) according to synthetic protocols B and C using acid **M**. $[\alpha]_D^{22}$ −11 (*c* 0.14, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H), 1.56 (d, *J* = 7.2 Hz, 3H), 2.06–2.19 (m, 1H), 2.55–2.58 (m, 2H), 2.93 (s, 3H) 3.34 (s, 3H), 3.74–3.79 (m, 2H), 4.16–4.25 (m, 1H), 4.21 (d, overlapped, *J* = 6.6 Hz, 1H), 4.25–4.33 (m, 1H), 4.41–4.47 (m, 2H), 4.52 (s, 2H), 5.23 (q, *J* = 7.2 Hz, 1H), 7.19–7.34 (m, 8H), 7.36–7.41 (m, 4H), 7.91 (s, 1H), 7.94 (s, 1H) 7.98–8.02 (m, 2H) 8.21 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.4, 19.7, 22.1, 31.4, 36.0, 38.4, 41.0, 43.7, 51.0, 55.6, 60.6, 69.8, 70.0, 74.2, 126.1, 127.3, 128.2, 128.5, 128.7, 129.0, 129.2, 129.3, 129.4, 129.6, 130.7, 131.0, 137.2, 137.4, 139.5, 143.7, 145.0, 145.3, 167.8, 168.5, 169.6, 173.9, 174.6. HRMS calcd (M+H)$^+$: 830.3435; found

830.3450. LC–MS Purity System A: t_R = 7.87 min, 100%; System B: t_R = 7.94 min, 100%.

4.10.7.1. (3*S*,4*S*)-4-Azido-5-benzyloxy-3-hydroxy-pentanoic acid methyl ester (18). To a cooled solution (0 °C) of acid **14** (550 mg, 2.07 mmol) in MeOH (10 mL) was added thionyl chloride (226 μL, 3.11 mmol) and the mixture was stirred for 16 h and concentrated. Purification by FC (toluene/ethyl acetate 4:1) gave methyl ester **18** (438 mg, 76%) as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ 2.53 (dd, J = 7.6, 15.6 Hz, 1H), 2.58 (dd, J = 5.6, 15.6 Hz, 1H), 3.57 (ddd, J = 3.8, 4.9, 6.9 Hz, 1H), 3.66 (s, 3H), 3.69 (dd, J = 6.9, 10.1 Hz, 1H), 3.74 (dd, J = 4.9, 10.1 Hz, 1H), 4.14 (ddd, J = 3.8, 5.6, 7.6 Hz, 1H), 4.52 (d, J = 11.9 Hz, 1H), 4.55 (d, J = 11.9 Hz, 1H), 7.23–7.37 (m, 5H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 39.8, 52.2, 66.0, 68.9, 71.2, 74.2, 128.7, 128.8, 129.4, 139.2, 173.3.

4.10.7.2. 4-{[(*S*)-2-((3*R*,4*S*)-4-Azido-5-benzyloxy-3-hydroxy-pentanoylamino)-3-methyl-butyrylamino]-methyl}-benzoic acid methyl ester ((*R*)-19). To a stirred solution of Dess–Martin periodinane (167 mg, 0.394 mmol) in dry DCM (2.5 mL) was added a solution of compound **18** (100 mg, 0.358 mmol) in dry DCM (2 mL). After stirring for 40 min the solution was diluted with ether (5 mL) and poured into a solution of saturated $NaHCO_3$ (15 mL) containing a sevenfold excess of $Na_2S_2O_3$ (435 mg, 2.75 mmol). Stirring was continued until the solid was dissolved and the layers were separated. The water phase was extracted once more with ether and the combined ether layers were washed twice with water, dried and concentrated. The crude ketone was dissolved in MeOH (6 mL). The solution was cooled to −15 °C before adding $NaBH_4$ (25 mg, 0.658 mmol). After 10 min the reaction was quenched with brine (10 mL) and the MeOH was evaporated. The remaining aqueous phase was extracted three times with EtOAc and the combined organic phases were dried and concentrated. The diastereomeric mixture of alcohols was dissolved in dioxane/water 1:1 (4 mL) and cooled to 0 °C before adding LiOH (580 μL, 0.580 mmol). After stirring for 1 h the solution was neutralized with 1 M HCl and concentrated and co-concentrated with toluene. The crude carboxylic acid and amine **A** (141 mg, 0.387 mmol) was dissolved in DMF (3 mL) and the solution was cooled to 0 °C. DIPEA (168 μL, 0.967 mmol) and HATU (140 mg, 0.368 mmol) were added and the mixture was stirred for 0.5 h and for an additional 2 h at room temperature. Concentration and purification by HPLC (MeOH/H_2O 80:20 + 0.2% TFA) gave 77 mg (white solid) of the first eluted compound (the *S*-isomer) and 64 mg (white solid) of the second eluted compound (*R*)-19, altogether in 74% total yield over four steps. ^{1}H NMR (*R*-isomer) (300 MHz, $CDCl_3$) δ 0.92 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 2.03–2.17 (m, 1H), 2.39 (dd, J = 9.1, 14.8 Hz, 1H), 2.51 (dd, J = 2.8, 14.8 Hz, 1H), 3.50–3.57 (m, 1H), 3.58–3.65 (m, 1H), 3.75 (dd, J = 3.7, 9.8 Hz, 1H), 3.88 (s, 3H), 3.93–4.02 (m, 1H), 4.26–4.36 (m, 2H), 4.44 (dd, J = 6.0, 15.4 Hz, 1H), 4.53 (s, 2H), 5.20 (br s, 1H), 7.01 (b, 1H), 7.20–7.38 (m, 8H), 7.93 (d, J = 8.2 Hz, 2H); ^{13}C NMR (*R*-isomer) (75.5 MHz, $CDCl_3$) δ 18.2, 19.2, 30.1, 39.1, 43.2, 52.1, 59.0, 64.6, 68.8, 69.8, 73.5, 127.4, 127.6, 127.9, 128.5, 129.3, 129.9, 137.4, 142.8, 166.7, 171.7, 172.4.

4.10.8. 5-((*S*)-2-{(3*S*,4*S*)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-isophthalic acid dimethyl ester (26)

Compound **26** (colorless solid) was synthesized in three steps from central core **5** according to synthetic protocols A and B using amine **B** and acid **M**. $[\alpha]_D^{22}$ −23 (*c* 0.11, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (d, J = 6.6 Hz, 3H), 1.03 (d, J = 6.6 Hz, 3H), 1.58 (d, J = 6.9 Hz, 3H), 2.14–2.28 (m, 1H), 2.57 (dd, J = 7.1, 14.6 Hz, 1H), 2.66 (dd, J = 6.9, 14.6 Hz, 1H), 2.98 (s, 3H), 3.37 (s, 3H), 3.88 (s, 6H), 4.15–4.27 (m, 2H), 4.31 (d, J = 6.6 Hz 1H), 4.40–4.48 (m, 1H), 4.50–4.58 (m, 1H), 5.25 (q, J = 7.0 Hz, 1H), 6.36–6.45 (m, 1H), 6.46–6.56 (m, 2H), 7.18–7.26 (m, 1H), 7.27–7.34 (m, 2H), 7.37–7.43 (m, 2H), 8.04 (s, 1H), 8.10 (s, 1H), 8.28–8.33 (m, 2H), 8.53 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.7, 19.7, 22.1, 31.8, 36.1, 38.4, 41.3, 51.0, 52.9, 54.1, 61.6, 68.7, 68.9, 97.2 (t, J_{CF} = 26.3 Hz), 99.5 (d, J_{CF} = 28.9 Hz, 2C), 126.2, 126.2, 126.7, 127.3, 128.2, 129.5, 129.5, 129.5, 132.3, 132.4, 137.2, 137.4, 140.4, 143.8, 145.0, 162.2, 164.9 (d, J_{CF} = 245.1 Hz), 165.2 (d, J_{CF} = 244.7 Hz), 167.3, 167.8, 169.2, 172.8, 173.9. HRMS calcd $(M+H)^+$: 910.3145; found 910.3165. LC–MS Purity System A: t_R = 9.84 min, 100%; System B: t_R = 9.39 min, 100%.

4.10.9. 5-((*S*)-2-{(3*S*,4*S*)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-isophthalic acid (27)

Compound **27** (colorless solid) was synthesized in four steps from central core **5** according to synthetic protocols A, B, and D using amine **B** and acid **M**. $[\alpha]_D^{22}$ −25 (*c* 0.12, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.01 (d, J = 6.6 Hz, 3H), 1.02 (d, J = 6.6 Hz, 3H), 1.57 (d, J = 7.2 Hz, 3H), 2.12–2.27 (m, 1H), 2.56 (dd, J = 7.1, 14.6 Hz, 1H), 2.64 (dd, J = 6.5, 14.6 Hz, 1H), 2.97 (s, 3H), 3.36 (s, 3H), 4.14–4.27 (m, 2H), 4.28–4.36 (m, 1H), 4.40–4.50 (m, 1H), 4.51–4.60 (m, 1H), 5.25 (q, J = 6.9 Hz, 1H), 6.38–6.48 (m, 1H), 6.49–6.59 (m, 2H), 7.17–7.26 (m, 1H), 7.27–7.36 (m, 2H), 7.37–7.44 (m, 2H), 8.03 (s, 1H), 8.09 (s, 1H), 8.29 (s, 1H), 8.35 (s, 1H), 8.26 (s, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.7, 19.7, 22.1, 31.9, 36.0, 38.4, 41.2, 51.0, 54.3, 61.5, 68.6, 68.8, 97.2 (t, J_{CF} = 26.2 Hz), 99.6 (d, J_{CF} = 28.9 Hz, 2C), 126.1, 126.5, 127.3, 127.4, 128.2, 129.4, 129.6, 133.0, 137.2, 137.4, 140.2, 143.8, 145.0, 162.2, 165.0 (d, J_{CF} = 245.1 Hz), 165.2 (d, J_{CF} = 245.4 Hz), 167.8, 168.5, 169.1, 172.8, 173.9. HRMS calcd $(M+H)^+$: 882.2832; found 882.2856. LC–MS Purity System A: t_R = 8.14 min, 100%; System B: t_R = 8.05 min, 100%.

4.10.10. 5-((*S*)-2-{(3*S*,4*S*)-5-(3,5-Difluoro-benzyloxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-isophthalic acid (28)

Compound **28** (colorless solid) was synthesized in four steps from central core **11** according to synthetic protocols A, B, and D using amine **B** and acid **M**. $[\alpha]_D^{22}$ −39 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.02 (d, J = 6.8 Hz, 3H), 1.03 (d, J = 6.8 Hz, 3H), 1.58 (d, J = 6.9 Hz, 3H), 2.13–2.26 (m, 1H), 2.52 (dd, J = 7.1, 14.6 Hz, 1H), 2.62 (dd, J = 6.8, 14.6 Hz, 1H), 2.96 (s, 3H), 3.36 (s, 3H), 3.69 (dd, J = 7.2, 10.0 Hz, 1H), 3.78 (dd, J = 6.5, 10.0 Hz, 1H), 4.28–4.40 (m, 2H), 4.43–4.47 (m, 1H), 4.47 (s, overlapped, 2H), 5.25 (q, J = 6.9 Hz, 1H), 6.69–6.78 (m, 1H), 6.81–6.86 (m, 2H), 7.20–7.25 (m, 1H), 7.28–7.35 (m, 2H), 7.38–7.43 (m, 2H), 8.02 (s, 1H), 8.09 (s, 1H), 8.28 (s, 1H), 8.34 (s, 1H), 8.52 (s, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.7, 19.7, 22.1, 31.9, 36.0, 38.4, 41.5, 51.0, 54.3, 61.5, 68.6, 70.8, 72.4, 101.3, 103.4 (t, J = 25.6 Hz), 110.9 (d, J = 25.2 Hz, 2C), 126.1, 126.5, 127.3, 128.2, 129.3, 129.6, 137.3, 137.4, 140.2, 143.8, 144.4, 145.0, 164.3 (d, J_{CF} = 247.1 Hz), 164.5 (d, J_{CF} = 247.1 Hz), 168.3, 169.1, 169.4, 172.8, 174.0. HRMS calcd $(M+H)^+$: 896.2988; found 896.3002. LC–MS Purity System A: t_R = 7.98 min, 99%; System B: t_R = 8.00 min, 98%.

4.10.11. 5-((*S*)-2-{(3*S*,4*S*)-5-Benzyloxy-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-isophthalic acid (29)

Compound **29** (colorless solid) was synthesized in four steps from central core **14** according to synthetic protocols A, B, and D using amine **B** and acid **M**. $[\alpha]_D^{22}$ −38 (*c* 0.1, MeOH); ^{1}H NMR

(300 MHz, CD_3OD) δ 1.02 (d, J = 6.8 Hz, 3H), 1.04 (d, J = 6.8 Hz, 3H), 1.58 (d, J = 7.1 Hz, 3H), 2.14–2.24 (m, 1H), 2.52 (dd, J = 7.3, 14.4 Hz, 1H), 2.62 (dd, J = 6.5, 14.4 Hz, 1H), 2.97 (s, 3H), 3.36 (s, 3H), 3.68 (dd, J = 7.0, 10.1 Hz, 1H), 3.75 (dd, J = 6.2, 10.1 Hz, 1H), 4.32 (d, J = 6.7 Hz, 1H), 4.34–4.46 (m, 2H), 4.48 (s, 2H), 5.26 (q, J = 7.1 Hz, 1H), 7.18–7.25 (m, 6H), 7.29–7.35 (m, 3H), 7.38–7.43 (m, 2H), 8.02–8.04 (m, 1H), 8.07–8.10 (m, 1H), 8.28 (s, 1H), 8.36 (s, 1H), 8.54 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.7, 19.7, 22.1, 31.9, 36.0, 38.4, 41.5, 51.0, 54.4, 61.5, 69.0, 70.4, 73.8, 126.1, 126.3, 126.6, 127.3, 127.4, 128.2, 128.6, 128.9, 129.2, 129.3, 129.6, 133.1, 137.3, 139.4, 140.2, 143.8, 145.0, 167.8, 168.5, 169.1, 172.8, 174.0. HRMS calcd $(M+H)^+$: 860.3177; found 860.3201. LC–MS Purity System A: t_R = 7.62 min, 100%; System B: t_R = 7.80 min, 100%.

4.10.12. 5-{(*S*)-2-[(3*S*,4*S*)-5-(3,5-Difluoro-benzyloxy)-4-(3-dipropylcarbamoyl-benzoylamino)-3-hydroxy-pentanoylamino]-3-methyl-butyrylamino}-isophthalic acid (30)

Compound **30** (colorless solid) was synthesized in four steps from central core **11** according to synthetic protocols A, B, and D using amine **B** and acid **N**. $[\alpha]_D^{22}$ −13 (c 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.71 (t, J = 7.2 Hz, 3H), 0.98 (t, J = 7.2 Hz, 3H), 1.02 (d, J = 6.8 Hz, 3H), 1.03 (d, J = 6.8 Hz, 3H), 1.47–1.62 (m, 2H), 1.64–1.77 (m, 2H), 2.16–2.28 (m, 1H), 2.52 (dd, J = 7.1, 14.4 Hz, 1H), 2.63 (dd, J = 6.8, 14.4 Hz, 1H), 3.14–3.24 (m, 2H), 3.43–3.52 (m, 2H), 3.71 (dd, J = 7.1, 10.0 Hz, 1H), 3.79 (dd, J = 6.3, 10.0 Hz, 1H), 4.32 (d, J = 6.6 Hz, 1H), 4.34–4.41 (m, 1H), 4.43–4.48 (m, 1H), 4.49 (s, 2H), 6.70–6.79 (m, 1H), 6.83–6.88 (m, 2H), 7.51–7.60 (m, 2H), 7.89–7.91 (m, 1H), 7.97–8.02 (m, 1H), 8.36 (s, 1H), 8.54 (s, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 11.3, 11.7, 18.6, 19.7, 21.7, 22.9, 31.8, 41.5, 52.3, 54.2, 61.4, 68.9, 70.8, 72.4, 103.3 (t, J = 25.8 Hz), 110.9 (d, J = 25.2 Hz, 2C), 126.6, 126.8, 127.4, 129.6, 130.5, 133.1, 136.1, 138.4, 140.2, 144.4, 164.3 (d, J = 247.4 Hz), 164.5 (d, J = 247.1 Hz), 168.5, 169.7, 172.8, 173.2, 174.1. HRMS calcd $(M+H)^+$: 769.3260; found 769.3286. LC–MS Purity System A: t_R = 8.41 min, 97%; System B: t_R = 7.93 min, 97%.

4.10.13. 5-{(*S*)-2-[(3*S*,4*S*)-5-Benzyloxy-4-(3-dipropylcarbamoyl-benzoylamino)-3-hydroxy-pentanoylamino]-3-methyl-butyrylamino}-isophthalic acid (31)

Compound **31** (colorless solid) was synthesized in four steps from central core **14** according to synthetic protocols A, B, and D using amine **B** and acid **N**. $[\alpha]_D^{22}$ −15 (c 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.71 (t, J = 7.4 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H), 1.02 (d, J = 6.8 Hz, 3H), 1.04 (d, J = 6.8 Hz, 3H), 1.48–1.60 (m, 2H), 1.65–1.77 (m, 2H), 2.14–2.28 (m, 1H), 2.52 (dd, J = 7.1, 14.8 Hz, 1H), 2.62 (dd, J = 6.9, 14.8 Hz, 1H), 3.15–3.23 (m, 2H), 3.43–3.52 (m, 2H), 3.69 (dd, J = 6.6, 10.2 Hz, 1H), 3.76 (dd, J = 6.5, 10.2 Hz, 1H), 4.33 (d, J = 6.5 Hz, 1H), 4.35–4.47 (m, 2H), 4.48 (s, 2H), 7.19–7.27 (m, 5H), 7.48–7.60 (m, 2H), 7.87–7.91 (m, 1H), 7.96–8.01 (m, 1H), 8.36 (s, 1H), 8.54 (s, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 11.3, 11.7, 18.6, 19.7, 21.7, 22.9, 31.8, 41.4, 52.3, 54.2, 61.4, 69.0, 70.4, 73.4, 126.6, 126.7, 127.4, 128.6, 128.8, 129.1, 129.3, 129.6, 129.9, 130.5, 133.1, 136.2, 138.4, 139.4, 140.2, 168.6, 169.7, 172.9, 173.2, 174.1.HRMS calcd $(M+H)^+$: 733.3449; found 733.3441. LC–MS Purity System A: t_R = 7.76 min, 99%; System B: t_R = 7.63 min, 98%.

4.10.14. 5-((*S*)-2-{(3*S*,4*S*)-5-(3,5-Difluoro-benzyloxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-methylcarbamoyl-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-isophthalic acid (32)

Compound **32** (colorless solid) was synthesized in four steps from central core **11** according to synthetic protocols A, B, and D using amine **B** and acid **O**. $[\alpha]_D^{22}$ +7.0 (c 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.03 (d, J = 6.9 Hz, 3H), 1.04 (d, J = 6.9 Hz, 3H), 2.14–2.27 (m, 1H), 2.54 (dd, J = 7.2, 14.4 Hz, 1H), 2.64 (dd, J = 6.9, 14.4 Hz, 1H), 2.95 (s, 3H), 2.97 (s, 3H), 3.37 (s, 3H), 3.71 (dd, J = 7.1, 10.2 Hz, 1H), 3.78 (dd, J = 6.5, 10.2 Hz, 1H), 4.32 (d, J = 6.9 Hz, 1H), 4.35–4.41 (m, 1H), 4.42–4.46 (m, 1H), 4.47 (s, 2H), 6.71–6.79 (m, 1H), 6.81–6.87 (m, 2H), 8.02 (d, J = 3.6 Hz, 1H), 8.08 (d, J = 3.6 Hz, 1H), 8.27 (s, 1H), 8.35 (s, 1H), 8.54 (s, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.7, 19.7, 27.0, 31.9, 36.0, 38.4, 41.5, 54.2, 61.5, 68.6, 70.8, 72.4, 103.4 (t, J = 26.0 Hz), 110.9 (d, J = 24.9 Hz, 2C), 126.0, 126.6, 127.4, 129.1, 129.3, 133.1, 137.1, 137.4, 140.2, 143.8, 164.3 (d, J = 247.1 Hz), 164.5 (d, J = 247.1 Hz), 168.5, 168.9, 169.2, 172.8, 174.0. HRMS calcd $(M+H)^+$: 806.2519; found 806.2529. LC–MS Purity System A: t_R = 4.83 min, 100%; System B: t_R = 6.74 min, 99%.

4.10.15. *N*-[(1*S*,2*S*)-3-Cyclopropylcarbamoyl-1-(3,5-difluoro-phenoxymethyl)-2-hydroxy-propyl]-5-(methanesulfonyl-methyl-amino)-*N*′-((*R*)-1-phenyl-ethyl)-isophthalamide (33)

Compound **33** (colorless solid) was synthesized in three steps from central core **5** according to synthetic protocols A and B using amine **C** and acid **M**. $[\alpha]_D^{22}$ −43 (c 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.44–0.52 (m, 2H), 0.64–0.74 (m, 2H), 1.59 (d, J = 7.1 Hz, 3H), 2.37–2.45 (m, 2H), 2.59–2.68 (m, 1H), 2.98 (s, 3H), 3.38 (s, 3H), 4.16 (dd, J = 6.9, 9.6 Hz, 1H), 4.25 (dd, J = 6.6, 9.6 Hz, 1H), 4.39–4.46 (m, 1H), 4.47–4.55 (m, 1H), 5.25 (q, J = 7.1 Hz, 1H), 6.47–6.57 (m, 1H), 6.58–6.67 (m, 2H), 7.20–7.28 (m, 1H), 7.29–7.38 (m, 2H), 7.39–7.45 (m, 2H), 8.04 (s, 1H), 8.06 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 5.2, 5.3, 20.9, 22.1, 34.7, 37.2, 40.2, 49.8, 53.2, 67.0, 67.4, 96.0 (t, J_{CF} = 26.5 Hz), 98.4 (d, J_{CF} = 29.2 Hz, 2C), 124.9, 126.1, 127.0, 128.2, 128.2, 128.4, 135.8, 136.2, 142.6, 143.9, 161.1, 163.9 (d, J_{CF} = 245.1 Hz), 164.1 (d, J_{CF} = 245.1 Hz), 166.6, 167.8, 173.4. HRMS calcd $(M+H)^+$: 659.2351; found 659.2367. LC–MS Purity System A: t_R = 7.88 min, 100%; System B: t_R = 8.20 min, 100%.

4.10.16. 4-({(3*S*,4*S*)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethyl-carbamoyl)-benzoylamino]-pentanoylamino}-methyl)-benzoic acid (34)

Compound **34** (colorless solid) was synthesized in four steps from central core **5** according to synthetic protocols A, B, and C using amine **D** and acid **M**. $[\alpha]_D^{22}$ −51 (c 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.57 (d, J = 7.1 Hz, 3H), 2.52–2.58 (m, 2H), 2.96 (s, 3H), 3.35 (s, 3H), 4.19 (dd, J = 6.7, 9.8 Hz, 1H), 4.27 (dd, J = 6.5, 9.8 Hz, 1H), 4.43 (d, J = 6.6 Hz, 2H), 4.45–4.52 (m, 1H), 4.56 (dt, J = 2.6, 6.6 Hz, 1H), 5.34 (q, J = 7.1 Hz, 1H), 6.45–6.53 (m, 1H), 6.57–6.64 (m, 2H), 7.14–7.26 (m, 1H), 7.27–7.35 (m, 2H), 7.36–7.42 (m, 4H), 7.94 (d, J = 8.4 Hz, 2H), 8.03 (s, 1H), 8.06 (s, 1H), 8.26 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 22.1, 36.0, 38.3, 41.6, 43.8, 51.0, 54.6, 64.3, 68.3, 68.7, 97.2 (t, J = 26.3 Hz), 99.6 (d, J = 29.2 Hz, 2C), 126.0, 127.2, 127.3, 128.2, 128.4, 129.4, 129.6, 130.7, 131.0, 137.0, 137.4, 143.8, 145.0, 145.3, 162.3,165.1 (d, J = 245.1 Hz), 165.3 (d, J = 245.1 Hz), 167.7, 169.0, 169.6, 173.6. HRMS calcd $(M+H)^+$: 753.2406; found 753.2399. LC–MS Purity System A: t_R = 7.96 min, 100%; System B: t_R = 8.16 min, 100%.

4.10.17. 4-[(2-{(3*S*,4*S*)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethyl-carbamoyl)-benzoylamino]-pentanoylamino}-acetylamino)-methyl]-benzoic acid (35)

Compound **35** (colorless solid) was synthesized in four steps from central core **5** according to synthetic protocols A, B, and C using amine **E** and acid **M**. $[\alpha]_D^{22}$ −49 (c 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.56 (d, J = 7.1 Hz, 3H), 2.54 (d, J = 7.2 Hz, 2H), 2.95 (s, 3H), 3.35 (s, 3H), 3.79 (d, J = 16.8 Hz, 1H), 4.01 (d, J = 16.8 Hz, 2H), 4.11–4.25 (m, 2H), 4.39–4.54 (m, 4H), 5.23 (q, J = 7.1 Hz, 1H), 6.44–6.52 (m, 1H), 6.53–6.58 (m, 2H), 7.17–7.24

(m, 1H), 7.26–7.41 (m, 6H), 7.90 (d, J = 8.1 Hz, 2H), 8.02 (s, 1H), 8.03 (s, 1H) 8.25 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 22.1, 36.0, 38.4, 41.4, 43.7, 43.8, 51.0, 54.0, 68.5, 68.9, 97.2 (t, J = 26.3 Hz), 99.6 (d, J = 28.9 Hz, 2C), 126.1, 127.2, 128.2, 128.3, 129.3, 129.6, 130.7, 131.0, 137.0, 137.4, 143.7, 145.0, 145.3, 162.2, 165.0 (d, J = 244.8 Hz), 165.2 (d, J = 245.1 Hz), 167.7, 169.0, 169.6, 172.0, 174.0. HRMS calcd $(M+H)^+$: 810.2620; found 810.2622. LC–MS Purity System A: t_R = 7.51 min, 100%; System B: t_R = 7.73 min, 100%.

4.10.18. *N*-[(1*S*,2*S*)-3-((*S*)-1-Benzylcarbamoyl-2-methyl-propylcarbamoyl)-1-(3,5-difluoro-phenoxymethyl)-2-hydroxy-propyl]-5-(methanesulfonyl-methyl-amino)-*N*′-((*R*)-1-phenyl-ethyl)-isophthalamide (36)

Compound **36** (colorless solid) was synthesized in three steps from central core **5** according to synthetic protocols A and B using amine **F** and acid **M**. $[\alpha]_D^{22}$ −41 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.92 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H), 1.57 (d, J = 6.9 Hz, 3H), 2.07–2.20 (m, 1H), 2.47–2.60 (m, 2H), 2.95 (s, 3H), 3.34 (s, 3H), 4.12–4.28 (m, 3H), 4.35–4.46 (m, 3H), 4.47–4.55 (m, 1H), 5.24 (q, J = 6.9 Hz, 1H), 6.44–6.55 (m, 1H), 6.55–6.64 (m, 2H), 7.12–7.35 (m, 8H), 7.40 (d, J = 7.1 Hz, 2H), 8.01–8.07 (m, 2H), 8.29 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.4, 19.7, 22.1, 31.5, 36.0, 38.4, 41.3, 44.1, 51.0, 54.2, 60.6, 68.5, 68.8, 97.2 (t, J_{CF} = 26.5 Hz), 99.6 (d, J_{CF} = 29.2 Hz, 2C), 126.2, 127.3, 128.1, 128.2, 128.6, 129.3, 129.4, 129.6, 129.6, 137.0, 137.4, 139.9, 143.7, 145.0, 162.3, 165.0 (d, J_{CF} = 245.1 Hz), 165.2 (d, J_{CF} = 245.3 Hz), 167.7, 168.9, 173.7, 173.7. HRMS calcd $(M+H)^+$: 808.3192; found 808.3212. LC–MS Purity System A: t_R = 9.55 min, 100%; System B: t_R = 9.07 min, 100%.

4.10.19. 4-((*S*)-2-{(3*S*,4*S*)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-5-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-benzoic acid (37)

Compound **37** (colorless solid) was synthesized in four steps from central core **5** according to synthetic protocols A, B, and C using amine **G** and acid **M**. $[\alpha]_D^{22}$ −20 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 0.89 (d, J = 6.6 Hz, 6H), 1.49 (d, J = 6.9 Hz, 3H), 1.95–2.11 (m, 1H), 2.30–2.40 (m, 1H), 2.43–2.54 (m, overlapped, 1H), 3.01 (s, 3H), 3.29 (s, 3H), 4.10–4.19 (m, 1H), 4.20–4.35 (m, 3H), 4.36–4.46 (m, 1H), 5.11–5.23 (m, 1H), 6.66–6.80 (m, 3H), 7.20 (t, J = 7.3 Hz, 1H), 7.25–7.34 (m, 2H), 7.38 (d, J = 7.7 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.85 (d, J = 8.5 Hz, 2H), 7.98–8.08 (m, 3H), 8.34 (s, 1H), 8.52 (b, 1H), 9.04 (b, 1H), 10.3 (br s, 1H); ^{13}C NMR (75.5 MHz, $CD_3)_2SO$) δ 18.2, 19.2, 21.1, 22.0, 30.5, 35.7, 37.7, 48.7, 53.1, 58.8, 66.8, 67.6, 96.2 (t, J_{CF} = 26.3 Hz), 98.8 (d, J_{CF} = 28.3 Hz, 2C), 118.6, 125.2, 126.1, 126.4, 126.7, 127.9, 128.2, 130.2, 135.3, 135.6, 141.7, 142.4, 144.6, 160.6, 162.9 (d, J_{CF} = 243.9 Hz), 163.2 (d, J_{CF} = 244.2 Hz), 164.5, 165.7, 167.2, 170.8, 172.0. HRMS calcd $(M+H)^+$: 838.2933; found 838.2948. LC–MS Purity System A: t_R = 8.52 min, 100%; System B: t_R = 8.39 min, 100%.

4.10.20. 4-[((*S*)-2-{(3*S*,4*S*)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-((*R*)-1-phenyl-ethylcarbamoyl)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-methyl]-benzoic acid (38)

Compound **38** (colorless solid) was synthesized in four steps from central core **5** according to synthetic protocols A, B, and C using amine **A** and acid **P**. $[\alpha]_D^{22}$ −17 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.95 (d, J = 6.9 Hz, 3H), 0.96 (d, J = 6.9 Hz, 3H), 1.57 (d, J = 7.1 Hz, 3H), 2.11–2.25 (m, 1H), 2.54 (dd, J = 7.3, 14.9 Hz, 1H), 2.61 (dd, J = 7.1, 14.9 Hz, 1H), 4.10–4.21 (m, 2H), 4.24 (d, J = 6.3 Hz, 1H), 4.40–4.52 (m, 4H), 5.24 (q, J = 7.1 Hz, 1H), 6.44–6.53 (m, 1H), 6.54–6.61 (m, 2H), 7.18–7.26 (m, 1H), 7.28–7.36 (m, 2H), 7.36–7.44 (m, 4H), 7.54 (t, J = 7.6 Hz, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.98 (d, J = 7.7 Hz, 2H), 8.32 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.4, 19.7, 22.2, 31.4, 41.2, 43.7, 50.8, 53.9, 60.8, 68.6, 68.9, 97.2 (t, J_{CF} = 26.5 Hz), 99.6 (d, J_{CF} = 28.9 Hz, 2C), 127.2, 127.5, 128.1, 128.5, 129.5, 129.8, 130.7, 130.9, 131.5, 131.6, 135.9, 136.3, 145.2, 145.4, 162.3, 165.0 (d, J_{CF} = 245.1 Hz), 165.3 (d, J_{CF} = 244.2 Hz), 168.8, 169.6, 169.9, 173.8, 174.0. HRMS calcd $(M+H)^+$: 745.3049; found 745.3045. LC–MS Purity System A: t_R = 8.47 min, 100%; System B: t_R = 8.15 min, 100%.

4.10.21. 4-[((*S*)-2-{(3*S*,4*S*)-5-(3,5-Difluoro-phenoxy)-3-hydroxy-4-[3-(methanesulfonyl-methyl-amino)-benzoylamino]-pentanoylamino}-3-methyl-butyrylamino)-methyl]-benzoic acid (39)

Compound **39** (colorless solid) was synthesized in four steps from central core **5** according to synthetic protocols A, B, and C using amine **A** and acid **Q**. $[\alpha]_D^{22}$ −31 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.94 (d, J = 6.9 Hz, 3H), 0.96 (d, J = 6.9 Hz, 3H), 2.12–2.25 (m, 1H), 2.53 (dd, J = 7.3, 14.8 Hz, 1H), 2.61 (dd, J = 6.9, 14.8 Hz, 1H), 2.92 (s, 3H), 3.32 (s, 3H), 4.05–4.26 (m, 4H), 4.39–4.51 (m, 3H), 6.44–6.52 (m, 1H), 6.53–6.60 (m, 2H), 7.39 (d, J = 8.3 Hz, 2H), 7.45–7.52 (m, 1H), 7.58–7.64 (m, 1H), 7.68–7.73 (m, 1H), 7.75–7.81 (m, 1H), 7.91 (d, J = 8.3 Hz, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.3, 19.7, 31.4, 35.7, 38.5, 41.2, 43.7, 53.9, 60.7, 68.6, 68.9, 97.2 (t, J_{CF} = 26.2 Hz), 99.6 (d, J_{CF} = 29.2 Hz, 2C), 126.7, 127.5, 128.4, 129.9, 130.4, 130.9, 132.4, 136.7, 143.4, 145.2, 161.1, 163.9 (d, J_{CF} = 244.9 Hz), 164.1 (d, J_{CF} = 244.9 Hz), 168.2, 169.7, 173.8, 174.0. HRMS calcd $(M+H)^+$: 705.2406; found 705.2406. LC–MS Purity System A: t_R = 7.80 min, 99%; System B: t_R = 7.44 min, 99%.

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