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Original article

Synthesis of potent BACE-1 inhibitors incorporating a hydroxyethylene isostere as central core

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ABSTRACT

We herein describe the design and synthesis of a series of BACE-1 inhibitors incorporating a P1-substituted hydroxylethylene transition state isostere. The synthetic route starting from commercially available carbohydrates yielded a pivotal lactone intermediate with excellent stereochemical control which subsequently could be diversified at the P1-position. The final inhibitors were optimized using three different amines to provide the residues in the P2′–P3′ position and three different acids affording the residues in the P2-P3 position. In addition we report on the stereochemical preference of the P1′-methyl substituent in the synthesized inhibitors. All inhibitors were evaluated in an in vitro BACE-1 assay where the most potent inhibitor, **34**-(*R*), exhibited a BACE-1 IC_{50} value of 3.1 nM.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease of the brain and is the most common form of dementia among the aging population in the world [1,2]. Symptoms of the disease are behavioral disturbances and loss of cognitive function. AD is the biggest unmet medicinal need in neurology with over 30 million people suffering from the disease worldwide with death occurring, on average, nine years after diagnosis [2,3]. Current available therapies only treat the symptoms and do not affect the underlying progression of the disease [4]. AD is characterized by the progressive formation of insoluble amyloid plaques and fibrillary tangles in the brain. Plaques, relatively specific for AD, are primarily a result from extracellular accumulation of aggregated amyloid β-peptide (Aβ). Aβ is a peptide fragment formed by proteolytic cleavage of the large transmembrane amyloid precursor protein (APP) [5–8]. The Aβ peptides ranges from 40 to 42 amino acids in length where amyloid-β42 (Aβ42) dominates in the plaque found in the brain of AD patients. From the amyloid cascade hypothesis, supported by genetic and pathological evidence, the formation of Aβ42 plays an early and vital role in all cases of AD [6,9]. BACE-1 (β-site APP cleaving enzyme) was identified in 1999 independently by several groups [10–14] as a novel membrane-bound aspartic protease belonging to the pepsin family. BACE-1 is responsible for the N-terminal cleavage of APP leading to Aβ after C-terminal intramembrane proteolysis of the formed fragment, C99, by γ-secretase [15–17]. BACE-1 knockout homozygote mice show complete absence of Aβ production and the animals appear to develop normally and have no discernable abnormalities [18]. Tissue cultures and animal studies indicated that BACE-1 is expressed in all tissues but at highest levels in the neurons in the brain. Therefore, in vivo inhibition of BACE-1 is likely to reduce the production of Aβ and is considered to be an attractive therapeutic target for the treatment and prevention of AD [19].

Analogues of peptides in which the scissile bond is replaced with a transition state isostere have proven to be effective inhibitors of this protease [20]. A key structural element in most transition state isosteres is a secondary hydroxyl moiety that interacts with the catalytic aspartic acids side chains of BACE-1 via hydrogen bonds.

A number of hydroxyl containing BACE-1 inhibitors have been reported[15,21–25] that contains *e.g.* statine [26], *tert*-hydroxy

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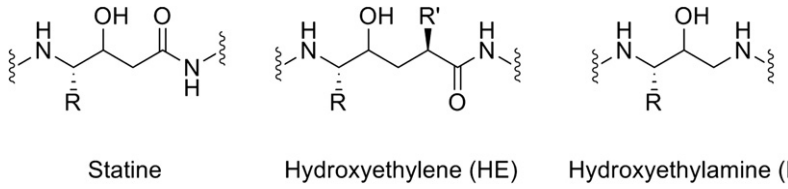


Fig. 1. Examples of transition state isosteres with a hyrdoxylgroup incorporated as stable peptide bond replacement.

statine [27], hydroxyethylene [28] (HE) and hydroxyethylamine [29] (HEA) as non-cleavable transition state isosteres (Fig. 1) and crystal structures have been reported revealing that the hydroxyl group of the inhibitors forms hydrogen bonds with the side chains of Asp32 and Asp228 in the active site [30].

Previously reports have shown that a 10-fold increase in BACE-1 inhibitory activity can be achieved using an HE template over statine motifs [31]. Tang and co-workers have reported on an eight residue peptidic transition state inhibitor, OM99-2, having an HE central core displaying a BACE-1 IC_{50} value of 2 nM [28]. Using X-ray crystal structure information a lower molecular weight inhibitor could be designed (**I** [30], Fig. 2) displaying an IC_{50} value of 2.5 nM. Recently a low nanomolar inhibitor **II** (Fig. 2) containing a C-terminal truncated HEA moiety was disclosed by Stachel et al. [29].

Previous work from our group has demonstrated that insertion of an oxygen atom in the appropriate position of a peptidomimetic or dipeptidomimetic structure not only simplified synthesis of diversified templates but also can provide increased potency against the desired proteases [32]. Encouraged by these results and based on molecular modeling we set out to explore the P1-position within the HE motif to identify easily diversified central cores for future use in the development of BACE inhibitors. In this paper we report on the development of a synthetic route from commercially available carbohydrates to yield potent HE containing BACE-1 inhibitors comprising *O*-benzyl- or *O*-phenyl-moieties in the tether.

2. Result and discussion

2.1. Chemistry

The synthesis of the pivotal intermediate **7** is outlined in Scheme 1. Starting from commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose compound **1** was generated in an overall yield of 73% over four steps [33–36]. The isopropylidene group in **1** was removed under acidic conditions to give a mixture of α- and β-anomers of the methyl glycoside **2** in a ratio of 11:2 [37]. The hydroxyl group in **2** was then deoxygenated as described by Barton[38,39] using thiocarbonyldiimidazole in refluxing THF yielding the thiocarbonyl derivative **3** (99% yield) which was subsequently reduced using tributyl tin hydride in refluxing toluene rendering the methyl glycosides **4** [40] in 72% yield. Oxidation of the glycoside **4** (anomeric mixture) applying the procedure reported by Grieco [41] using *m*-chloroperbenzoic acid in methylene chloride at 0 °C in the presence of boron trifluoride etherate gave the corresponding γ-lactone **5** in quantitative yield. Stereoselective methylation at the *C*2 position was achieved by treating γ-lactone **5** with LDA for 30 min at −78 °C followed by addition of methyl iodide [42] furnishing the desired alkylated lactone **6** in 66% yield. Only one epimer of compound **6** was observed after column chromatographic purification, probably due to steric hindrance from the bulky benzyloxy groups at *C*5 and *C*6. Catalytic hydrogenolysis of compound **6** gave the diol **7** in almost quantitative yield. At this stage the stereochemical assignment was confirmed by NOESY experiments on **7** [43]. The NOESY spectrum of the diol **7** affirmed that the hydrogen $H^{3\alpha}$ was in proximity to the hydrogen atoms in both the methyl group and H^4 (Fig. 3). Furthermore $H^{3\beta}$ was spatially correlated with H^2, which indicated that the methyl group at *C*2 in **7** has the desired (*R*) configuration [43].

Synthesis of final products was performed according to two similar protocols starting from the diol **7** furnishing compounds with either *O*-benzyl- or *O*-phenyl-moieties in the *C*6 position (Scheme 2). For the synthesis of the *O*-benzyl substituted derivatives regioselective O-alkylation of the primary hydroxyl group in compound **7** was achieved by reacting the diol with dibutyltin oxide in toluene to form the corresponding tin acetal, which was allowed to react with 3,5-difluorobenzylbromide or benzylbromide in the presence of tetrabutylammonium bromide to afford the desired 6-*O*-benzylated compounds **8** and **9** in 81% and 79% yield [44]. Compounds **8** and **9** were then converted to their corresponding azides **10** and **11** with inversion of the configuration at *C*5

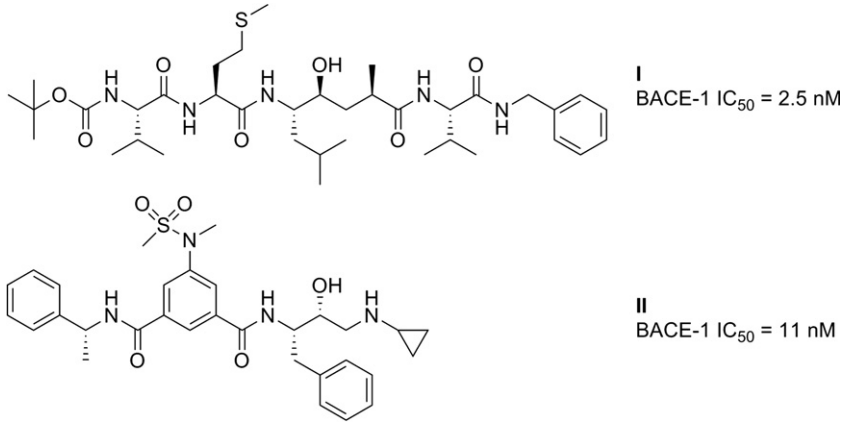
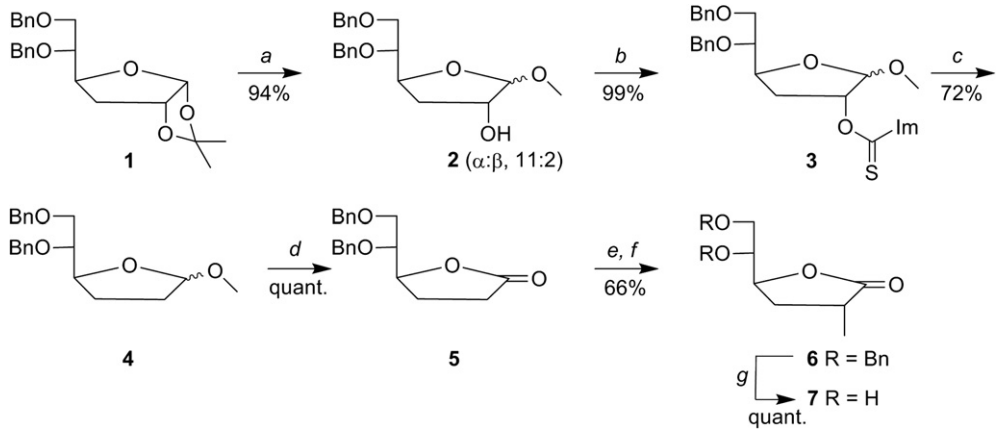


Fig. 2. Inhibitors from the literature containing the HE-(**I**), and the HEA-isostere (**II**).



Scheme 1. Reagents and conditions. (a) H_2SO_4, MeOH, 0 °C; (b) thiocarbonyldiimidazole, THF, reflux; (c) tributyl tin hydride, toluene, reflux; (d) *m*-CPBA, BF_3OEt, DCM; (e) LDA, THF, −78 °C; (f) MeI; (g) Pd/C, H_2, EtOH (95%).

using Mitsunobu conditions with DIAD, PPh_3, and DPPA in THF in 93% and 90% yield [45].

For the synthesis of the *O*-phenyl substituted derivatives the diol **7** was reacted with DIAD and PPh_3 in refluxing chloroform to provide the epoxide **12** in 63% yield [37,38]. Regioselective opening of the epoxide **12** at the least hindered position by 3,5-difluorophenol in warm DMF in the presence of potassium carbonate furnished mixture **13** in 91% yield [45]. This clean reaction was however accompanied by epimerization at the *C*2 position of **13**. Due to difficulties in separation at this stage, using either flash column chromatography or HPLC, the diastereomeric mixture was taken to the next reaction step where separation could be achieved. The mixture **13** was thus converted to the corresponding azides with inversion of configuration at *C*5 under Mitsunobu-like conditions, vide supra, to yield the separable diastereomers **14**-(*R*) and **15**-(*S*) in 32% and 42% yield [45]. To corroborate the assigned stereochemistry ^1H–^1H-NOESY experiments were performed on both **14**-(*R*) and **15**-(*S*). The NOESY spectrum of **14**-(*R*) revealed that H^4 is spatially correlated with the methyl hydrogen at *C*2, which is consistent with the (*R*) configuration of the methyl group in the lactone **14**-(*R*) (Fig. 4). In the NOESY spectrum of **15**-(*S*), H^4 is correlated with H^2, which indicates that the lactone **15**-(*S*) has the presumed (*S*) configuration [43].

The lactones **10**, **11**, **14**-(*R*), and **15**-(*S*) were then further reacted with a selected set of amines (**A**–**C**) and carboxylic acid derivatives (**D**–**F**). Two different protocols were used for the conversion of the lactones to the amides **16**–**21**. For the synthesis of **16** and **18**–**21** the lactones **10**, **11**, **14**-(*R*) and **15**-(*S*) were ring opened with (*S*)-2-amino-*N*-benzyl-3-methyl-butyramide (**A**) or 4-fluorobenzylamine (**C**) in the presence of diisopropylethylamine (DIPEA) and 2-hydroxypyridine in refluxing THF to give amides **16** and **18**–**21** in 36–88% yield [46]. For the synthesis of **17** a protocol described by Weinreb et al. was used [47]. Reacting trimethylaluminium with cyclopropylamine (**B**) in methylene chloride afforded the corresponding dimethylaluminium amide in situ. The lactone **10** was then added to the solution to furnish amide **17** in 53% yield. The amine **A** was synthesized from Boc–Val–OH and benzylamine whereas the amines **B** and **C** were commercially available [30,43]. Reduction of the azide group in **16**–**21** with PPh_3 in methanol containing a few drops of water provided the corresponding amines **22**–**27** in 62–100% yield [48]. These amines, **22**–**27**, were then subjected to standard peptide coupling chemistry with three different carboxylic acids, (*S*)-2-((*S*)-2-*tert*-butoxycarbonylamino-3-methyl-butyrylamino)-4-methylsulfanyl-butyric acid (**E**), 5-(methanesulfonyl-methyl-amino)-*N*-((*R*)-1-phenyl-ethyl)-isophthalamide (**D**) or 5-(methanesulfonyl-methyl-amino)-*N*-methyl-isophthalamic acid (**F**), in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and DIPEA in DMF to yield the final products **28**–**35** in 17–77% yield. The acid **E** was synthesized from commercially available amino acids (Boc–Val–OH and Met–OMe) and the acids **D** and **F** were synthesized according to previously reported literature procedure [29,43,49].

2.2. Biological data and structure activity relationships

All synthesized inhibitors were screened against BACE-1 to determine IC_{50} values (Table 1) [32]. For the most potent inhibitors the BACE cellular activities were determined measuring production of secreted soluble Aβ-40 in cultured HEK-293 cells (Table 2). In parallel the inhibitors were screened for inhibition of the anti-target human cathepsin D [32].

All BACE-1 inhibitors, vide infra, contain the novel HE moiety containing a methyl substituent in the P1′-position previously reported to be the preferred side chain [31]. To optimize the central core, Val–benzylamide (**A**) were incorporated as P2′–P3′ groups and combined with a selected set of P2–P3 substituents (Table 1). The first inhibitor, **33**, carrying benzyloxymethyl as P1-substituent furnished a BACE-1 IC_{50} value of 3.6 nM and a cathepsin D IC_{50} value of 41 nM. When the benzyloxymethyl group in the P1-position was replaced with a difluorobenzyloxymethyl group as in **28** the BACE-1 IC_{50} value increases marginally from 3.6 nM to 6.6 nM. A small improvement in selectivity over cathepsin D was observed, i.e. **33** and **28** furnishing cathepsin D IC_{50} values of 41 and 210 nM. Contraction of the P1-substituent with one carbon to a difluorophenyloxymethyl group as in **34**-(*R*) gave a small improvement in BACE-1 activity, displaying an IC_{50} value of 3.1 nM.

In addition we studied the effect of the stereochemistry of the substituent at the P1′-position. Whilst the *R* stereochemistry of the methyl group in the P1′-position of **34**-(*R*) is more potent than diastereomer **35**-(*S*) the potency loss of **35**-(*S*) is quite modest, IC_{50}

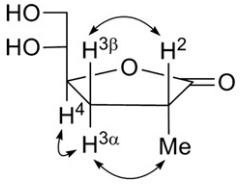
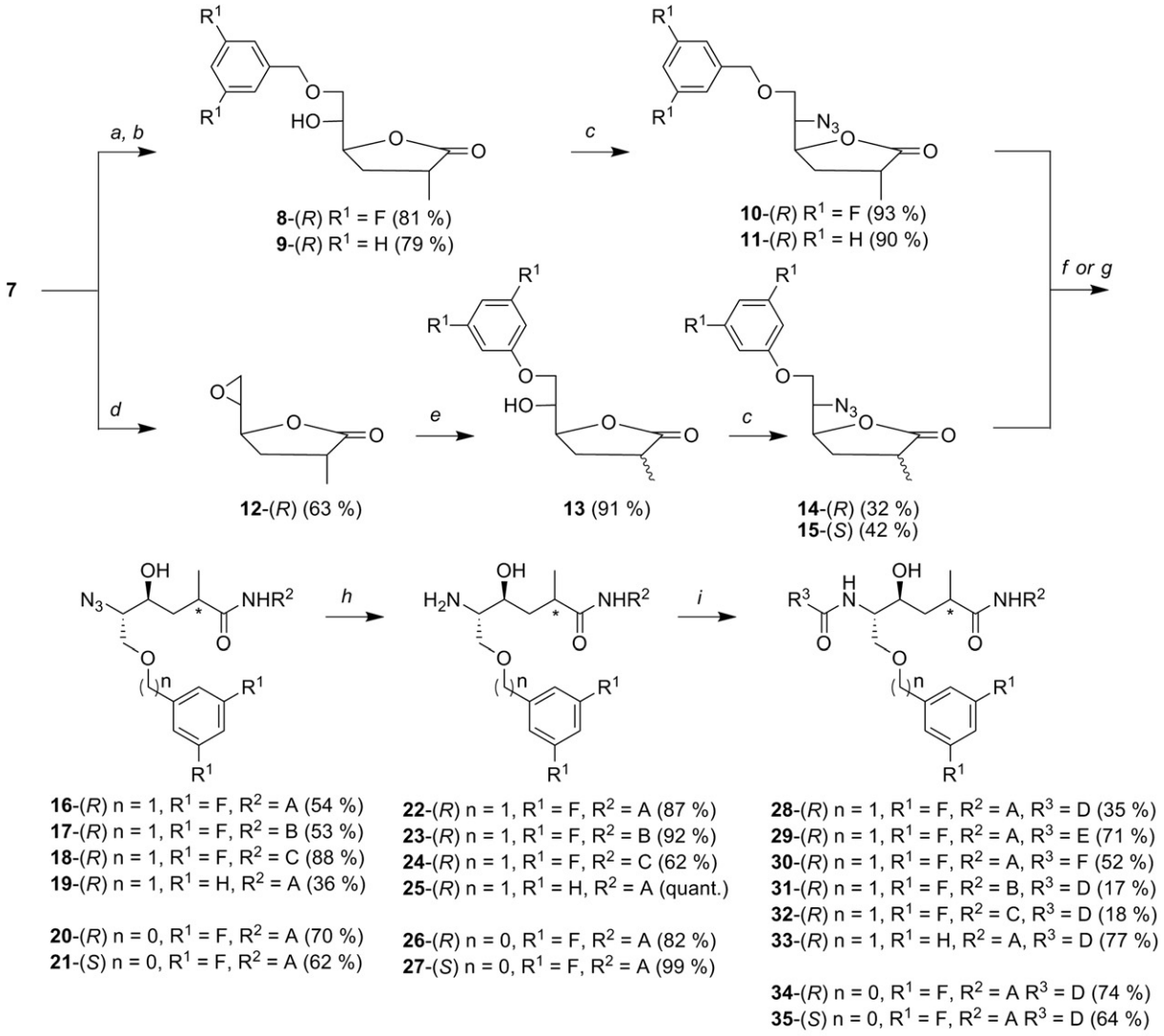


Fig. 3. Relevant NOE correlations in the diol **7**.



Scheme 2. Reagents and conditions. (a) Bu_2SnO, toluene, reflux; (b) tetrabutylammonium bromide, benzylbromide or 3,5-difluorobenzylbromide, toluene, 90 °C; (c) PPh_3, DIAD, DPPA, THF; (d) DIAD, PPh_3, $CHCl_3$, reflux; (e) 3,5-difluorophenol, K_2CO_3, DMF, 110 °C; (f) R^2NH_2 (**A** or **C**), DIPEA, 2-hydroxypyridine, THF (dry), reflux; (g) R^2NH_2 (**B**), Me_3Al, DCM; (h) PPh_3, MeOH, H_2O; (i) R^3COOH (**D**, **E** or **F**), DIPEA, HATU, DMF.

values of 3.1 nM and 10 nM for **34**-(*R*) and **35**-(*S*). This indicates that the S1′-pocket could be further explored for improvements in both potency and selectivity.

In the exploration of this HE moiety we have evaluated a selected set of P2–P3 groups and modifications in the P2′-position. Introducing the Met–Val [50] (**E**) moiety in the P2–P3 position, **29**, surprisingly resulted in a BACE-1 IC_{50} of 10 μM. Interestingly, **29** is a highly potent cathepsin D inhibitor with an IC_{50} value of 0.63 nM. Removal of the P3 methylbenzylamine group of inhibitor **28** furnishing the truncated compound **30** resulted in loss of activity and a BACE-1 IC_{50} value of 1260 nM compared to 6.6 nM for **28**, validating that the P3 methylbenzylamine substituent is essential for activity. When replacing the Val–benzylamide (**A**) in the P2′–P3′ position with cyclopropylamine (**B**)

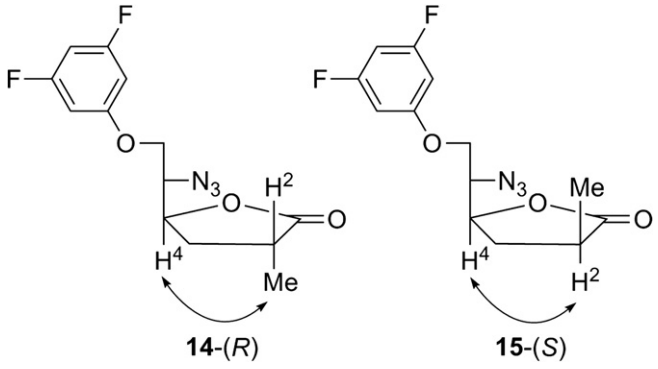


Fig. 4. Relevant NOE correlations in compound **14**-(*R*) and **15**-(*S*).

Table 1
BACE-1 and cathepsin D enzyme data.

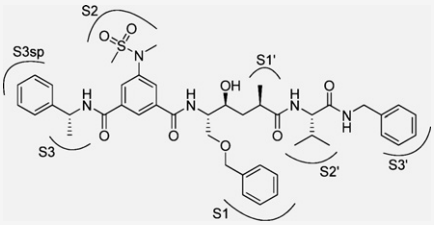
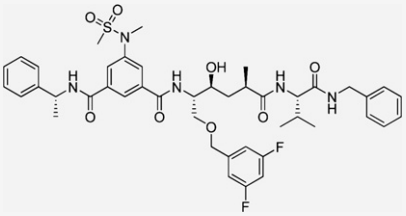
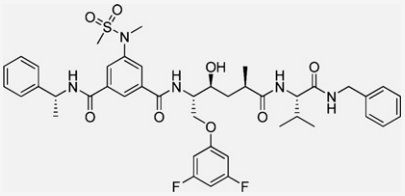
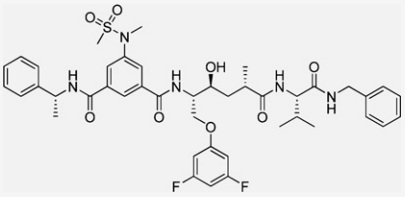
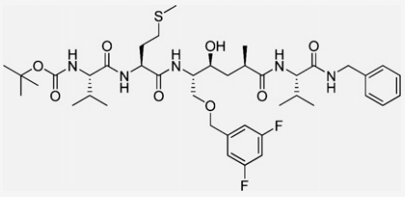
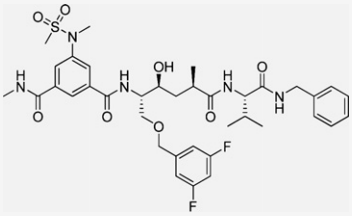
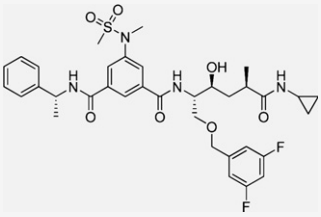
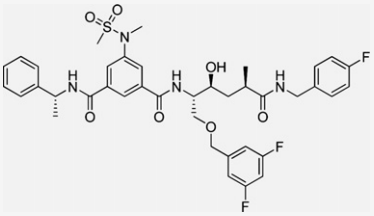
Compound	Structure	IC_{50} (nM) BACE-1	K_i (nM) Cat D
33		3.6	41
28		6.6	210
34-(*R*)		3.1	59
35-(*S*)		10	120
29		10 000	0.63
30		1260	>5000
31		>10 000	>5000

Table 1 (*continued*)

Compound	Structure	IC_{50} (nM) BACE-1	K_i (nM) Cat D
32		3600	>5000

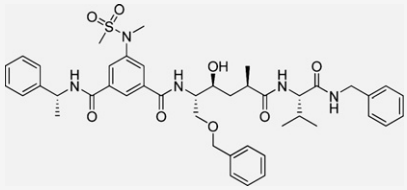
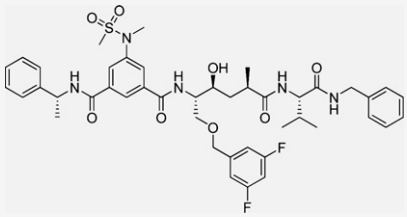
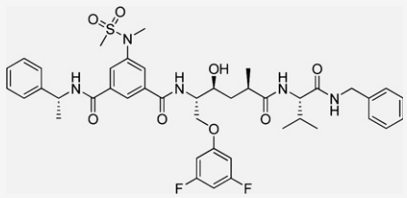
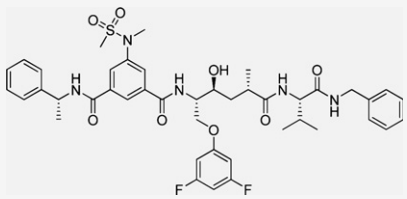
or 4-fluorobenzylamine (**C**), important interactions with BACE-1 were lost, delivering **31** and **32** displaying BACE-1 IC_{50} values of >10 μM and 3.6 μM.

Some BACE-1 inhibitors were evaluated for their activity in a cell culture assay. Whereas the inhibitors showed good inhibition of BACE-1 in cell-free systems, they all lost considerable activity in the cell-based assay (Table 2). Most promising was inhibitor **34**-(*R*) furnishing a BACE-1 IC_{50} value of 3.1 nM and an IC_{50} value of 160 nM in the cell-based assay.

2.3. X-ray crystal structure results

To characterize the binding mode of these novel BACE-1 inhibitors compound **28** was co-crystallized with human BACE-1 providing an X-ray crystal structure determined to 2.8 Å resolution (PDB-code: 3IXK, Fig. 5) [32]. Structural analysis reveals a compact binding including numerous hydrogen bonds that coordinates inhibitor **28** with the BACE-1 enzyme active site. The hydroxyl group of the hydroxyethylene transition state isostere in the inhibitor is positioned between the two catalytic residues Asp32 and Asp228, forming a hydrogen bond network. Furthermore the inhibitor makes several hydrogen bonds to the backbone of BACE-1. These bonds are; P3 NH to Gly230, P3 carbonyl to Thr232, P2 carbonyl to the backbone of the flap residue Gln73, P1 NH to Gly230, P1′ carbonyl to the flap residue Thr72, P2′ NH to Gly34, P2′ carbonyl to the side chain hydroxyl of Tyr198 and the P3′ NH to Pro70. The sulphonamide of the P2 substituent makes hydrogen bond interactions with the backbone NH of Thr232 and Asn233 and with the side chain of Arg235.

Table 2
Cellular inhibition data.

Compound	Structure	Cell IC_{50} (HEK-293) (nM)
33		1000
28		4500
34-(*R*)		160
35-(*S*)		340

The P2′ valine side chain interacts mainly with Ser35, Val69, Ile126, Arg128 in the S2′ pocket and the P3′ capping benzyl is binding on top of the flap and interacts with Pro70, Tyr71 and Thr72.

Hydrophobic interactions are additionally an important component of the potency of **28**. The P3 capping phenyl group makes close interactions with several residues in the S3 sub pocket. It is stacked between Thr232 and Gly13 from top to 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, Ile110 and Trp115. The aromatic ring of P2 is stacked between Thr231 and with the side chain of the flap residue Gln73.

The aromatic moiety of the P1 side chain in **28** is surrounded by both aromatic and hydrophobic residues in the S1 pocket, notably Phe108, Ile110, Trp115 and the flap residue Tyr71. Additionally there is a close contact interaction between one of the P1 *meta*-

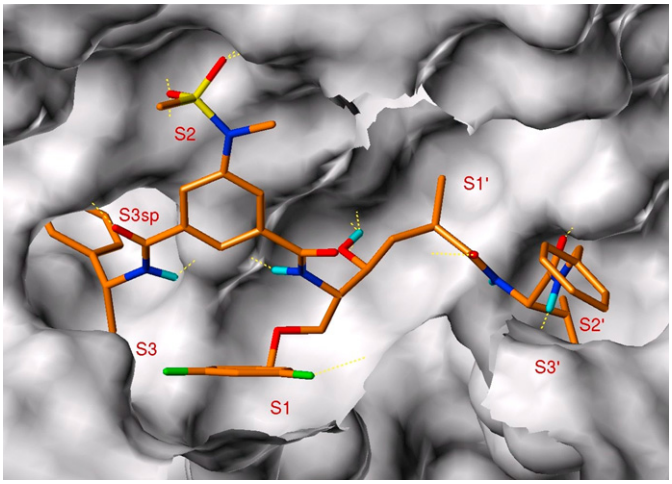


Fig. 5. X-ray crystal structure of compound **28** in the active site of BACE-1.

fluoro substituents with the backbone amide of Gln73 and Gly74 of the flap inducing an amide flip whereby these more extended P1 side chain of *e.g.* inhibitor **28** also can be accomodated into the S1 pocket of BACE-1.

3. Conclusion

We have developed a promising class of BACE-1 inhibitors comprising the HE as transition state isostere with different extensions in the P1-position readily available from an inexpensive carbohydrate starting material. The synthetic route yielded the pivotal intermediate **7** with excellent stereochemical control which was corroborated by NOESY experiments. Variations in the P1-position with three different substituents were evaluated and revealed that the 3,5-difluorophenyl moiety was the most suitable in combination with this HE isostere. The two stereochemical configurations at the methyl substituent in the P1′-position were investigated and from the BACE-1 inhibitory data the (*R*) stereochemistry of the methyl group is preferred. Furthermore, the HE isostere was evaluated together with different P2–P3 and P2′–P3′ substituents selected from the literature based on good inhibitory properties for other transition state isosteres. The most potent inhibitor **34**-(*R*) exhibits a promising BACE-1 IC_{50} value of 3.1 nM (Fig. 6). The described inhibitors provides a novel and easily diversified HE central core that can be further used use for the development BACE-1 inhibitors as well as inhibitors of other aspartic proteases.

4. Experimental section

4.1. A-beta cell-based assay

Human embryonic kidney 293 cells (HEK-293) stably expressing Swedish mutant APP (swAPP751)[51] were cultured in DMEM supplemented with 10% FCS, PEST (50 U penicillin/50 μg/mL streptomycin) and 200 μg Hygromycin/mL at 37 °C, 5% CO_2. Cells were seeded at a density of 2×10^4 cells/well into 96-well plates and left overnight to settle. Thereafter cells were washed with fresh media once to reduce background. Inhibitor was added (max 1% DMSO final conc.). After 24 h cell culture media was harvested and analysed by Aβ 1–40 ELISA according to the manufacturer instruction (The Genetics Company, Schwitzerland). The results were treated with the curve fitting package in GraphPad Prism 5.

4.2. General methods

All chemicals were purchased from commercial suppliers and used directly without further purification. Standard ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 300 instrument using

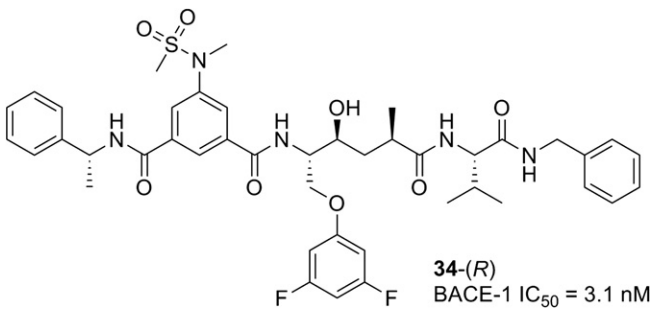


Fig. 6. BACE inhibition value for the most potent inhibitor, compound **34**-(*R*) from this series.

$CDCl_3$ or methanol-d_4 (CD_3OD) with TMS as an internal standard. NOESY spectra that were used to determine the structure of compound **7**, **14**-(*R*) and **15**-(*S*) were recorded on Varian 500 instrument using $CDCl_3$ or CD_3OD with TMS as an internal standard. In the recorded NMR spectra of the diastereomeric mixture **13** 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 ^{1}H NMR. TLC was carried out on Merck precoated 60 F_{254} plates using UV-light and charring with ethanol/sulfuric acid/acetic acid/p-aninsaldehyde (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). Preparative isocratic HPLC was performed on a Gynkotek (pump: P580, detector: UVD 170S, software: Chromeleon) using a Kromasil 100-10-C18 (250 × 20 mm) column. Mixtures of deionized water and methanol with 0.1% TFA or 0.1% TEA were used as mobile phase. Gradient LC/MS was performed on a Gilson system (Column: Phenomenex C18 250 × 15 mm for preparative and Phenomenex C18 150 × 4.6 mm for analytical runs; Pump: Gilson gradient pump 322; UV/VIS-detector: Gilson 155; MS detector: Thermo Finnigan Surveyor MSQ; Gilson Fraction Collector FC204, software: Gilson UniPoint ver. 4.0 and Xcalibur ver. 1.3) using methanol with 0.1% formic acid and deionized water with 0.1% formic acid as mobile phase. Optical rotations were measured using a Perkin–Elmer 141 polariometer. Concentrations were performed in vacuo below 40 °C. All low degree temperature reactions were accomplished by submerging the reaction vessels into an ethanol bath that had been cooled by additions of liquid nitrogen. All degassing of solvents were achieved using ultra sonification (Ultrasonik 104×) for at least 1 h. Drying of solvents: THF was refluxed over sodium/benzophenone and distilled onto 4 Å MS, toluene and dichloromethane was refluxed over calcium hydride and distilled onto 4 Å MS. Organic extracts were dried over magnesium sulfate monohydrate and filtered. Filtrating was achieved using filter paper Munktell, OOH quality. Combustion analysis was performed by Analytische Laboratorien, Lindlar, Germany.

4.2.1. 5,6-Di-O-benzyl-3-deoxy-1,2-O-isopropylidene-α-D-glucofuranoside (**1**)

Compound **1** was synthesized from commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose in a four-step protocol according to literature procedure in an overall yield of 73% [33–36].

4.2.2. Methyl 5,6-di-O-benzyl-3-deoxy-α-D-glucofuranoside (**2**α) and methyl 5,6-di-O-benzyl-3-deoxy-β-D-glucofuranoside (**2**β)

Compound **1** (6.21 g, 16.2 mmol) was dissolved in methanol and cooled in an ice bath (0 °C) and conc. H_2SO_4 (2.69 mL, 48.5 mmol) was slowly added to the solution. The reaction mixture was stirred overnight at room temperature and then neutralized with $NaHCO_3$ and concentrated. The residue was extracted with ethyl acetate (3 × 50 mL) and H_2O (50 mL). The combined organic layers were dried, filtered and concentrated. Purification by flash column chromatography (toluene/ethyl acetate 3:1) yielded the α- and β-anomers in an 11:2 ratio (**2**α:**2**β) as transparent oils (4.69 g, 80% and 0.84 g, 14% for the α- and β-anomers). **2**α: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.0 (dd, J = 6.6, 13.7 Hz, 1H), 2.14 (dq, J = 4.7, 13.5 Hz, 1H), 3.30 (s, 3H), 3.57–3.69 (m, 2H), 3.80 (dd, J = 2.7, 10.2 Hz, 1H), 4.23 (t, J = 4.9 Hz, 1H), 4.39–4.47 (m, 1H), 4.53–4.66 (m, 3H), 4.75–4.81 (m, 2H), 7.22–7.38 (m, 10H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 35.5, 54.9, 71.3, 73.2, 73.7, 76.0, 79.0, 81.8, 109.8, 127.8, 127.9, 128.1, 128.6, 128.7, 138.7, 139.0. **2**β: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.76–1.88 (m, 1H), 2.20–2.30 (m, 1H), 2.40 (d, J = 11.8 Hz, 1H), 3.47 (s, 3H), 3.56 (d, J = 5.5 Hz, 1H), 3.68–3.75 (m, 1H), 4.20–4.30 (m, 1H), 4.31–4.40 (m, 1H), 4.54 (s, 2H), 4.57–4.64 (m, 1H), 4.70 (d, J = 17.9 Hz, 1H), 4.75–

4.85 (m, 1H), 7.23–7.39 (m, 10H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 32.8, 55.2, 70.4, 72.0, 73.3, 73.4, 76.0, 79.6, 102.5, 127.3, 127.5, 127.6, 127.7, 128.2, 128.3, 128.4, 129.0, 138.1, 138.7.

4.2.3. Methyl 5,6-di-O-benzyl-3-deoxy-2-O-(imidazolethiocarbonyl)-D-glucofuranoside (**3**)

The alcohol **2** (α- and β-anomer mixture) (2.24 g, 6.25 mmol) and 1,1′-thiocarbonyldiimidazole (1.67 g, 9.37 mmol) were dissolved in THF (31 mL) and the mixture was refluxed overnight. The crude residue was concentrated and purified by flash column chromatography (toluene/ethyl acetate 3:1) to yield compound **3** (2.92 g, 99%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.29–2.50 (m, 2H), 3.37 (s, 3H), 3.61–3.73 (m, 2H), 3.76–3.84 (m, 1H), 4.43–4.53 (m, 1H), 4.60 (dd J = 7.4, 12.1 Hz, 2H), 4.64 (d, J = 11.5 Hz, 1H), 4.80 (d, J = 11.5 Hz, 1H), 5.10 (s, 1H), 5.74 (d, J = 4.1 Hz, 1H), 7.05–7.07 (m, 1H), 7.27–7.39 (m, 10H), 7.60–7.62 (m, 1H), 8.33–8.34 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 32.1, 54.9, 70.1, 72.7, 73.4, 79.2, 80.8, 86.1, 106.0, 117.7, 127.7, 127.5, 127.6, 128.2, 128.3, 130.9, 136.7, 138.0, 138.3, 183.0. MS m/z 469.3 [$(M+H)^+$ calcd for $C_{25}H_{29}N_2O_5S^+$ 469.18].

4.2.4. Methyl 5,6-di-O-benzyl-2,3-dideoxy-D-glucofuranoside (**4**)

Tributyl tin hydride (5.18 g, 17.79 mmol) was dissolved in dry toluene (35 mL) under N_2-atmosphere and refluxed for 5 min. Compound **3** (5.56 g, 11.86 mmol) dissolved in dry toluene (35 mL) was added drop wise to the solution during 30 min. The combined solution was stirred at 110 °C and after 2 h the mixture was concentrated. Purification by flash column chromatography (toluene/ethyl acetate 18:1) yielded compound **4** (2.94 g, 72%) as colourless semi-crystals. ^{1}H NMR (300 MHz, CD_3OD): δ 1.87–1.98 (m, 4H), 3.25 (s, 3H), 3.55–3.66 (m, 2H), 3.81 (dd, J = 2.2, 9.9 Hz, 1H), 4.10 (dt, J = 6.5, 8.2 Hz, 1H), 4.45 (s, 1H), 4.61 (d, J = 11.6 Hz, 1H), 4.74 (d, J = 11.6 Hz, 1H), 4.80 (s, 1H), 4.92 (t, J = 2.4 Hz, 1H), 7.24–7.38 (m, 10H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 27.4, 33.5, 55.0, 71.8, 73.8, 74.4, 80.7, 82.7, 106.7, 128.6, 128.7, 128.8, 129.0, 129.2, 129.3, 139.8, 140.0. MS m/z 365.4 [$(M+Na)^+$ calcd for $C_{21}H_{26}NaO_4^+$ 365.17].

4.2.5. 5,6-Di-O-benzyl-2,3-dideoxy-D-glucono-1,4-lactone (**5**)

The methyl-acetal **4** (2.79 g, 8.16 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and cooled to 0 °C in an ice bath. BF_3OEt (0.52 mL, 2.04 mmol) and *m*-chloroperbenzoic acid (2.20 g, 9.79 mmol) were added to the solution and the mixture was kept at 0 °C for 2 h before it was allowed to reach room temperature. After 4 h the mixture was concentrated and extracted with ethyl acetate (3 × 50 mL) and saturated $NaHCO_3$ (50 mL). The combined organic layers were dried, filtered and concentrated. The crude residue was purified by flash column chromatography (toluene/ethyl acetate 18:1) to yield the lactone **5** (2.66 g, quant.) as white crystals. ^{1}H NMR (300 MHz, CD_3OD): δ 2.14–2.25 (m, 2H), 2.44–2.50 (m, 2H), 3.60 (d, J = 5.3 Hz, 2H), 3.86 (dt, J = 3.6, 5.3 Hz, 1H), 4.49 (d, J = 12.0 Hz, 1H), 4.53 (d, J = 12.0 Hz, 1H), 4.56 (d, J = 11.5 Hz, 1H), 4.67 (d, J = 11.5 Hz, 1H), 4.68–4.75 (m, 1H), 7.25–7.34 (m, 10H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 23.2, 29.3, 69.9, 74.3, 74.5, 80.0, 81.9, 128.7, 128.8, 128.9, 129.0, 129.3, 129.4, 139.4, 139.6, 180.2. MS m/z 327.3 [$(M+H)^+$ calcd for $C_{20}H_{23}O_4^+$ 327.39].

4.2.6. 5,6-Di-O-benzyl-2,3-dideoxy-2-methyl-D-glucono-1,4-lactone (**6**)

The lactone **5** (1.31 g, 4.01 mmol) was dissolved in dry THF (40 mL) and cooled to −78 °C. After 15 min a solution of 2.0 M LDA (2.47 mL, 4.01 mmol) was added drop wise. After 30 min at −78 °C methyl iodide (2.5 mL, 40.1 mmol) dissolved in dry THF (5 mL) was slowly added. After further 2 h at −78 °C the reaction was allowed to attain room temperature and quenched with saturated ammonium chloride (4 mL). The mixture was diluted with H_2O (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried, filtered and concentrated. Purification by flash column chromatography (toluene/ethyl acetate 18:1) gave compound **6** (899 mg, 66%) as an pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (d, J = 7.42, 3H), 1.78–1.91 (m, 1H), 2.43–2.53 (m, 1H), 2.66–2.81 (m, 1H), 3.53–3.65 (m, 2H), 3.81–3.90 (m, 1H), 4.47–4.73 (m, 5H), 7.23–7.42 (m, 10H); ^{13}C NMR (75.5 MHz, $CDCl_3$): d 16.2, 30.4, 34.0, 68.9, 73.4, 73.5, 77.5, 78.5, 127.6, 127.7, 127.8, 127.9, 128.3, 128.4, 137.7, 137.8, 180.3. MS m/z 341.4 [$(M+H)^+$ calcd for $C_{21}H_{25}O_4^+$ 341.17].

4.2.7. 2,3-Dideoxy-2-methyl-D-glucono-1,4-lactone (**7**)

Compound **6** (174 mg, 0.51 mmol) was dissolved in ethanol (2.5 mL, 95%) and Pd-C (∼5 mg) was added. The reaction was performed under H_2-atmosphere, which was refilled until the reaction was complete. The mixture was filtered though a pad of celite and concentrated. This gave after co-concentration with methanol and toluene the diol **7** (83 mg, quant.) as white crystals. ^{1}H NMR (300 MHz, CD_3OD): δ 1.23 (d, J = 7.4 Hz, 3H), 1.85–1.98 (m, 1H), 2.45–2.57 (m, 1H), 2.72–2.86 (m, 1H), 3.52–3.59 (m, 2H), 3.74–3.84 (m, 1H), 4.53–4.61 (m, 1H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 15.3, 30.0, 34.3, 62.7, 72.4, 78.7, 181.8. HRMS m/z 183.0631 [$(M+Na)^+$ calcd for $C_7H_{12}NaO_4^+$ 183.0628].

4.2.8. 2,3-Dideoxy-6-O-(3,5-difluorobenzyl)-2-methyl-D-glucono-1,4-lactone (**8**)

The diol **7** (201 mg, 1.25 mmol) and dibutyltin oxide (405 mg, 1.63 mmol) was dissolved in toluene (7 mL). The mixture was refluxed for 5 h before the temperature was lowered to 90 °C and tetrabutylammonium bromide (464 mg, 1.44 mmol) and 3,5-difluorobenzylbromide (0.186 mL, 1.44 mmol) were added. The mixture was allowed to stir at 90 °C overnight and thereafter concentrated. Purification by flash column chromatography (toluene/ethyl acetate 6:1) gave compound **8** (291 mg, 81%) as a colourless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.22 (d, J = 7.3 Hz, 3H), 1.92 (dt, J = 8.6, 13.0 Hz, 1H), 2.52 (ddd, J = 3.6, 9.5, 13.0 Hz, 1H), 2.79 (ddq, J = 7.3, 8.6, 9.5 Hz, 1H), 3.54 (dd, J = 5.7, 10.8 Hz, 1H), 3.58 (dd, J = 5.0, 10.8 Hz, 1H), 3.95 (dt, J = 4.7, 5.7 Hz, 1H), 4.55 (s, 2H), 4.59 (ddd, J = 3.6, 4.7, 8.6 Hz, 1H), 6.78–6.86 (m, 1H), 6.92–7.00 (m, 2H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 16.4, 31.3, 35.3, 71.9, 72.5, 72.9, 79.7, 103.4 (t, J_{CF} = 25.8 Hz), 110.9 (d, J_{CF} = 25.5 Hz, 2C), 144.4 (t, J_{CF} = 8.9 Hz), 164.3 (d, J_{CF} = 247.5 Hz), 164.5 (d, J_{CF} = 247.5 Hz), 182.8. MS m/z 287.2 [$(M+H)^+$ calcd for $C_{14}H_{17}F_2O_4^+$ 287.11].

4.2.9. 2,3-Dideoxy-6-O-benzyl-2-methyl-D-glucono-1,4-lactone (**9**)

Compound **9** (413 mg, 79%) was synthesized from **7** (336 mg, 2.09 mmol) according to the method of the preparation of **8**, with the exception that benzylbromide was used instead of 3,5-difluorobenzylbromide. After purification compound **9** was collected as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (d, J = 7.28 Hz, 3H), 1.86 (dt, J = 8.3, 13.0 Hz, 1H), 2.49 (ddd, J = 3.9, 9.4, 13.0 Hz, 1H), 2.72 (ddq, J = 7.3, 8.5, 9.4, 1H), 2.94 (bs, 1H), 3.51 (dd, J = 5.2, 9.8 Hz, 1H), 3.58 (dd, J = 4.5, 9.8 Hz, 1H), 3.90 (dt, J = 4.5, 5.6 Hz, 1H), 4.47 (ddd, J = 3.9, 5.6, 8.3 Hz, 1H), 4.51 (d, J = 11.8 Hz, 1H), 4.56 (d, J = 11.8 Hz, 1H), 7.28–7.38 (m, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 16.0, 30.6, 33.7, 70.5, 70.7, 73.4, 77.5, 127.7, 127.8, 128.4, 137.5, 180.3.

4.2.10. 5-Azido-2,3,5-trideoxy-6-O-(3,5-difluorobenzyl)-2-methyl-L-iodono-1,4-lactone (**10**)

Compound **8** (150 mg, 0.53 mmol) was dissolved in dry THF (5 mL) and cooled to −15 °C (ice/acetone 1:1). Triphenyl phosphine (207 mg, 0.79 mmol) and diisopropyl azodicarboxylate (0.156 mL, 0.79 mmol) were added. After 10 min the mixture was allowed to reach 0 °C and diphenylphosphoryl azide (217 mg, 0.79 mmol) was added. After 30 min of stirring at 0 °C the mixture was allowed to

attain room temperature and was stirred overnight. The solvent was evaporated, and the crude mixture was purified by flash column chromatography (toluene/ethyl acetate 18:1) yielding the azide **10** (152 mg, 93%) as a colourless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.22 (d, $J = 7.4$ Hz, 3H), 2.05 (dt, $J = 8.4$, 13.2 Hz, 1H), 2.42 (ddd, $J = 4.1$, 9.6, 13.2 Hz, 1H), 2.83 (ddq, $J = 7.4$, 8.4, 9.6 Hz, 1H), 3.73 (dd, $J = 8.0$, 9.9 Hz, 1H), 3.79 (dd, $J = 4.0$, 9.9 Hz, 1H), 3.80–3.88 (m, 1H), 4.58 (s, 2H), 4.65 (dt, $J = 4.1$, 8.4 Hz, 1H), 6.77–6.86 (m, 1H), 6.91–6.99 (m, 2H); ^{13}C NMR (75.5 MHz, CD_3OD): 16.4, 33.5, 34.9, 65.4, 71.6, 72.8, 72.9, 103.6 (t, $J_{CF} = 25.8$ Hz), 110.9 (d, $J_{CF} = 25.2$ Hz, 2C), 143.9 (t, $J_{CF} = 8.9$ Hz), 164.4 (d, $J_{CF} = 247.2$ Hz), 164.6 (d, $J_{CF} = 247.2$ Hz), 182.0.

4.2.11. 5-Azido-2,3,5-trideoxy-6-O-benzyl-2-methyl-L-idono-1,4-lactone (**11**)

Compound **11** (403 mg, 90%) was synthesized from **9** (408 mg, 1.63 mmol) according to the method of the preparation of **10**. After purification compound **11** was collected as a colourless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.22 (d, $J = 7.4$ Hz, 3H), 2.02 (dt, $J = 8.4$, 13.1 Hz, 1H), 2.39 (ddd, $J = 4.1$, 9.6, 13.1 Hz, 1H), 3.83 (ddq, $J = 7.4$, 8.4, 9.6 Hz, 1H), 3.67–3.82 (m, 3H), 4.51–4.66 (m, 3H), 7.26–7.38 (m, 5H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 16.4, 33.5, 35.0, 65.4, 71.1, 74.4, 78.2, 128.8, 129.4, 139.2, 182.1.

4.2.12. 5,6-Anhydro-2,3-dideoxy-2-methyl-D-glucono-1,4-lactone (**12**)

The diol **7** (119 mg, 0.75 mmol) was dissolved in chloroform (37 mL), triphenyl phosphine (293 mg, 1.12 mmol) and diisopropyl azodicarboxylate (220 μL, 1.12 mmol) were added. The mixture was refluxed overnight before the solvent was evaporated. The crude mixture was purified by flash column chromatography (toluene/ethyl acetate 9:1) to give the epoxide **12** (66 mg, 63%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (d, $J = 6.32$ Hz, 3H), 1.76–1.87 (m, 1H), 2.06–2.17 (m, 1H), 2.50–2.56 (m, 1H), 2.57–2.68 (m, 1H), 2.74 (t, $J = 4.67$ Hz, 1H), 3.06–3.12 (m, 1H), 4.40–4.49 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 15.3, 30.2, 33.3, 44.2, 51.5, 76.5, 179.1.

4.2.13. 2,3-Dideoxy-6-O-(3,5-difluorophenyl)-2-methyl-D-glucono-1,4-lactone and 2,3-dideoxy-6-O-(3,5-difluorophenyl)-2-methyl-D-manono-1,4-lactone (**13**)

To the epoxide **12** (66 mg, 0.47 mmol) in DMF (2.4 mL) were 3,5-difluorophenol (92 mg, 0.71 mmol) and K_2CO_3 (37 mg, 0.24 mmol) added. The reaction mixture was heated to 110 °C for 4 h. The DMF was removed by co-evaporation with toluene (3 × 15 mL). The crude residue was purified by flash column chromatography (toluene/ethyl acetate 9:1) to yield a diastereomeric mixture of compound **13** (116 mg, 91%) as a colourless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.21–1.29 (m, 3H), 1.87–2.02 (m, 1H), 2.41–2.63 (m, 1H), 2.64–2.85 (m, 1H), [3.31 (d, $J = 4.94$ Hz, 0.5H) & 3.47 (d, $J = 5.22$ Hz, 0.5 Hz, 0.5H)], 3.98–4.04 (m, 2H), 4.10–4.20 (m, 1H), [4.46–4.53 (m, 0.5H), 4.55–4.63 (m, 0.5H)], 6.38–6.48 (m, 3H); ^{13}C NMR (75.5 MHz, CD_3OD): δ [15.4 & 16.4], [31.5 & 32.8], [35.3 & 36.5], 70.8, [71.2 & 71.4], 79.3, 97.2 (t, $J_{CF} = 25.7$ Hz), 99.5 (d, $J_{CF} = 28.9$ Hz, 2C), 162.3 (t, $J_{CF} = 13.7$ Hz), 165.0 (d, $J_{CF} = 247.1$ Hz), 165.5 (d, $J_{CF} = 247.1$ Hz), [181.7 & 182.6]. MS m/z 272.8 [$(M + H)^+$ calcd for $C_{13}H_{15}F_2O_4^+$ 273.09].

4.2.14. 5-Azido-2,3,5-trideoxy-6-O-(3,5-difluorobenzyl)-2-methyl-L-idono-1,4-lactone (**14**-(*R*)) and 5-azido-2,3,5-trideoxy-6-O-(3,5-difluorobenzyl)-2-methyl-L-gulono-1,4-lactone (**15**-(*S*))

A diastereomeric mixture of **13** (116 mg, 0.42 mmol) and triphenyl phosphine (168 mg, 0.64 mmol) was dissolved in dry THF (4.3 mL). The mixture was cooled to −15 °C (acetone/ice 1:1) and diisopropyl azodicarboxylate (126 μL, 0.64 mmol) was added. The mixture was stirred for 30 min at −15 °C (acetone/ice 1:1) before the temperature was raised to 0 °C and diphenylphosphoryl azide (142 μL, 0.64 mmol) was added. The reaction mixture was allowed to attain room temperature and was stirred overnight. The solvent was evaporated and the crude diastereomeric mixture was purified by flash column chromatography (toluene/ethyl acetate 18:1). The two diastereomers **14**-(*R*) (40 mg, 32%) and **15**-(*S*) (53 mg, 42%) were separated and collected as transparent oils. **14**-(*R*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (d, $J = 7.1$ Hz, 3H), 2.02–2.16 (m, 1H), 2.40–2.53 (m, 1H), 2.82–2.98 (m, 1H), 3.86–3.95 (m, 1H), 4.21 (d, $J = 6.3$ Hz, 2H), 4.63–4.71 (m, 1H), 6.38–6.52 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 16.2, 32.6, 33.4, 63.3, 68.4, 75.2, 97.4 (t, $J_{CF} = 25.8$ Hz), 98.5 (d, $J_{CF} = 28.9$ Hz, 2C), 159.6 (t, $J_{CF} = 13.7$ Hz), 163.5 (d, $J_{CF} = 247.2$ Hz), 163.7 (d, $J_{CF} = 247.2$ Hz) 179.1. $[\alpha]_D^{22} = +63.5$ (chloroform). MS m/z 319.6 [$(M + Na)^+$ calcd for $C_{13}H_{13}F_2N_3NaO_3^+$ 320.08]. **15**-(*S*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (d, $J = 4.1$ Hz, 3H), 1.87–2.01 (m, 1H), 2.43–2.56 (m, 1H), 2.66–2.80 (m, 1H), 3.80–3.87 (m, 1H), 4.17 (m, 2H), 4.52–4.62 (m, 1H), 6.41–6.52 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 15.1, 32.9, 35.0, 62.0, 68.2, 76.0, 97.4 (t, $J_{CF} = 26.1$ Hz), 98.5 (d, $J_{CF} = 29.2$ Hz, 2C), 163.5 (d, $J_{CF} = 247.4$ Hz), 163.8 (d, $J_{CF} = 247.4$ Hz), 177.9. $[\alpha]_D^{22} = +46.3$ (chloroform). MS m/z 319.6 [$(M + Na)^+$ calcd for $C_{13}H_{13}F_2N_3NaO_3^+$ 320.08].

4.2.15. (2R,4S,5S)-5-Azido-6-(3,5-difluoro-benzyloxy)-4-hydroxy-2-methyl-hexanoic acid ((S)-1-benzylcarbamoyl-2-methyl-propyl)-amide (**16**)

Compound **16** (133 mg, 54%) was synthesized from **10** (141 mg, 0.51 mmol) according to the method of the preparation of **19**. Compound **16** was collected as crystals after purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90–0.99 (m, 6H), 1.16 (d, $J = 7.01$ Hz, 3H), 1.67–1.77 (m, 2H), 2.06–2.19 (m, 1H), 2.58–2.72 (m, 1H), 3.05 (bs, 1H), 3.39–3.47 (m, 1H), 3.64–3.78 (m, 3H), 4.17–4.24 (m, 1H), 4.32–4.50 (m, 2H), 4.52 (s, 2H), 6.30–6.38 (m, 1H), 6.41–6.49 (m, 1H), 6.67–6.77 (m, 1H), 6.81–6.89 (m, 2H), 7.20–7.36 (m, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.0, 17.2, 17.9, 29.7, 36.5, 37.2, 42.1, 58.3, 65.1, 68.1, 69.8, 70.9, 101.5 (t, $J_{CF} = 25.2$ Hz), 108.9 (d, $J_{CF} = 25.2$ Hz, 2C), 126.2, 126.5, 127.5, 137.5, 141.9 (t, $J_{CF} = 8.8$ Hz), 162.4 (d, $J_{CF} = 251.2$ Hz), 162.6 (d, $J_{CF} = 251.2$ Hz), 171.5, 176.7. MS m/z 518.4 [$(M + H)^+$ calcd for $C_{26}H_{34}F_2N_5O_4^+$ 518.26].

4.2.16. (2R,4S,5S)-5-Azido-6-(3,5-difluoro-benzyloxy)-4-hydroxy-2-methyl-hexanoic acid cyclopropylamide (**17**)

Cyclopropylamine (**B**) (49 μL, 0.71 mmol) was dissolved in CH_2Cl_2 (2 mL) and 2 M Me_3Al (710 μL, 1.41 mmol) was added drop wise to the solution. The reaction was stirred for 15 min before the lactone **10** (110 mg, 0.35 mmol) dissolved in CH_2Cl_2 (1 mL) slowly was added. The reaction mixture was stirred at 40 °C for 1 h and then quenched with 1 M HCl to pH 5. The crude mixture was extracted with H_2O (10 mL) and ethyl acetate (3 × 10 mL). The combined organic layers were dried, filtered and concentrated. Purification by flash column chromatography (toluene/ethyl acetate 2:1) gave the azide **17** (69 mg, 53%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (d, $J = 6.9$ Hz, 3H), 1.17 (s, 2H), 1.25 (s, 2H), 1.51–1.62 (m, 1H), 1.64–1.78 (m, 2H), 2.38–2.48 (m, 1H), 2.43 (bs, 1H), 3.54 (ddd, $J = 3.6$, 4.7, 6.6 Hz, 1H), 3.75 (dd, $J = 4.7$, 9.9 Hz, 1H), 3.79 (dd, 6.6, 9.9 Hz, 1H), 3.86–3.92 (m, 1H), 4.56 (s, 2H), 6.68–6.77 (m, 1H), 6.84–6.92 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 16.0, 25.6, 29.1, 36.8, 40.4, 65.1, 69.7, 71.4, 72.3, 73.3, 103.0 (t, $J_{CF} = 25.2$ Hz), 109.9 (d, $J_{CF} = 25.2$ Hz, 2C), 141.8 (t, $J_{CF} = 8.9$ Hz), 163.0 (d, $J_{CF} = 249.0$ Hz), 163.2 (d, $J_{CF} = 249.0$ Hz), 184.7.

4.2.17. (2R,4S,5S)-5-Azido-6-(3,5-difluoro-benzyloxy)-4-hydroxy-2-methyl-hexanoic acid 4-fluoro-benzylamide (**18**)

Compound **18** (188 mg, 88%) was synthesized from **10** (152 mg, 0.49 mmol) according to the method of the preparation of **19** using the amine 4-fluorobenzylamine (**C**) instead of the amine (*S*)-2-

Amino-*N*-benzyl-3-methyl-butyramide (**A**). Compound **18** was collected as white crystals after purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (d, $J = 6.7$ Hz, 3H), 1.56–1.59 (m, 1H), 1.70–1.79 (m, 2H), 2.52–2.67 (m, 1H), 2.89–2.95 (m, 1H), 3.41–3.48 (m, 1H), 3.61–3.79 (m, 2H), 4.40 (d, $J = 5.8$, 2H), 4.54 (s, 2H), 6.69–6.78 (m, 1H), 6.82–6.91 (m, 2H), 6.95–7.04 (m, 2H), 7.20–7.26 (m, 3H). ^{13}C NMR (75.5 MHz, CDCl3): δ 18.3, 37.7, 38.4, 42.9, 65.8, 69.1, 71.5, 72.4, 103.3 (t, $J_{CF} = 25.3$ Hz, 1C), 110.1 (d, $J_{CF} = 25.3$ Hz, 2C), 115.7 (d, $J_{CF} = 21.5$ Hz, 2C), 129.5 (d, $J_{CF} = 8.0$ Hz, 2C), 134.3 (d, $J_{CF} = 3.3$ Hz, 1C), 141.9 (t, $J_{CF} = 8.9$ Hz, 1C), 162.3 (d, $J_{CF} = 245.9$ Hz, 1C), 163.2 (d, $J_{CF} = 248.8$ Hz, 1C), 163.4 (d, $J_{CF} = 248.8$ Hz, 1C), 176.7. MS *m*/*z* 437.1 [$(M + H)^+$ calcd for $C_{21}H_{24}F_3N_4O_3^+$ 437.2].

4.2.18. (2R,4S,5S)-5-Azido-6-benzyloxy-4-hydroxy-2-methyl-hexanoic acid ((S)-1-benzylcarbamoyl-2-methyl-propyl)-amide (**19**)

Compound **11** (59 mg, 0.21 mmol) and (*S*)-2-amino-*N*-benzyl-3-methyl-butyramide (**A**) (71 mg, 0.34 mmol) were dissolved in dry THF (2 mL). DIPEA (74 μL, 0.43 mmol) and 2-hydroxy-pyridine (81 mg, 0.86 mmol) were added and the mixture was refluxed for 4 days. Purification by HPLC chromatography (80% MeOH, 20% H_2O + 0.2% TFA) gave **19** (37 mg, 36%) as a colourless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 0.92 (d, $J = 6.8$ Hz, 3H), 0.94 (d, $J = 6.8$ Hz, 3H), 1.13 (d, $J = 7.0$ Hz, 3H), 1.55 (ddd, $J = 3.9$, 10.2, 13.9 Hz, 1H), 1.82 (ddd, $J = 2.9$, 10.4, 13.9 Hz, 1H), 1.98–2.11 (m, 1H), 2.70 (ddd, $J = 3.9$, 7.0, 10.4 Hz, 1H), 3.42–3.49 (m, 1H), 3.59 (ddd, $J = 2.9$, 3.9, 10.2 Hz, 1H), 3.64 (dd, $J = 7.2$, 10.1 Hz, 1H), 3.70 (dd, $J = 4.6$, 10.1 Hz, 1H), 4.12–4.19 (m, 1H), 4.35 (dd, $J = 5.8$, 15.0 Hz, 1H), 4.55 (dd, $J = 6.0$, 15.0 Hz, 1H), 4.55 (s, 2H), 7.20–7.37 (m, 10H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 19.0, 19.1, 19.8, 31.8, 38.5, 39.2, 44.1, 60.7, 67.2, 70.3, 71.4, 74.3, 128.2, 128.6, 128.7, 128.8, 129.4, 129.5, 139.4, 139.8, 173.8, 179.0. MS *m*/*z* 482.4 [$(M + H)^+$ calcd for $C_{26}H_{36}N_5O_4^+$ 482.6].

4.2.19. (2R,4S,5S)-5-Azido-6-(3,5-difluoro-phenoxy)-4-hydroxy-2-methyl-hexanoic acid ((S)-1-benzylcarbamoyl-2-methyl-propyl)-amide (**20**-(*R*))

Compound **20**-(*R*) (94 mg, 70%) was synthesized from **14**-(*R*) (77 mg, 0.26 mmol) according to the method of the preparation of **19**. Compound **20**-(*R*) was collected as crystals after purification by flash column chromatography (toluene/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ (1:1, v/v)): δ 0.92 (d, $J = 6.8$ Hz, 3H), 0.93 (d, $J = 6.6$ Hz, 3H), 1.14 (d, $J = 6.9$ Hz, 3H), 1.56–1.68 (m, 1H), 1.78–1.91 (m, 1H), 1.97–2.10 (m, 1H), 2.62–2.75 (m, 1H), 3.60–3.70 (m, 2H), 4.02–4.20 (m, 3H), 4.33 (d, $J = 14.9$ Hz, 1H), 4.40 (d, $J = 14.9$ Hz, 1H), 6.39–6.52 (m, 3H), 7.16–7.31 (m, 5H); ^{13}C NMR (75.5 MHz, $CD_3OD/CDCl_3$ (1:1, v/v)): δ 18.2, 18.3, 19.1, 30.8, 37.6, 38.1, 43.2, 59.2, 65.4, 68.8, 68.9, 96.6 (t, $J_{CF} = 26.1$ Hz), 98.5 (d, $J_{CF} = 28.6$ Hz, 2C), 127.3, 127.5, 128.5, 138.3, 160.6 (t, $J_{CF} = 13.7$ Hz), 163.8 (d, $J_{CF} = 264.1$ Hz), 164.0 (d, $J_{CF} = 264.1$ Hz), 172.3, 177.5. MS *m*/*z* 504.5 [$(M + H)^+$ calcd for $C_{25}H_{32}F_2N_5O_4^+$ 504.24].

4.2.20. (2S,4S,5S)-5-Azido-6-(3,5-difluoro-phenoxy)-4-hydroxy-2-methyl-hexanoic acid ((S)-1-benzylcarbamoyl-2-methyl-propyl)-amide (**21**-(*S*))

Compound **21**-(*S*) (93 mg, 62%) was synthesized from **15**-(*S*) (86 mg, 0.29 mmol) according to the method of the preparation of **19**. Compound **21**-(*S*) was collected as crystals after purification by flash column chromatography (toluene/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ (1:1, v/v)): δ 0.91 (d, $J = 6.9$ Hz, 3H), 0.92 (d, $J = 6.9$ Hz, 3H), 1.15 (d, $J = 6.9$ Hz, 3H), 1.49–1.60 (m, 1H), 1.82–1.96 (m, 1H), 2.05–2.19 (m, 1H), 2.50–2.64 (m, 1H), 3.61–3.70 (m, 1H), 3.70–3.79 (m, 1H), 3.99 (m, 3H), 4.35 (s, 2H), 6.38–6.51 (m, 3H), 7.15–7.32 (m, 5H); ^{13}C NMR (75.5 MHz, $CD_3OD/CDCl_3$ (1:1, v/v)): δ 17.7, 17.8, 19.1, 30.5, 37.7, 43.3, 58.9, 65.1, 69.1, 69.2, 96.7 (t, $J_{CF} = 26.1$ Hz), 98.6 (d, $J_{CF} = 28.9$ Hz, 2C), 127.3, 127.6, 128.6, 138.3, 160.6 (t, $J_{CF} = 13.5$ Hz), 163.8 (d, $J_{CF} = 246.3$ Hz), 164.0 (d, $J_{CF} = 246.3$ Hz), 172.4, 178.2. MS *m*/*z* 504.5 [$(M + H)^+$ calcd for $C_{25}H_{32}F_2N_5O_4^+$ 504.24].

4.2.21. (2R,4S,5S)-5-Amino-6-(3,5-difluoro-benzyloxy)-4-hydroxy-2-methyl-hexanoic acid ((S)-1-benzylcarbamoyl-2-methyl-propyl)-amide (**22**)

The azide **16** (42 mg, 0.08 mmol) and triphenyl phosphine (32 mg, 0.12 mmol) was dissolved in MeOH (4 mL). Three drops of water were added and the reaction mixture was stirred overnight at room temperature. Removal of the solvent and purification by LC/MS (50% MeOH, 6 min ramp time to 100% MeOH, 10 min) gave the amine **22** (34.8 mg, 87%) as white crystals. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88–1.01 (m, 6H), 1.15 (d, $J = 7.01$ Hz, 3H), 1.42–1.54 (m, 1H), 1.85–1.97 (m, 1H), 1.99–2.11 (m, 1H), 2.69–2.84 (m, 1H), 3.09–3.19 (m, 1H), 3.53–3.73 (m, 3H), 4.16 (d, $J = 7.76$ Hz, 1H), 4.37 (d, $J = 2.68$ Hz, 2H), 4.56 (s, 2H), 6.80–6.90 (m, 1H), 6.95–7.05 (m, 2H), 7.20–7.34 (m, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.9, 18.1, 18.6, 30.6, 37.1, 37.8, 42.8, 56.2, 59.4, 67.0, 68.4, 71.9, 102.5 (t, $J_{CF} = 26.1$ Hz), 110.1 (d, $J_{CF} = 25.5$ Hz, 2C), 127.0, 127.4, 128.3, 138.6, 142.6 (t, $J_{CF} = 8.9$ Hz), 163.3 (d, $J_{CF} = 247.4$ Hz), 163.5 (d, $J_{CF} = 247.4$ Hz), 172.4, 177.5. MS *m*/*z* 477.9 [$(M + H)^+$ calcd for $C_{25}H_{34}F_2N_3O_4^+$ 478.3].

4.2.22. (2R,4S,5S)-5-Amino-6-(3,5-difluoro-benzyloxy)-4-hydroxy-2-methyl-hexanoic acid cyclopropylamide (**23**)

Compound **23** (58 mg, 92%) was synthesized from **17** (69 mg, 0.19 mmol) according to the method of the preparation of **22**. After purification compound **23** was collected as a colourless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 0.99 (d, $J = 6.9$ Hz, 3H), 1.05–1.12 (m, 1H), 1.11 (s, 2H), 1.19 (s, 2H), 1.23–1.35 (m, 2H), 1.58–1.66 (m, 1H), 1.83–1.92 (m, 1H), 3.67–3.73 (m, 2H), 3.81–3.88 (m, 1H), 4.59 (s, 2H), 6.82–6.90 (m, 1H), 6.97–7.04 (m, 2H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 17.1, 24.8, 28.8, 37.5, 42.2, 56.9, 68.8, 69.3, 73.1, 73.9, 103.7 (t, $J_{CF} = 25.7$ Hz), 110.3 (d, $J_{CF} = 25.2$ Hz, 2C), 144.0 (t, $J_{CF} = 8.8$ Hz), 163.5 (d, $J_{CF} = 247.2$ Hz), 163.7 (d, $J_{CF} = 247.2$ Hz), 178.4.

4.2.23. (2R,4S,5S)-5-Amino-6-(3,5-difluoro-benzyloxy)-4-hydroxy-2-methyl-hexanoic acid 4-fluoro-benzylamide (**24**)

Compound **24** (96.5 mg, 62%) was synthesized from **18** (149 mg, 0.57 mmol) according to the method of the preparation of **22**. After purification compound **24** was collected as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (d, $J = 6.9$, 3H), 1.43–1.54 (m, 1H), 1.66–1.87 (m, 1H), 2.32–2.53 (m, 3H), 2.53–2.67 (m, 1H), 2.73–2.82 (m,1H), 3.30–3.42 (m, 2H), 3.48 (dd, $J = 4.1$, 9.4 Hz, 1H), 4.36 (t, $J = 5.4$ Hz, 1H), 4.44 (s, 2H), 6.41–6.50 (m, 1H), 6.66–6.75 (m, 1H), 6.77–6.86 (m, 2H), 6.88–7.02 (m, 2H), 7.14–7.31 (m, 2H). ^{13}C NMR (75.5 MHz, CDCl3): δ 18.6, 37.8, 39.1, 42.9, 55.4, 69.2, 72.3, 73.6, 103.2 (t, $J_{CF} = 25.4$ Hz, 1C), 110.1 (d, $J_{CF} = 25.1$, 2C), 115.6 (d, $J_{CF} = 21.5$ Hz, 2C), 129.6 (d, $J_{CF} = 8.2$ Hz, 2C), 134.6 (d, $J_{CF} = 3.3$ Hz, 1C), 142.3 (t, $J_{CF} = 8.8$ Hz, 1C), 162.3 (d, $J_{CF} = 246.2$ Hz, 1C), 163.2 (d, $J_{CF} = 248.9$ Hz, 1C), 163.4 (d, $J_{CF} = 248.9$ Hz, 1C), 176.6. MS *m*/*z* 411.1 [$(M + H)^+$ calcd for $C_{21}H_{26}F_3N_2O_3^+$ 411.2].

4.2.24. (2R,4S,5S)-5-Amino-6-benzyloxy-4-hydroxy-2-methyl-hexanoic acid ((S)-1-benzylcarbamoyl-2-methyl-propyl)-amide (**25**)

Compound **25** (35 mg, quant.) was synthesized from **19** (37 mg, 0.077 mmol) according to the method of the preparation of **22**. After purification compound **25** was collected as white powder. ^{1}H NMR (300 MHz, CD_3OD): δ 0.91 (d, $J = 6.8$ Hz, 3H), 0.93 (d, $J = 6.8$ Hz, 3H), 1.11 (d, $J = 7.0$ Hz, 3H), 1.48 (ddd, $J = 4.1$, 10.2, 13.9 Hz, 1H), 1.84 (ddd, $J = 3.3$, 10.3, 13.9 Hz, 1H), 1.98–2.10 (m, 1H), 2.63–2.81 (m, 2H), 3.43 (dd, $J = 6.3$, 9.4 Hz, 1H), 3.50–3.58 (m, 2H), 4.16 (d, $J = 7.8$ Hz, 1H), 4.34 (d, $J = 14.9$ Hz, 1H), 4.40 (d, $J = 14.9$ Hz, 1H),

4.50 (s, 2H), 7.23–7.34 (m, 10H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 19.0, 19.8, 31.8, 38.6, 39.3, 44.0, 56.4, 60.5, 70.6, 72.9, 74.3, 128.2, 128.6, 128.8, 128.9, 129.4, 129.5, 139.6, 139.8, 173.7, 179.1. MS *m*/*z* 456.4 [$(M+H)^+$ calcd for $C_{26}H_{37}N_3O_4^+$ 456.3].

4.2.25. (2R,4S,5S)-5-Amino-6-(3,5-difluoro-phenoxy)-4-hydroxy-2-methyl-hexanoic acid ((S)-1-benzylcarbamoyl-2-methyl-propyl)-amide (**26**-(R))

Compound **26**-(*R*) (77 mg, 82%) was synthesized from **20**-(*R*) (97 mg, 0.19 mmol) according to the method of the preparation of **22**, white powder after purification. ^{1}H NMR (300 MHz, CD_3OD): δ 0.93 (d, $J = 6.7$ Hz, 3H), 0.95 (d, $J = 6.7$ Hz, 3H), 1.14 (d, $J = 7.14$ Hz, 3H), 1.51–1.63 (m, 1H), 1.82–1.93 (m, 1H), 1.98–2.12 (m, 1H), 2.66–2.79 (m, 1H), 2.90–2.98 (m, 1H), 3.58–3.65 (m, 1H), 3.85 (dd, $J = 6.3$, 9.3 Hz, 1H), 3.99 (dd, $J = 5.8$, 9.3 Hz, 1H), 4.19 (d, $J = 7.7$ Hz, 1H), 4.37 (d, $J = 2.48$ Hz, 2H), 6.44–6.60 (m, 3H), 7.16–7.32 (m, 5H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 17.8, 18.7, 30.7, 37.5, 38.0, 42.9, 54.5, 59.3, 69.0, 70.4, 95.8 (t, $J_{CF} = 26.3$ Hz), 98.3 (d, $J_{CF} = 28.9$ Hz, 2C), 127.0, 127.4, 128.3, 138.6, 161.3 (t, $J_{CF} = 13.7$ Hz), 163.9 (d, $J_{CF} = 244.8$ Hz), 164.1 (d, $J_{CF} = 244.8$ Hz), 172.5, 177.9. MS *m*/*z* 477.9 [$(M+H)^+$ calcd for $C_{25}H_{34}F_2N_3O_4^+$ 478.25].

4.2.26. (2S,4S,5S)-5-Amino-6-(3,5-difluoro-phenoxy)-4-hydroxy-2-methyl-hexanoic acid ((S)-1-benzylcarbamoyl-2-methyl-propyl)-amide (**27**-(S))

Compound **27**-(*S*) (66 mg, 99%) was synthesized from **21**-(*S*) (70 mg, 0.14 mmol) according to the method of the preparation of **22**, white powder after purification. ^{1}H NMR (300 MHz, CD_3OD): δ 0.94 (d, $J = 6.9$ Hz, 6H), 1.16 (d, $J = 6.9$ Hz, 3H), 1.52–1.63 (m, 1H), 1.80–1.92 (m, 1H), 2.07–2.21 (m, 1H), 2.59–2.71 (m, 1H), 2.93–3.01 (m, 1H), 3.67–3.75 (m, 1H), 3.84 (dd, $J = 9.1$, 6.6 Hz, 1H), 3.96 (dd, $J = 9.1$, 5.2 Hz, 1H), 4.20 (d, $J = 6.9$ Hz, 1H), 4.36 (s, 2H), 6.44–6.60 (m, 3H), 7.16–7.32 (m, 5H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 17.3, 18.6, 30.4, 37.6, 37.8, 42.9, 54.5, 59.0, 69.6, 70.5, 95.8 (t, $J_{CF} = 26.6$ Hz), 98.3 (d, $J_{CF} = 29.2$ Hz, 2C), 127.0, 127.4, 128.3, 138.3, 161.4 (t, $J_{CF} = 13.7$ Hz), 163.9 (d, $J_{CF} = 244.7$ Hz), 164.1 (d, $J_{CF} = 244.7$ Hz), 172.6, 178.7. MS *m*/*z* 477.9 [$(M+H)^+$ calcd for $C_{25}H_{34}F_2N_3O_4^+$ 478.25].

4.2.27. N-[(1S,2S,4R)-4-((S)-1-Benzylcarbamoyl-2-methyl-propylcarbamoyl)-1-(3,5-difluoro-benzyloxymethyl)-2-hydroxy-pentyl]-5-(methanesulfonyl-methyl-amino)-N′-((R)-1-phenyl-ethyl)-isophthalamide (**28**)

The amine **22** (17 mg, 0.034 mmol) was dissolved in DMF (2 mL) and cooled to 0 °C. 5-(Methanesulfonyl-methyl-amino)-*N*-((*R*)-1-phenyl-ethyl)-isophthalamide (**D**) (25 mg, 0.064 mmol), diisopropylethylamine (12 μL, 0.067 mmol) and *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (25 mg, 0.067 mmol) were added and the reaction was stirred at room temperature overnight. The crude product was purified with LC/MS (50% MeOH, 15 min ramp time to 100% MeOH, 10 min) to give **28** (10 mg, 35%) as white crystals after lyophilization. ^{1}H NMR (300 MHz, CD_3OD): δ 0.86 (d, $J = 6.8$ Hz, 3H), 0.87 (d, $J = 6.8$ Hz, 3H), 1.14 (d, $J = 6.9$ Hz, 3H), 1.48–1.57 (m, 1H), 1.58 (d, $J = 7.0$ Hz, 3H), 1.84–2.05 (m, 2H), 2.70–2.81 (m, 1H), 2.95 (s, 3H), 3.36 (s, 3H), 3.68 (dd, $J = 7.0$, 9.8 Hz, 1H), 3.77 (dd, $J = 6.3$, 9.8 Hz, 1H), 3.85–3.91 (m, 1H), 4.14 (d, $J = 7.7$ Hz, 1H), 4.32–4.40 (m, 1H), 4.33 (d, $J = 15.0$ Hz, 1H), 4.39 (d, $J = 15.0$ Hz, 1H), 4.56 (s, 2H), 5.25 (q, $J = 7.1$ Hz, 1H), 6.76–6.84 (m, 1H), 6.91–6.96 (m, 2H), 7.19–7.42 (m, 10H), 8.02–8.07 (m, 2H), 8.27 (t, $J = 1.58$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.6, 18.3, 19.3, 21.7, 30.8, 35.5, 37.7, 37.9, 38.5, 43.5, 49.7, 52.9, 59.0, 69.7, 71.2, 72.3, 103.2 (t, $J_{CF} = 25.2$ Hz), 110.0 (d, $J_{CF} = 24.9$ Hz, 2C), 124.2, 126.2, 127.5, 127.5, 127.6, 127.8, 128.0, 128.6, 128.7, 135.3, 135.9, 137.8, 141.6 (t, $J_{CF} = 8.6$ Hz), 142.3, 142.9, 163.0 (d, $J_{CF} = 249.4$ Hz), 163.2 (d, $J_{CF} = 249.4$ Hz), 164.6, 166.0, 171.2, 176.9. HRMS *m*/*z* 850.3675 [$(M+H)^+$ calcd for $C_{44}H_{54}F_2N_5O_8S^+$ 850.3656]. Anal. ($C_{44}H_{53}F_2N_5O_8S$) C, H, N.

4.2.28. ((S)-1-[(S)-1-[(1S,2S,4R)-4-((S)-1-Benzylcarbamoyl-2-methyl-propylcarbamoyl)-1-(3,5-difluoro-benzyloxymethyl)-2-hydroxy-pentylcarbamoyl]-3-methylsulfanyl-propylcarbamoyl]-2-methyl-propyl)-carbamic acid tert-butyl ester (**29**)

Compound **29** (27 mg, 71%) was synthesized from **22** (25 mg, 0.051 mmol) according to the method of the preparation of **28** using (*S*)-2-((*S*)-2-*tert*-butoxycarbonylamino-3-methyl-butyrylamino)-4-methylsulfanyl-butyric acid (**E**) instead of 5-(methanesulfonyl-methyl-amino)-*N*-((*R*)-1-phenyl-ethyl)-isophthalamide (**D**). Compound **29** was collected as white powder after lyophilization. ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ (2:1, v/v)): 0.88 (d, $J = 6.8$ Hz, 3H), 0.89 (d, $J = 6.8$ Hz, 6H), 0.90 (d, $J = 6.8$ Hz, 3H), 1.10 (d, $J = 6.9$ Hz, 3H), 1.42 (s, 9H), 1.49 (dq, $J = 4.8$, 13.9 Hz, 1H), 1.71 (ddd, $J = 3.8$, 5.8, 13.9 Hz, 1H), 1.91–2.10 (m, 4H), 2.05 (s, 3H), 2.47–2.54 (m, 2H), 2.55–2.67 (m, 1H), 3.50 (dd, $J = 6.0$, 9.6 Hz, 1H), 3.56 (dd, $J = 6.6$, 9.6 Hz, 1H), 3.76–3.83 (m, 1H), 3.83 (d, $J = 6.6$ Hz, 1H), 3.95–4.04 (m, 1H), 4.12 (d, $J = 7.8$ Hz, 1H), 4.32 (d, $J = 14.9$ Hz, 1H), 4.40 (d, $J = 14.9$ Hz, 1H), 4.43 (d, $J = 12.4$ Hz, 1H), 4.48 (d, $J = 12.4$ Hz, 1H), 4.48–4.53 (m, 1H), 6.68–6.74 (m, 1H), 6.82–6.86 (m, 2H), 7.17–7.31 (m, 5H); ^{13}C NMR (75.5 MHz, $CD_3OD/CDCl_3$ (2:1, v/v)): δ 15.3, 18.1, 18.2, 18.7, 19.5, 28.5, 30.5, 31.2, 31.6, 37.8, 38.7, 43.7, 53.2, 53.5, 59.5, 60.8, 68.1, 70.4, 72.3, 80.6, 103.1 (t, $J_{CF} = 25.5$ Hz), 110.3 (d, $J_{CF} = 25.2$ Hz, 2C), 127.7, 128.0, 129.0, 138.5, 142.9 (t, $J_{CF} = 8.9$ Hz), 157.1, 161.9 (d, $J_{CF} = 248.2$ Hz), 165.2 (d, $J_{CF} = 247.9$ Hz), 172.5, 173.4, 177.8. HRMS *m*/*z* 822.4293 [$(M+H)^+$ calcd for $C_{41}H_{62}F_2N_5O_8S^+$ 822.4282].

4.2.29. N-[(1S,2S,4R)-4-((S)-1-Benzylcarbamoyl-2-methyl-propylcarbamoyl)-1-(3,5-difluoro-benzyloxymethyl)-2-hydroxy-pentyl]-5-(methanesulfonyl-methyl-amino)-N′-methyl-isophthalamide (**30**)

Compound **30** (18 mg, 52%) was synthesized from **22** (25 mg, 0.051 mmol) according to the method of the preparation of **28** using 5-(methanesulfonyl-methyl-amino)-*N*-methyl-isophthalamic acid (**F**) instead of 5-(methanesulfonyl-methyl-amino)-*N*-((*R*)-1-phenyl-ethyl)-isophthalamide (**D**). Compound **30** was collected as white powder after lyophilization. ^{1}H NMR (300 MHz, CD_3OD): δ 0.87 (d, $J = 6.8$ Hz, 6H), 1.14 (d, $J = 6.9$ Hz, 3H), 1.48–1.58 (m, 1H), 1.83–1.92 (m, 1H), 1.93–2.05 (m, 1H), 2.72–2.80 (m, 1H), 2.92 (s, 3H), 2.95 (s, 3H), 3.37 (s, 3H), 3.69 (dd, $J = 7.0$, 9.8 Hz, 1H), 3.78 (dd, $J = 6.3$, 9.8 Hz, 1H), 3.85–3.92 (m, 1H), 4.14 (d, $J = 7.7$ Hz, 1H), 4.33 (d, $J = 15.0$ Hz, 1H), 4.41 (d, $J = 15.0$ Hz, 1H), 4.34–4.45 (m, 1H), 4.56 (s, 2H), 6.76–6.84 (m, 1H), 6.92–6.97 (m, 2H), 7.21–7.32 (m, 5H), 8.02–8.05 (m, 2H), 8.23–8.25 (m, 1H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 18.5, 19.0, 19.8, 27.0, 31.9, 35.9, 38.4, 39.5, 40.4, 44.0, 55.0, 60.4, 69.5, 70.7, 72.6, 103.4 (t, $J_{CF} = 25.8$ Hz), 111.0 (d, $J_{CF} = 25.5$ Hz, 2C), 125.9, 128.2, 128.6, 129.1, 129.5, 137.1, 137.2, 139.8, 143.8, 144.5 (t, $J_{CF} = 9.0$ Hz), 162.9 (d, $J_{CF} = 247.3$ Hz), 166.1 (d, $J_{CF} = 247.1$ Hz), 168.9, 173.7, 180.0. HRMS *m*/*z* 760.3196 [$(M+H)^+$ calcd for $C_{37}H_{48}F_2N_5O_8S^+$ 760.3186].

4.2.30. N-((1S,2S,4R)-1-Benzyloxymethyl-4-cyclopropylcarbamoyl-2-hydroxy-pentyl)-5-(methanesulfonyl-methyl-amino)-N′-((R)-1-phenyl-ethyl)-isophthalamide (**31**)

Compound **31** (13 mg, 17%) was synthesized from **23** (36 mg, 0.105 mmol) according to the method of the preparation of **28**. Compound **31** was collected as white powder after lyophilization. ^{1}H NMR (300 MHz, CD_3OD): δ 0.99 (d, $J = 6.9$ Hz, 3H), 1.11 (d, $J = 11.1$ Hz, 4H), 1.23–1.33 (m, 2H), 1.58 (d, $J = 7.1$ Hz, 3H), 1.59–1.68 (m, 1H), 1.83–1.92 (m, 1H), 2.96 (s, 3H), 3.37 (s, 3H), 3.68 (dd, $J = 6.8$, 9.6 Hz, 1H), 3.77 (dd, $J = 6.7$, 9.6 Hz, 1H), 4.01–4.09 (m, 1H), 4.41 (dt, $J = 2.4$, 6.6 Hz, 1H), 4.55 (d, $J = 12.9$ Hz, 1H), 4.61 (d, $J = 12.9$ Hz, 1H), 5.25 (q, $J = 7.1$ Hz, 1H), 6.77–6.82 (m, 1H), 6.90–6.99 (m, 2H), 7.22–7.44 (m, 5H), 8.02 (d, $J = 1.6$ Hz, 2H), 8.21 (t, $J = 1.6$ Hz, 1H); ^{13}C NMR

(75.5 MHz, CD_3OD): δ 15.7, 22.1, 26.3, 27.4, 35.9, 37.4, 38.3, 42.1, 50.9, 53.6, 70.3, 71.3, 72.6, 73.8, 103.4 (t, J_{CF} = 25.8 Hz), 110.9 (d, J_{CF} = 25.2 Hz, 2C), 125.8, 127.3, 128.2, 129.2, 129.3, 129.6, 137.2, 137.5, 143.8, 144.6, 145.0, 164.4 (d, J_{CF} = 247.5 Hz), 164.6 (d, J_{CF} = 247.5 Hz), 167.8, 167.9, 168.9. HRMS *m/z* 676.2863 [$(M+H)^+$ calcd for $C_{37}H_{48}F_2N_5O_8S^+$ 676.2842].

4.2.31. *N-[(1S,2S,4R)-1-(3,5-Difluoro-benzyloxymethyl)-4-(4-fluoro-benzylcarbamoyl)-2-hydroxy-pentyl]-5-(methanesulfonyl-methyl-amino)-N′-((R)-1-phenyl-ethyl)-isophthalamide (**32**)*

Compound **32** (9.4 mg, 18%) was synthesized from **24** (27.9 mg, 0.068 mmol) according to the method of the preparation of **28**. Compound **32** was collected as white powder after lyophilization. ^{1}H NMR (300 MHz, CD_3OD): δ 1.17 (d, *J* = 7.1 Hz, 3H), 1.21–1.54 (m, 3H), 1.58 (d, *J* = 6.9 Hz, 3H), 1.86–1.99 (m, 1H), 2.65 (s, 3H), 2.95 (s, 3H), 3.61–3.86 (m, 4H), 4.19–4.40 (m, 2H), 4.49–4.62 (m, 2H), 5.19–5.32 (m, 1H), 6.74–6.86 (m, 1H), 6.87–7.04 (m, 4H), 7.06–7.50 (m, 10H), 7.95–8.09 (m, 2H), 8.20–8.23 (m, 1H). ^{13}C NMR (75.5 MHz, CD_3OD): δ 17.8, 20.9, 34.7, 37.1, 37.6, 38.0, 42.1, 49.8, 54.2, 68.5, 69.5, 71.5, 102.3 (t, J_{CF} = 28.8 Hz, 1C), 109.8 (d, J_{CF} = 25.5 Hz, 2C), 114.9 (d, J_{CF} = 21.8 Hz, 2C), 124.7, 126.1, 127.0, 128.0, 128.1, 128.4, 129.1 (d, J_{CF} = 8.0 Hz, 2C), 135.0, 136.1, 142.6, 143.3 (t, J_{CF} = 9.2 Hz, 1C), 143.8, 162.1 (d, J_{CF} = 243.9, 1C), 163.2 (d, J_{CF} = 247.1, 1C), 163.4 (d, J_{CF} = 247.1, 1C), 166.5, 167.8, 177.7. HRMS *m/z* 769.2868 [$(M+H)^+$ calcd for $C_{39}H_{44}F_3N_4O_7S^+$ 769.2877].

4.2.32. *N-[(1S,2S,4R)-4-((S)-1-Benzylcarbamoyl-2-methyl-propylcarbamoyl)-1-benzyloxymethyl-2-hydroxy-pentyl]-5-(methanesulfonyl-methyl-amino)-N′-((R)-1-phenyl-ethyl)-isophthalamide (**33**)*

Compound **33** (27 mg, 77%) was synthesized from **25** (20 mg, 0.044 mmol) according to the method of the preparation of **28**. Compound **33** was collected as white powder after lyophilization. ^{1}H NMR (300 MHz, CD_3OD): δ 0.92 (d, 6.8 Hz, 3H), 0.93 (d, 6.8 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.51–1.62 (m, 1H), 1.61 (d, *J* = 7.1 Hz, 3H), 1.89–1.99 (m, 1H), 2.00–2.10 (m, 1H), 2.74–2.84 (m, 1H), 3.00 (s, 3H), 3.40 (s, 3H), 3.69–3.82 (m, 2H), 3.90–3.97 (m, 1H), 4.19 (d, *J* = 7.7 Hz, 1H), 4.38 (d, *J* = 15.0 Hz, 1H), 4.38–4.44 (m, 1H), 4.44 (d, *J* = 15.0 Hz, 1H), 4.57 (d, *J* = 11.9 Hz, 1H), 4.62 (d, *J* = 11.9 Hz, 1H), 5.30 (q, *J* = 7.0 Hz, 1H), 7.25–7.48 (m, 15H), 8.07–8.11 (m, 2H), 8.31 (t, *J* = 1.58 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 18.4, 19.0, 19.8, 22.1, 31.9, 36.0, 38.4, 38.5, 39.5, 44.0, 50.9, 55.0, 60.4, 69.7, 70.4, 74.1, 126.0, 127.3, 128.2, 128.6, 128.7, 128.9, 129.4, 129.5, 129.6, 137.2, 137.4, 139.5, 139.8, 143.8, 145.0, 167.7, 168.9, 173.7, 179.0. HRMS *m/z* 814.3847 [$(M+H)^+$ calcd for $C_{44}H_{56}N_5O_8S^+$ 814.3844]. Anal. ($C_{43}H_{55}N_5O_8S$) C, H, N.

4.2.33. *N-[(1S,2S,4R)-4-((S)-1-Benzylcarbamoyl-2-methyl-propylcarbamoyl)-1-(3,5-difluoro-phenoxymethyl)-2-hydroxy-pentyl]-5-(methanesulfonyl-methyl-amino)-N′-((R)-1-phenyl-ethyl)-isophthalamide (**34**-(*R*))*

Compound **34**-(*R*) (34 mg, 74%) was synthesized from **26**-(*R*) (26 mg, 0.055 mmol) according to the method of the preparation of **28**. White powder after lyophilization. ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$ (1:1, v/v)): δ 0.84 (d, *J* = 6.9 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 1.56 (d, *J* = 7.2 Hz, 3H), 1.50–1.64 (m, 1H), 1.77–1.88 (m, 1H), 1.90–2.03 (m, 1H), 2.62–2.75 (m, 1H), 2.92 (s, 3H), 3.33 (s, 3H), 3.89–3.97 (m, 1H), 4.05–4.20 (m, 3H), 4.29–4.37 (m, 2H), 4.40–4.45 (m, 1H), 5.25 (q, *J* = 6.9 Hz, 1H), 6.34–6.51 (m, 3H), 7.16–7.39 (m, 10H), 7.99 (t, *J* = 1.6 Hz, 1H) 8.01 (t, *J* = 1.6 Hz, 1H), 8.26 (t, *J* = 1.6 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD/$CDCl_3$ (1:1, v/v)): δ 17.7, 18.2, 19.1, 21.4, 31.0, 35.6, 37.6, 37.9, 38.5, 43.3, 49.8, 53.4, 59.1, 67.4, 67.7, 96.5 (t, J_{CF} = 26.1 Hz), 98.5 (d, J_{CF} = 28.9 Hz, 2C), 125.0, 126.3, 127.3, 127.4, 127.6, 128.5, 128.6, 128.7, 135.6, 136.3, 138.2, 142.4, 143.5, 160.8 (t, J_{CF} = 14.0 Hz), 163.8 (d, J_{CF} = 245.9 Hz), 164.0 (d, J_{CF} = 245.9 Hz), 166.0, 167.3, 172.2, 177.5. HRMS *m/z* 836.3476 [$(M+H)^+$ calcd for $C_{43}H_{52}F_2N_5O_8S^+$ 836.3499]. Anal. ($C_{43}H_{51}F_2N_5O_8S\cdot\frac{1}{2}H_2O$) C, H, N: calcd, 8.29; found, 7.36.

4.2.34. *N-[(1S,2S,4S)-4-((S)-1-Benzylcarbamoyl-2-methyl-propylcarbamoyl)-1-(3,5-difluoro-phenoxymethyl)-2-hydroxy-pentyl]-5-(methanesulfonyl-methyl-amino)-N′-((R)-1-phenyl-ethyl)-isophthalamide (**35**-(*S*))*

Compound **35**-(*S*) (28 mg, 64%) was synthesized from **27**-(*S*) (25 mg, 0.052 mmol) according to the method of the preparation of **28**. White powder after lyophilization. ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$ (1:1, v/v)): δ 0.92 (d, *J* = 6.8 Hz, 6H), 1.14 (d, *J* = 6.9 Hz, 3H), 1.48–1.56 (m, 1H), 1.58 (d, *J* = 7.0 Hz, 3H), 1.85–1.97 (m, 1H), 2.05–2.19 (m, 1H), 2.55–2.67 (m, 1H), 2.93 (s, 3H), 3.33 (s, 3H), 3.96–4.03 (m, 1H), 4.05–4.12 (m, 2H), 4.13–4.22 (m, 1H), 4.28–4.32 (m, 2H), 4.40–4.47 (m, 1H), 5.25 (q, *J* = 7.0 Hz, 1H), 6.36–6.55 (m, 3H), 7.11–7.41 (m, 10H), 7.98–8.01 (m, 2H), 8.23 (t, *J* = 1.58 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD/$CDCl_3$ (1:1, v/v)): δ 17.7, 18.2, 19.0, 21.3, 30.5, 35.4, 37.7, 38.0, 38.1, 43.2, 49.8, 53.6, 58.9, 67.6, 68.3, 96.4 (t, J_{CF} = 26.1 Hz), 98.5 (d, J_{CF} = 28.9 Hz, 2C), 124.8, 126.2, 127.2, 127.3, 127.5, 128.4, 128.5, 128.6, 128.7, 135.7, 136.3, 138.3, 142.4, 143.6, 160.9 (t, J_{CF} = 13.6 Hz), 163.8 (d, J_{CF} = 245.9 Hz), 164.0 (d, J_{CF} = 245.9 Hz), 165.6, 166.2, 167.6, 178.4. HRMS *m/z* 836.3502 [$(M+H)^+$ calcd for $C_{43}H_{52}F_2N_5O_8S^+$ 836.3499]. Anal. ($C_{43}H_{51}F_2N_5O_8S\cdot H_2O$) C, H, N: calcd, 8.20; found, 7.58.

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Appendix. Supplementary data

Experimental details and spectroscopic data for the compound **A** and **D**. ^{1}H and 2D-NMR (COSY and NOESY) on compound **7**, **14**-(*R*) and **15**-(*S*). MS, HPLC purity on all the final compounds **29**–**32**, together with combustion analysis of the final compounds **28**, **34**-(*R*), **35**-(*S*) and **33**. This material is available at Science Direct. Supplementary data associated with this article can be found in online version at doi:10.1016/j.ejmech.2009.11.013.

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