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Original article

# Design and synthesis of novel P2 substituents in diol-based HIV protease inhibitors

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ABSTRACT

The synthesis and SAR of HIV-1 protease inhibitors containing novel P2 structural elements are presented. The inhibitors were designed having hydrogen bond accepting P2 substituents to probe potential favorable interactions to Asp-29/Asp-30 of the HIV-1 protease backbone utilizing inhibitor **3** as a model template. Several inhibitors were synthesized from an L-Val methyl amide P2 motif by appending hydrogen bonding moieties from either the isopropyl side-chain or from the methyl amide portion. The most promising inhibitors **4a** and **4e** displayed $K_i$ values of 1.0 nM and 0.7 nM respectively and $EC_{50}$ values in the MT4 cell-based assay of 0.17 μM and 0.33 μM respectively, a slight loss in potency compared to lead inhibitor **3**. These inhibitors were also tested against an HIV protease inhibitor resistant strain carrying the M46I, V82F, and I84V mutations. Inhibitors **4a** and **4e** displayed a 3 and 4 fold change respectively compared with HIV wild type, whereas lead inhibitor **3** showed a higher 9 fold change. This study further demonstrate the chemical tractability of the approach where various P2 substituents can be introduced in just one chemical step from lactone **21** enabling facile modifications of the overall properties in this inhibitor class.



## 1. Introduction

Over two decades ago the human immunodeficiency virus (HIV) was identified as the etiologic agent of AIDS [1,2] and in 2008 it was estimated by UNAIDS [3] that 33 million people were afflicted by this disease. The HIV-1 genome encodes an aspartic protease [4,5] (HIV-1 PR) that is essential for the production of structural and functional viral proteins by processing of the *gag*- and *gag-pol* viral gene products [4,6]. Inhibition of the HIV-1 PR leads to the production of immature virus particles [7,8] and in clinical practice HIV-1 PR inhibitors has demonstrated high efficacy which has led to the development of efficacious treatment regimens.

Today ten HIV-1 protease inhibitors are approved by the FDA; Saquinavir, [9] Ritonavir, [10] Indinavir, [11,12] Nelfinavir, [13] Amprenavir, [14] Fosamprenavir, [15] Lopinavir, [16] Atazanavir, [17] Tiprinavir [18] and Darunavir [19] (compound **1**, Fig. 1). HIV-1 PR inhibitors in combination with nucleoside analogues, which targets the HIV-1 reverse transcriptase (RT), has emerged as one of the two major Highly Active Antiretroviral Therapy, HAART, treatment regimens [20,21].

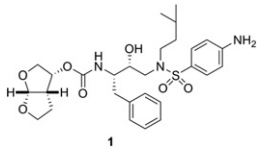


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**Fig. 1.** Compound **1**, Darunavir.

Current drug discovery efforts are now focusing on once daily HIV-1 PR inhibitors displaying improved efficacy, minor cross resistance towards clinical mutant resistant strains, high genetic barrier, low drug–drug interactions and an advantageous side effect profile. Cost is also a major issue with current HIV therapies and globally only ~20% of the HIV infected population is receiving adequate treatment [3].

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We have previously reported on the discovery of novel potent HIV–1 PR inhibitors, i.e. **2** [22] and **3** [23] (Fig. 2), displaying a $C_2$-symmetric diol-type central scaffold carrying P1/P1′ benzyloxy residues. In the $C_2$-symmetrical inhibitor **2** there are two amino-indanol P2/P2′ residues whereas in inhibitor **3** one of the amino-indanols has been replaced by L-Val methyl amide.

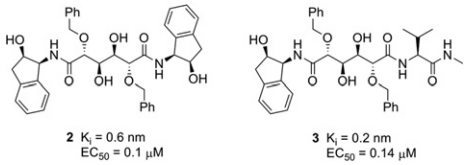



**Fig. 2.** Lead inhibitors **2** and **3**.

We now report on the synthesis and anti-HIV activities of a series of L-Val methyl amide analogues as P2 residues in inhibitor structure **3**, (i.e. **4a**–**h**, Fig. 3) containing potentially hydrogen interacting or accepting elements such as fluorine, methoxy groups and oxetane, with the aim to form positive interactions between these P2 residues and the N–H moiety in Asp-29 or Asp-30 of the HIV-1 protease backbone. The most promising inhibitor structures **4a** and **4e** displayed $K_i$ values of 1.0 nM and 0.7 nM, respectively and $EC_{50}$ values in the MT4 cell-based assay of 0.17 μM and 0.33 μM, respectively, a slight loss in potency compared to lead inhibitor **3**. Interestingly, these inhibitors displayed a 3 and 4 fold change in $EC_{50}$ value, respectively, over HIV wild type when tested against an HIV PI multiple resistant strain, whereas inhibitor **3** showed a higher 9 fold change.

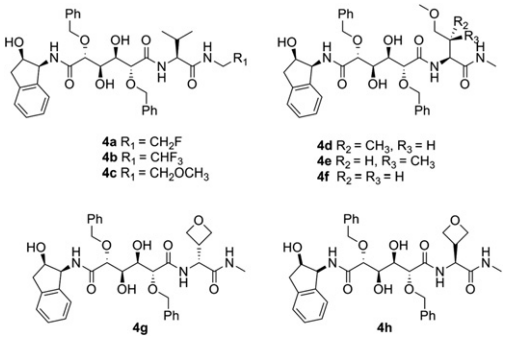
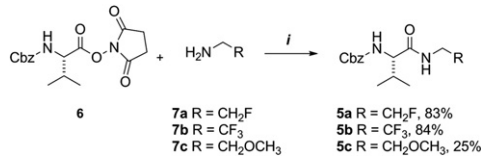



**Fig. 3.** Target structures **4a**–**h**.

## 2. Results and discussion

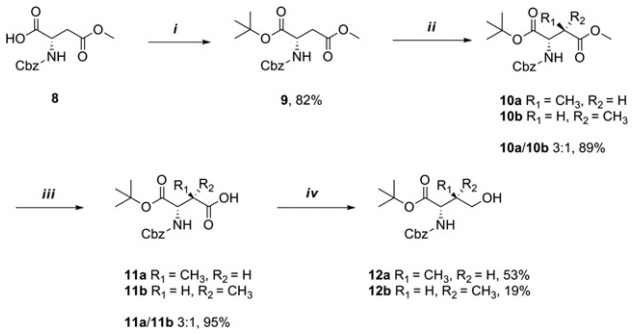
### 2.1. Chemistry

For the synthesis of the Cbz protected amines **5a**–**c** (Scheme 1) commercially available Cbz-L-Val-O-succinimide **6** was coupled with amines **7a**–**c** to generate **5a**–**c** [24] in 25–84% yield [22].



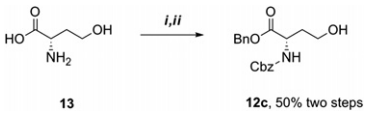

**Scheme 1.** Reagents and conditions: i: NMM, **7a**, **b** or **c**, THF.

Commercially available Cbz-L-Asp-OMe **8** was converted into its corresponding β-*tert*-butyl ester (Scheme 2) using isobutylene and a catalytic amount of $H_2SO_4$ [25] furnishing **9** [26] in 82% yield. Compound **9** was methylated [27,28] using LiHMDS and iodomethane to generate **10a**/**10b** [29] as an inseparable mixture of diastereomers in a 3:1 ratio in 89% yield. Selective hydrolysis [25] with NaOH (1 M) rendered the acids **11a**/**11b** as an inseparable mixture in 95% yield. The carboxylic acids **11a** and **11b** were activated with ethyl chloroformate and then reduced with sodium borohydride to generate the alcohols **12a** [30] and **12b** [30] that were easily separated by column chromatography in 53% and 19% yield, respectively.



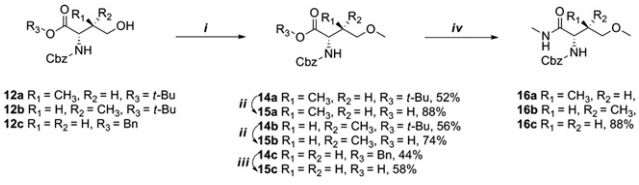

**Scheme 2.** Reagents and conditions: i: Isobutylene, $H_2SO_4$ (cat), $CH_2Cl_2$; ii: HMDS, BuLi, MeI, THF, 0 °C; iii: NaOH 1 M, MeOH; iv: a) Ethyl chloroformate, NMM, THF; b) $NaBH_4$, $H_2O$.

Commercially available L-homoserine **13** (Scheme 3) was Cbz and Bn protected [31] using $NaHCO_3$ and benzyl chloroformate, followed by treatment with NaOH and benzyl bromide to generate compound **12c** in 50% yield over two steps.




**Scheme 3.** Reagents and conditions: i: benzyl chloroformate, $NaHCO_3$; ii: NaOH, benzyl bromide.

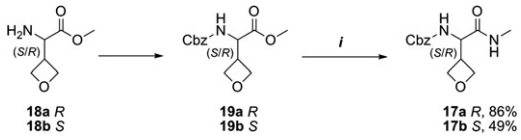
The free hydroxyl groups of **12a**–**c** were converted into the corresponding methyl ethers **14a**–**c** (Scheme 4) in 44–56% yield using methyl triflate [32] and 2,6-di-*tert*-butyl-4-methylpyridine.




**Scheme 4.** Reagents and conditions: i: MeOTf, 2,6-di-*tert*-butyl-4-methylpyridine, $CH_2Cl_2$; ii: TFA, TES, $CH_2Cl_2$; iii: NaOH (aq), THF/MeOH (13:10); iv: PyBOP, DIPEA, $MeNH_2$, $CH_2Cl_2$.

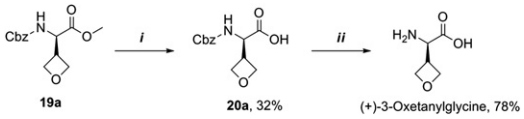
The *tert*-butyl esters **14a** and **14b** were cleaved using TFA [33] to render **15a** and **15b** in 88% and 74% yield respectively. The benzyl ester of **14c** was cleaved [34] with NaOH (0.59 M) to generate **14c** in 58% yield. The methylamides **16a**–**c** were obtained in 62–88% yield from **15a**–**c** using PyBOP [35], DIPEA and methylamine.

For the synthesis of the Cbz protected amines **17a** and **17b** (Scheme 5) a racemic mixture of oxetanylglycine methyl ester **18a**/**18b** [36] was Cbz protected and separated by HPLC to generate the protected amines **19a** and **19b**. The methyl ester was converted to the corresponding methyl amide using methylamine in ethanol (33%) to generate **17a** and **17b** in 86% and 49% yield respectively.



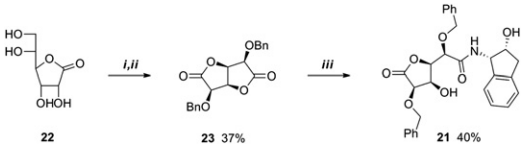

**Scheme 5.** Reagents and conditions: i: $MeNH_2$ 33% in ethanol.

In order to confirm the stereochemistry of **17a**, the methyl ester of **19a** was cleaved to generate the acid **20a** in 32% yield (Scheme 6). The Cbz-group was removed by hydrogenolysis over a catalytic amount of palladium on charcoal to yield (+)-3-oxetanylglycine in 78% yield, measurement of the optical rotation confirmed the stereochemistry [36].



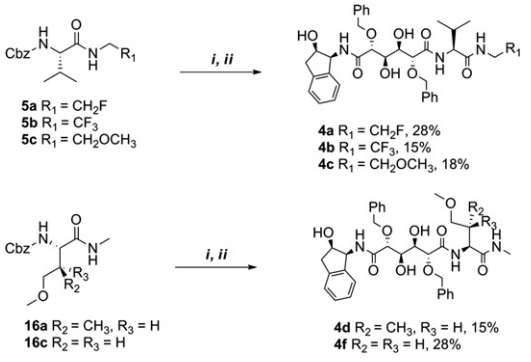

**Scheme 6.** Reagents and conditions: i: NaOH (1 M), MeOH; ii: $H_2$, Pd/C, MeOH.

The monolactone **21** (Scheme 7) was synthesized in three steps from L-mannaric acid γ-lactone **22** according to the published method [37].



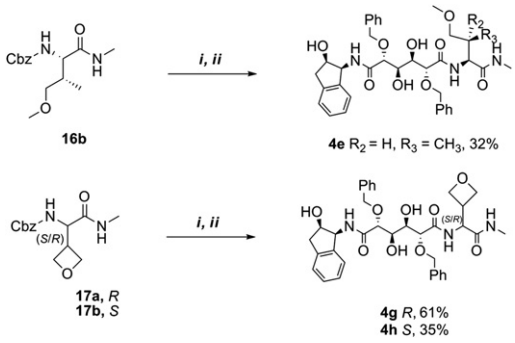

**Scheme 7.** Reagents and conditions: i: $HNO_3$, $H_2O$; ii: benzyl-2,2,2-trichloroacetimidate, TfOH, 1,4-dioxane; iii: (1*S*,2*R*)-1-amino-2-indanol, 2-hydroxypyridine, $CH_2Cl_2$.

The Cbz protection group of **5a**–**c**, **16a**–**c** and **17a** and **17b** was removed by hydrogenolysis over a catalytic amount of palladium on charcoal (Schemes 8 and 9), delivering the corresponding amines which were used without further purification in the subsequent step. The amines of **5a**–**c**, **16a** and **16c** were coupled with the lactone **21** in refluxing 1,2-dichloroethane to give the products **4a**–**d** and **4f** in 15–28% yield (Scheme 8).



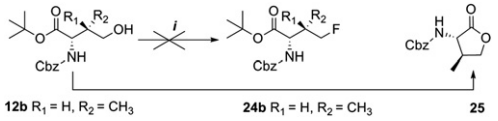

**Scheme 8.** Reagents and conditions: i: $H_2$, Pd/C, MeOH; ii: **21**, 1,2-dichloroethane, reflux, 16 h.

Due to the low yields obtained a modified method using 2 equiv of 2-hydroxypyridine and DIPEA as solvent was used to open lactone **21** with the amines of **16b**, **17a** and **17b** (Scheme 9) furnishing products **4e**, **4g** and **4h** in improved 32–65% yield.



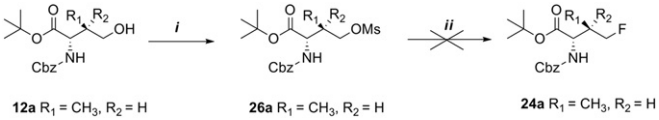

**Scheme 9.** Reagents and conditions: i: $H_2$, Pd/C, MeOH; ii: **21**, DIPEA, 2-hydroxypyridine, 70°, 16 h.

Based on the promising results obtained from inhibitor **4e**, the synthesis of γ-fluorinated amino acid analogues from amino acids **12b** (Scheme 10) were attempted. A range of fluorination reagents were examined e.g. DAST [38] Deoxofluor [39] and perfluoro-1-butanesulfonyl fluoride [40] (PBSF) together with triethylamine trihydrofluoride (TREAT) and TEA without success with only the lactonized by-product **25b** [41] isolated.




**Scheme 10.** Reagents and conditions: i: DAST, Deoxofluor or PBSF, TEA and TREAT, DCM or THF.

**12a** was converted into its corresponding mesylate [42] **26a** (Scheme 11), which was reacted with e.g. TBAF, CsF and KF with 18-crown-6 in aprotic solvents without providing the desired product.




**Scheme 11.** Reagents and conditions: i: MsCl, TEA, DMAP, 0 °C, ii: TBAF, CsF or KF and 18-crown-6.

### 2.2. Structure activity relationships

The well known amino-indanol moiety is a remarkably efficient P2/P2′ substituent [12,22,43] very neatly filling out the S2/S2′ pockets and forming a hydrogen bond to the backbone of Asp-29/129 in the HIV–PR. It is however highly susceptibility to oxidative metabolism, mainly mediated by the 3A4 isozyme of the hepatic cytochrome P-450 system, resulting in rapid hepatic clearance and poor oral bioavailability, [44] which has stimulated the search for more drugable P2–P3 amines. We were intrigued by the observation that whilst the P2 residue in inhibitor **3** does not occupy the S2 pocket to the same extent as the amino-indanol in inhibitor **2**, it is still a highly potent inhibitor. It was thus considered that modifications in the P2 position of inhibitor **3** might provide scope for additional potency and overcoming some of the shortcomings of the amino-indanol moiety.

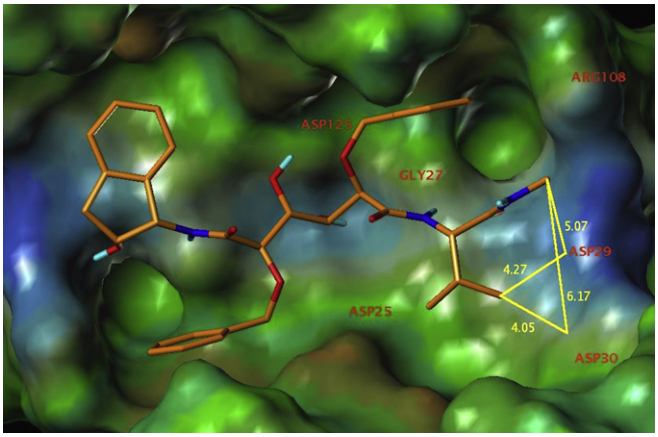


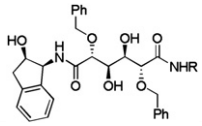

**Fig. 4.** 1EBZ PDB X-ray crystal structure of inhibitor **3** co-crystallized with the HIV-1 protease. The flap residues (Met46 – Ile54 and Met146 – Ile154) have been deleted to improve visibility of the inhibitor binding and the surface color coded by lipophilic potential.

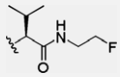
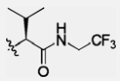
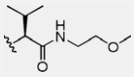
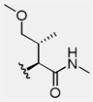
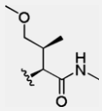
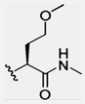
Inhibitors containing a "bis-THF" like P2 motif, e.g. inhibitor **1** [19] (Fig. 1), display high potency and moreover, broad-spectrum activity against multi-PI resistant HIV-1 variants. A 3D structure of inhibitor **1** bound to the HIV protease [45] display a tight binding between the "bis-THF" residue and the main chains of the protease active site amino acids (Asp-29 and Asp-30). As the main chains are less sensitive to mutations this binding is postulated to be a key attributing factor to maintaining potency against multi-drug-resistant HIV-1 [46].

From examining the 3D structure of inhibitor **3** bound to HIV-1 protease (Fig. 4) [47] two design alternatives were selected: a) building from the methyl group of the methyl amide which is 5.07 Å from the backbone nitrogen of Asp-29, but significantly further (6.17 Å) from that of Asp-30 and b) extending from the isopropyl group which is somewhat closer to both potential H-bond donors, 4.05 Å from the backbone nitrogen of Asp-30 and 4.27 Å from that of Asp-29.

Three compounds, **4a**–**c**, (Scheme 8) were designed to interact with Asp-29 and five compounds, **4d**–**h**, with Asp-30 (Schemes 8 and 9). The inhibitors were evaluated for their antiviral potency against the HIV protease ($K_i$) and anti-HIV activity in an MT4 cell-based assay ($EC_{50}$) against both wild type virus and an HIV protease inhibitor resistant strain carrying the M46I, V82F, and I84V mutations (Table 1).

**Table 1**
HIV-1 protease inhibitory activities.




| Entry | Compound | R= | $K_i$ (nM) | $EC_{50}$ (μM) | $EC_{50}$ resistant clone (μM) | $EC_{50}$ ratio |
|---|---|---|---|---|---|---|
| 1 | **3** |  | 0.2 | 0.14 | 1.2 | 9 |
| 2 | **4a** |  | 1.0 | 0.17 | 0.44 | 3 |
| 3 | **4b** |  | 1.9 | 0.15 | 0.92 | 5 |
| 4 | **4c** |  | 2.3 | 0.25 | 1.2 | 5 |
| 5 | **4d** |  | 18 | 3.5 | >10 | >2 |
| 6 | **4e** |  | 0.7 | 0.33 | 1.3 | 4 |
| 7 | **4f** |  | 10 | 1.8 | 6.9 | 3 |
| 8 | **4g** |  | 99 | >10 | >10 | – |
| 9 | **4h** |  | 10 | 5.4 | >10 | >1.6 |

Inhibitors **4a**–**c** all displayed similar potencies ($K_i$ values of 1.0, 1.9 and 2.3 nM, respectively) and antiviral activity ($EC_{50}$ values of 0.17, 0.15 and 0.25 μM, respectively) and with slightly less loss in cell-based activity against a resistant mutant strain than the lead inhibitor **3** (Table 1). Inhibitors **4a** and **4b** were also evaluated for their apparent permeability (Papp) in a Caco-2 cell assay, and compared with inhibitor **3** they displayed a somewhat increased permeability (Table 2).

**Table 2**
HIV-1 protease inhibitor permeability in a Caco-2 cell assay.

| Entry | Compound | Papp(Caco-2), $10^{-6}$ cm/s |
|---|---|---|
| 1 | **3** | 1.4 |
| 2 | **4a** | 1.6 |
| 3 | **4b** | 2.9 |

Log *P* and the topological polar surface area (PSA) (Table 3) were calculated for compounds **4a**–**h** where the increase in calculated log *P* for compounds **4a** and **4b** compared to inhibitor **3** supports the slight increase in permeability observed. Inhibitors **4d**–**f** each

**Table 3**
Calculated log *P* and topological polar surface areas for the inhibitors.

| Entry | Compound | Log *P* | PSA |
|---|---|---|---|
| 1 | **3** | 2.68 | 166 |
| 2 | **4a** | 2.84 | 166 |
| 3 | **4b** | 3.66 | 166 |
| 4 | **4c** | 2.52 | 176 |
| 5 | **4d** | 1.86 | 176 |
| 6 | **4e** | 1.86 | 176 |
| 7 | **4f** | 1.36 | 176 |
| 8 | **4g** | 1.22 | 176 |
| 9 | **4h** | 1.22 | 176 |

have a 2-methoxy-ethyl P2 residue, where **4e** having a 1*R*-methyl substituent on the P2 residue is the most potent, displaying a $K_i$ value of 0.7 nM and antiviral activity $EC_{50}$ value 0.33. Finally, the smaller size and more rigid oxetane compounds **4g–h** lost considerable in potencies both on enzyme and in the cell-based anti-HIV assay.

### 2.3. Modeling

Docking of compounds **4a** and **4b** in the crystal structure of 1EBZ gave high scoring poses (entries 2 and 3, Table 4), although further inspection indicated that neither **4a** nor **4b** would achieve polar interactions with Asp-29 or Asp-30. For these two inhibitors the substituent added to the terminal methyl amide group lies in the solvent channel and directed away from the polar groups in the S2 pocket (Fig. 5).

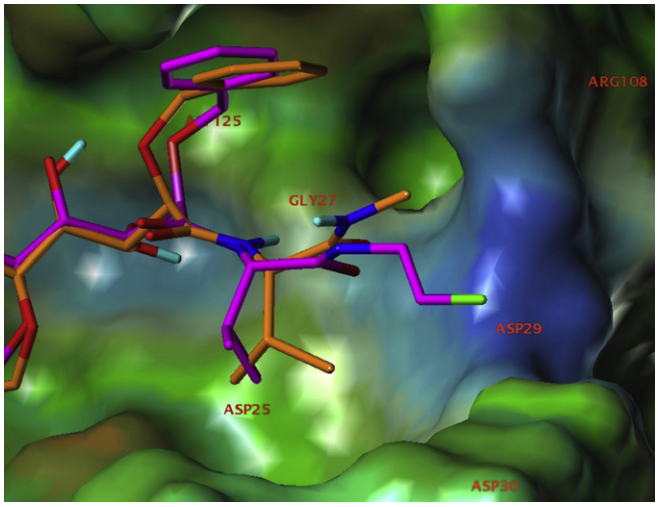



**Fig. 5.** Docked structure of inhibitor **4a** (magenta) overlaid with that of inhibitor **3** (orange) from the 1EBZ crystal structure presented as in Fig. 3.

The introduction of fluorine lead to a minor increase in calculated log *P* (entry 1, Table 3), which is reflected in a corresponding slight improvement in Caco-2 permeability (entry 2, Table 2) relative to inhibitor **3** (entry 1, Table 2). The effect of fluorination on permeability is more marked in the trifluorinated analogue **4b** (entry 3, Table 2).

Docking of **4d** and **4e** gave similar high scoring top poses of which the highest ranked are disclosed in Table 4. Furthermore, the subsequent four docks for **4d** have markedly lower scores (90.19–92.95) which are in contrast to the docks for **4e**, where the scores (95.37–96.48) are comparable with the top pose (Table 4).

**Table 4**
GoldScores of the highest ranked poses.

| Entry | Compound | GoldScore |
|---|---|---|
| 1 | **3** | 96.45 |
| 2 | **4a** | 99.05 |
| 3 | **4b** | 101.51 |
| 4 | **4c** | 102.11 |
| 5 | **4d** | 98.45 |
| 6 | **4e** | 97.34 |
| 7 | **4f** | 94.90 |
| 8 | **4g** | 81.58 |
| 9 | **4h** | 85.72 |

Visual inspections of the **4d** docks reveal the top pose to be a miss-dock with disrupted interactions with the catalytic aspartates. Some of the lower ranked docks, and pose 3 in particular, show a weak H-bond to Asp-30 (3.41 Å) and a much more tenuous one to Asp-29 (4.00 Å) (Fig. 6). Unfortunately this is achieved at the expense of a close contact (3.46 Å) between the unsubstituted P2 methyl group and the flap Ile-37 side-chain which may account for the low potency observed. All five docks of **4e** show that the methoxy group can be accommodated along the binding channel towards bulk solvent, but fail to make either of the desired polar S2 interactions. This result is compatible with the minor effect on activity resulting from this substitution.

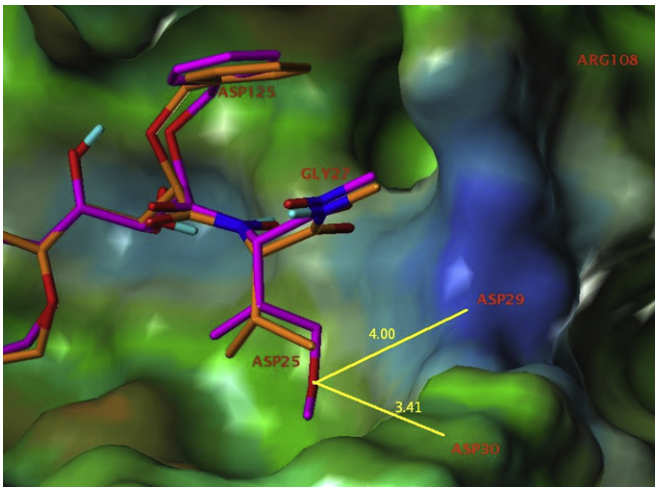



**Fig. 6.** Docked pose of inhibitor **4e** (magenta) overlaid with that of inhibitor **3** (orange) from the 1EBZ crystal structure presented as in Fig. 3.

The significantly lower biological activity of **4f** is puzzling since this inhibitor should be able to adopt a conformation similar to that of **4e**. Despite the slightly lower docking score, this possibly reflects the likely entropic cost of constraining a highly mobile side-chain.

The oxetane moiety in **4g** and **4h** is a small, rigid oxygen containing ring in the equivalent position to the methoxy substituents in **4d–f**. It is also a conformationally constrained analogue of the γ-methoxy-L-Val found in **4d** and **4e**.

However the activities of these analogues were found to be disappointingly low. This is in agreement with the markedly lower docking scores (Table 4) and is perhaps unsurprising for the "unnatural" enantiomer **4g** and could also be rationalized for **4h** on inspection of the modeled structure where the effect of the ring system is to "tie back" the oxygen atom away from both the Asp-29 and Asp-30 N–Hs and from solvent.

These inhibitors were also tested against an HIV protease inhibitor resistant strain carrying the M46I, V82F, and I84V mutations (Table 1). Inhibitors **4a** (entry 2, Table 1) and **4e** (entry 6, Table 1) in particular displayed substantially lower losses of activity against the resistant clone than does lead inhibitor **3** (entry 1, Table 1). However, whilst the I84V mutation is on the edge of the S2 pocket and might influence the binding, there is no clear overall pattern in the fold changes observed for these inhibitors against the mutant clone.

## 3. Conclusion

Eight novel inhibitors have been synthesized where inhibitor **4a** and inhibitor **4e** furnished the most potent inhibition of the HIV-1

protease and where inhibitors **4a** and **4b** were roughly equipotent to lead **3** ($EC_{50} = 0.1$ μM) in regards to anti-HIV activity in the MT4 cell-based assay. Noteworthy, the introduction of one or more fluorines, i.e. inhibitors **4a** and **4b**, increases the cell permeability, as expected and supported by Caco-2 data and calculated log *P* data and this may contribute to the overall favorable anti-HIV activity.

Inhibitors **4d**, **4e**, **4g** and **4h**, have extensions from the P2 isopropyl group and inhibitor **4f** have a P2 methoxyethyl group. Of these compounds inhibitor **4e** is believed to interact favorably with Asp-30. However, extensions with polar groups from the isopropyl side-chain of the P2 Val appear to be less well accommodated in the S2 pocket.

Attempts to make a fluorine analogue of **4e** to evaluate a smaller electronegative substituent and its interactions in the S2 pocket were not successful. When tested against a multi resistant clone, in particular two of the analogues prepared, **4a** and **4e**, displayed favorable lower fold changes compared with lead inhibitor **3**.

The advantageous cellular anti-HIV activities for the fluorinated compounds **4a** and **4b** as well as the short synthesis routes from the appropriate P2 amino acids (one chemical step) warrants further explorations of fluorinated P2-substituents.

## 4. Experimental section

### 4.1. HIV-1 protease inhibition

HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* [48] and $K_i$-values were determined using a fluorometric assay [49].

### 4.2. In vitro anti-HIV activity

The anti-HIV activity was measured in an HIV cytopathic assay in MT-4 cells where the effect was quantified using vital dye XTT [50]. The mutant virus was generated in MT4 cells when HIV, IIIb was replicating in the presence of increasing concentrations of Ritonavir. Resistant virus containing M46I, V82F, and I84V was isolated after 20 passages and used for cross resistance studies.

Briefly, test compounds in 5-fold serial dilutions were added to MT4 cells ($2 \times 10^4$ cells/well) in a micro plate and at the same time was added 20–50 tissue culture infectious doses (TCID) of wild type or mutant HIV, IIIb.

The viability of the cells was determined with XTT after five days of incubation and the 50% effective concentrations ($EC_{50}$) were calculated from dose response curves of the percent cytoprotection for individual compound concentrations.

### 4.3. Modeling

Ligand building, and general modeling and visualization with performed with Sybyl 8.0 [51]. The docking receptor was prepared from the 1EBZ RCSB PDB file in Sybyl by addition of hydrogen atoms and charges and the deletion of the ligand and of all water molecules with the exception of the one between the flaps which H-bonds to the ligand. Receptor residues were ionized as appropriate for pH 7.2 and in particular the two catalytic aspartyl residues were both ionized. The ligand molecules were sketched in 2D and protonated and converted to 3D with Concord and minimized in Sybyl with MMFF94S force field using the default parameters.

Docking calculations were performed with GOLD v4.0 [52]. The binding site was defined as receptor atoms within 10 Å of the oxygen atom of the inter-flap water and the GOLD cavity detection algorithm applied. The genetic algorithm was run 25 times using the automatic genetic algorithm parameters and the GoldScore fitness function. Because of the symmetric nature of the enzyme H-bond constraints from the ligand to the conserved water were applied to ensure that all docking solutions were bound in a consistent orientation. From each run the five highest ranked poses were retained for assessment both on the basis of the docking score and by visual inspection.

Calculations of log *P* and topological polar surface area were performed with ChemAxon's calculators [53,54].

### 4.4. General methods

All glassware was dried over an open flame before use in connection with an inert atmosphere. Concentrations were performed under reduced pressure at <40 °C (bath temperature). Thin layer chromatography was performed using Merck silica gel 60 F-254 plates with detection by UV, charring with 8% sulphuric acid or ammonium molybdate (100 g): Ce(IV)sulphate (2 g): sulphuric acid (10%, 2 L). Column chromatography was performed on silica (0.035–0.070 mm). NMR spectra was recorded at 25 °C on a Varian 300 MHz or 400 MHz instrument using the solvent residual peak ($CDCl_3$ $^1H$ $\delta = 7.26$ ppm and $^{13}C$ $\delta = 77.16$ ppm or $CD_3OD$ $^1H$ $\delta = 3.31$ and $^{13}C$ $\delta = 49.0$ ppm) as standard. Optical rotations were measured on a Perkin–Elmer 241 polarimeter using a 10 cm, 1 mL cell. LC–MS was performed on a Waters (micro mass ZQ) preparative LC–MS, using a reverse phase column: ACE C-8, 21 × 100 mm, 5 μm particles, 100A from ACE (UK) and the mobile phases were based on water/acetonitrile containing 0.1% TFA. Mass spectra were recorded with a micrOTOF 125 spectrometer. Unless stated otherwise, all materials were obtained from commercial suppliers and used without further purification. $CH_2Cl_2$ and THF were distilled from $CaH_2$ before use.

### 4.5. Synthetic experiments

#### 4.5.1. General method for the preparation of compounds ***5a–c***

To a cooled, −15 C, stirred suspension of Cbz-Val-OSu (1 equiv) in dry THF (5 mL) was added 4-methylmorpholine (3 equiv). After careful addition of the amine (1.5 equiv) the stirred solution was allowed to attain room temperature and left for 16 h. The solvent was removed and the crude product purified by silica gel chromatography (light petroleum (40–60)–ethyl acetate, gradient 3:1 – 1:1) to yield the title compounds **5a–c**.

*4.5.1.1. (**S**)-benzyl 1-(2-fluoroethylamino)-3-methyl-1-oxobutan-2-yl carbamate (**5a**).* The compound **5a** was prepared from 2-fluorethyl amine, according to general procedure, vide supra, in 84% yield (250 mg, 0.84 mmol) as a crystalline white solid. $[\alpha]_D$ – 12.1 (*c* 0.66, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$,) $\delta$ 7.29 (m, 5H), 5.76 (d, 1H, $J = 8.0$ Hz), 5.05 (s, 2H), 4.49 (t, 1H, $J = 4.5$, 9.0 Hz), 4.37 (t, 1H, $J = 4.5$, 9.0 Hz), 3.89 (dd, 1H, $J = 6.7$, 9.0 Hz), 3.48 (m, 2H), 2.55 (d, 1H $J = 7.2$ Hz), 2.02 (m, 1H), 0.90 (dd, 6H, $J = 6.8$, 11.3 Hz). $^{13}C$ NMR (100 MHz, $CDCl_3$,) $\delta$ 172.2, 156.8, 136.2, 128.6, 128.3, 128.0, 83.2, 81.6, 67.1, 60.5, 40.1, 31.9, 31.2, 19.2, 18.0; MS (ESI) *m/z* 319.1 ($[M + Na]^+$ calcd for $C_{15}H_{21}FN_2NaO_3^+$ 319.1).

*4.5.1.2. (**S**)-benzyl 3-methyl-1-oxo-1-(2,2,2-trifluoroethylamino) butan-2-ylcarbamate (**5b**).* The compound **5b** was prepared from 2,2,2-trifluorethyl amine according to general procedure, vide supra, in 83% yield (300 mg, 0.84 mmol) as a crystalline white solid. $[\alpha]^{20}_D$ – 8.5 (*c* 1.00, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$,) $\delta$ 8.07 (bs, 1H), 7.25 (m, 5H), 5.00 (s, 2H), 3.95 (m, 1H), 3.88 (d, 1H, $J = 6.7$ Hz), 1.94 (m, 1H), 0.90 (dd, 6H, $J = 6.8$, 9.6 Hz); $^{13}C$ NMR (100 MHz, $CDCl_3$,) $\delta$ 172.8, 156.8, 136.1, 128.5, 128.2, 127.9, 125.4, 122.7, 67.0,

60.2, 40.6, 40.2, 31.2, 18.8, 17.7; MS (ESI) $m/z$ 355.1 ($[M+Na]^+$ calcd for $C_{15}H_{19}F_3N_2NaO_3^+$ 355.1).

*4.5.1.3. (**S**)-benzyl 1-(2-methoxyethylamino)-3-methyl-1-oxobutan-2-ylcarbamate (**5c**).* $[\alpha]^{20}_D$ – 8.3 (*c* 1.00, MeOH); MS (ESI) $m/z$ 331.2 ($[M+Na]^+$ calcd for $C_{16}H_{24}N_2NaO_4^+$ 331.2); $^1$H NMR data and $^{13}$C NMR data were in agreement with published values [24].

*4.5.1.4. (**S**)-1-tert-butyl 4-methyl 2-(benzyloxycarbonylamino) succinate (**9**), **8**.* (15 g, 53.3 mmol) was dissolved in $CH_2Cl_2$ (200 mL) in a 500 mL three necked round bottom flask that was equipped with a gas inlet and a cooling finger. After treatment with $H_2SO_4$ (conc., 0.80 mL) the solution was cooled to −78 °C and isobutene (694 mmol, 66 mL) was condensed into the mixture. The reaction was sealed, allowed to reach room temperature and then left stirring for 16 h. After neutralisation with $NaHCO_3$ (aq., sat.), the organic phase was washed with brine, dried over $Na_2SO_4$ and concentrated. After purification by silica gel chromatography (toluene–ethyl acetate, gradient 50:1–30:1) compound **9** was obtained as a clear oil in 82% yield (14.65 g, 43.4 mmol). MS (ESI) $m/z$ 374.2 ($[M+Na]^+$ calcd for $C_{18}H_{25}NNaO_6^+$ 374.2); $^1$H NMR data and $^{13}$C NMR data were in agreement with published values [26].

*4.5.1.5. (**2S**,**3R**)-3-(benzyloxycarbonylamino)-4-tert-butoxy-2-methyl-4-oxobutanoic acid (**11a**) and (**2S**,**3S**)-3-(benzyloxy carbonylamino)-4-tert-butoxy-2-methyl-4-oxobutanoic acid (**11b**).* To a stirred mixture of **10a** and **10b** (2.13 g, 6.06 mmol) in MeOH (10 mL) was added NaOH (1 M, 6.7 mL). After stirring for 2 h in room temperature the mixture was concentrated to approx. 4 mL thereafter diluted with $H_2O$ (15 mL). The aqueous solution was extracted with $Et_2O$ (2 × 15 mL) before being transferred to a round bottom flask and $Et_2O$ (15 mL) was added. During vigorous stirring of the mixture, the pH was set to ~2 using HCl (conc.). The phases were separated and the aqueous phase was extracted with $Et_2O$ (15 mL). The pooled organic phases were washed with brine, dried ($Na_2SO_4$), concentrated and filtered through a short silica gel column (toluene–ethyl acetate, gradient 10:1–1:1) to yield the crude product as a clear syrup (1.94 g, 5.75 mmol, 95%), which was used in the next step without further purification.

*4.5.1.6. (**2S**,**3R**)-tert-butyl 2-(benzyloxycarbonylamino)-4-hydroxy-3-methylbutanoate (**12a**) and (**2S**,**3S**)-tert-butyl 2-(benzyloxy carbonylamino)-4-hydroxy-3-methylbutanoate (**12b**).* To a cooled (−10 °C) stirred solution of **11a** and **11b** (717 mg, 2.12 mmol) in dry THF (4 mL) was added NMM (240 μl, 2.14 mmol). After an additional 3 min ethyl chloroformate (205 μl, 2.14 mmol) was added drop wise. The mixture was allowed to reach room temperature during 15 min and was then filtered. The filtrate was added drop wise during 30 min to a cooled (3 °C) solution of $NaBH_4$ (176 mg, 4.66 mmol) in $H_2O$ (2 mL). The mixture was allowed to slowly reach room temperature. After 3 h, the mixture was again cooled on an ice bath, and acidified to pH ~2 with HCl (conc.). After extraction with ethyl acetate (3 × 7.5 mL), the organic phases was washed with brine, dried ($Na_2SO_4$) and concentrated to yield the crude product. After silica gel chromatography (toluene–ethyl acetate, gradient 100:1–40:1) **12b** was obtained (130 mg, 0.40 mmol, 19%) followed by **12a** (362 mg, 1.12 mmol, 53%). *tert*-Butyl (**2S**,**3R**)-2-[(benzyloxycarbonyl)amino]-3-methyl-4-hydroxybutanoate (**12a**). $[\alpha]^{20}_D$ + 2.5 (*c* 1.00, $CHCl_3$); $^{13}$C NMR (75 MHz, $CDCl_3$,) $\delta$ 171.3, 156.7, 136.3, 128.6, 128.3, 128.2, 82.5, 67.2, 64.4, 57.0, 38.8, 28.1, 13.8. MS (ESI) $m/z$ 346.2 ($[M+Na]^+$ calcd for $C_{17}H_{25}NNaO_5^+$ 346.2). $^1$H NMR data were in agreement with published values [30]. *tert*-Butyl (**2S**,**3S**)-2-[(benzyloxycarbonyl)amino]-3-methyl-4-hydroxybutanoate (**12b**). $[\alpha]^{20}_D$ + 62.7 (*c* 1.00, $CHCl_3$); $^{13}$C NMR (75 MHz, $CDCl_3$,) $\delta$ 171.2, 157.7, 136.0, 128.7, 128.4, 128.3, 82.6, 67.5, 64.0, 54.2, 38.9, 28.1, 9.9. MS (ESI) $m/z$ 346.2 ($[M+Na]^+$ calcd for $C_{17}H_{25}NNaO_5^+$ 346.2). $^1$H NMR data were in agreement with published values [30].

*4.5.2. General method for preparation of compounds **14a–c***

The alcohol (1 equiv) was dissolved in dry $CH_2Cl_2$ (3 mL) and stirred under argon when 2,6-di-*tert*-butyl-4-methylpyridine (3 equiv) was added. The mixture was cooled on an ice bath and MeOTf (3 equiv) was added. The reaction mixture was allowed to reach room temperature and was then left stirring for 16 h. After addition of $NaHCO_3$ (aq., sat., 3 mL), the organic phase was separated and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 3 mL). The organic phases were combined and washed with brine, dried ($MgSO_4$), concentrated and purified by silica column chromatography (toluene–ethyl acetate, gradient 100:1–20:1) to give the title compounds **14a–c**.

*4.5.2.1. (**2S**,**3R**)-tert-butyl 2-(benzyloxycarbonylamino)-4-methoxy-3-methylbutanoate (**14a**).* The compound **14a** was prepared from **12a**, according to general procedure, vide supra, in 52% yield (53 mg, 0.16 mmol). $[\alpha]^{20}_D$ + 2.5 (*c* 1.00, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$,) $\delta$ 7.38–7.29 (m, 5H), 5.69 (d, 1H, $J$ = 8.5 Hz), 5.10 (s, 2H), 4.27 (dd, 1H, $J$ = 4.2, 8.8 Hz), 3.36–3.24 (m, 4H). 2.34 (m, 1H), 1.45 (s, 9H), 1.00 (d, 3H, $J$ = 7.1 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$,) $\delta$ 171.0, 156.6, 136.6, 128.6, 128.2, 81.9, 74.6, 66.9, 59.0, 57.7, 35.9, 28.1, 14.4; MS (ESI) $m/z$ 360.2 ($[M+Na]^+$ calcd for $C_{18}H_{27}NNaO_5^+$ 360.2).

*4.5.2.2. (**2S**,**3S**)-tert-Butyl 2-(benzyloxycarbonylamino)-4-methoxy-3-methylbutanoate (**14b**).* The compound **14b** was prepared from **12b**, according to general procedure, vide supra, in 56% yield (100 mg, 0.29 mmol). $[\alpha]^{20}_D$ + 6.7 (*c* 1.00, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$,) $\delta$ 7.39–7.29 (m, 5H), 5.51 (d, 1H, $J$ = 8.7 Hz), 5.11 (s, 2H), 4.27 (dd, 1H, $J$ = 2.7, 8.9 Hz), 3.35–3.18 (m, 4H). 2.30 (m, 1H), 1.46 (s, 9H), 0.88 (d, 3H, $J$ = 7.1 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$,) $\delta$ 171.0, 156.3, 136.6, 128.6, 128.2, 82.1, 74.9, 67.0, 59.0, 56.3, 36.6, 28.1, 12.3; MS (ESI) $m/z$ 360.2 ($[M+Na]^+$ calcd for $C_{18}H_{27}NNaO_5^+$ 360.2).

*4.5.2.3. (**S**)-Benzyl 2-(benzyloxycarbonylamino)-4-methoxybutanoate (**14c**).* The compound **14c** was prepared from **12c**, according to general procedure, vide supra, in 44% yield (321 mg, 0.90 mmol). $[\alpha]^{20}_D$ − 11.8 (*c* 1.00, MeOH); $^1$H NMR (300 MHz, $CDCl_3$,) $\delta$ 7.40–7.29 (m, 10H), 5.77 (d, 1H, $J$ = 7.8 Hz), 5.17 (s, 2H), 5.11 (s, 2H), 4.53 (m, 1H), 3.48–3.32 (m, 2 H), 3.23 (s, 3H), 2.20–1.93 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$,) $\delta$ 172.1, 156.1, 136.5, 135.6, 128.7, 128.6, 128.5, 128.4, 128.2, 69.0, 67.2, 67.0, 58.9, 52.6, 31.9; MS (ESI) $m/z$ 380.1 ($[M+Na]^+$ calcd for $C_{20}H_{23}NNaO_5^+$ 380.1).

*4.5.3. General method for preparation of compounds **15a** and **15b***

The *tert*-butyl ester (1 equiv) was dissolved in dry $CH_2Cl_2$ (32 equiv) and while stirring TFA (13 equiv) was added followed by $Et_3SiH$ (2.5 equiv). After 45 min of stirring in room temperature the reaction mixture was concentrated and toluene was then co-evaporated three times from the residue, which was then purified on a short silica gel column (toluene–ethyl acetate, gradient: 10:1–1:1) to yield compounds **15a** and **15b** which were used without further purification in the next step.

*4.5.3.1. (**2S**,**3R**)-2-(benzyloxycarbonylamino)-4-methoxy-3-methylbutanoic acid (**15a**).* The compound **15a** was prepared from **14a**, according to general procedure, vide supra, in 88% yield (158 mg,

0.56 mmol). $[\alpha]^{20}_D$ + 13.7 (*c* 0.2, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$,) $\delta$ 7.39–7.29 (m, 5H), 5.92 (d, 1H, *J* = 7.1 Hz), 5.12 (s, 2H), 4.41 (bs, 1H), 3.56 (s, 1H) 3.40–3.23 (m, 5H). 2.44 (bs, 1H), 1.05 (d, 3H, *J* = 7.1 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$,) $\delta$ 156.8, 136.4, 128.7, 128.3, 128.3, 75.2, 67.2, 59.3, 35.5, 29.8, 14.0; MS (ESI) *m*/*z* 304.1 ($[M + Na]^+$ calcd for $C_{14}H_{19}NNaO_5^+$ 304.1).

(**2*S*,3*S***)-2-(benzyloxycarbonylamino)-4-methoxy-3-methylbutanoic acid (**15b**)

The compound **15b** was prepared from **14b**, according to general procedure, vide supra, in 74% yield (62 mg, 0.22 mmol). $[\alpha]^{20}_D$ − 4.1 (*c* 1.00, MeOH); $^1$H NMR (400 MHz, $CD_3OD$,) $\delta$ 7.40–7.27 (m, 5H), 5.10 (s, 2H), 4.43 (d, 1H, *J* = 3.9 Hz), 3.29–3.20 (m, 5H). 2.36 (m, 1H), 0.89 (d, 3H, *J* = 7.1 Hz); $^{13}$C NMR (100 MHz, $CD_3OD$,) $\delta$ 158.8, 138.2, 129.4, 129.0, 128.8, 75.5, 67.6, 59.0, 49.2, 36.8, 12.2; MS (ESI) *m*/*z* 304.1 ($[M + Na]^+$ calcd for $C_{14}H_{19}NNaO_5^+$ 304.1).

*4.5.3.2. (**S**)-2-(benzyloxycarbonylamino)-4-methoxybutanoic acid (**15c**).* To a stirred solution of **14c** (370 mg, 1.04 mmol) in THF (5 mL) was added NaOH (1 M, 3.13 mL) and MeOH (2 mL). After stirring at room temperature for 25 min. the THF solution was reduced in volume on a rotavapor to 2 mL, diluted with $H_2O$ (5 mL) and extracted with $Et_2O$. The pH was set to ~2 using HCl (1 M), before extraction with $CH_2Cl_2$ (4 × 5 mL). The combined organic phases were washed with brine, dried ($Na_2SO_4$), concentrated and purified on a short silica gel column (toluene–ethyl acetate gradient 10:1–1.1) to obtain **17c** in 58% yield (164 mg, 0.61 mmol). $^1$H NMR (300 MHz, $CDCl_3$,) $\delta$ 10.72 (bs, 1H), 7.39–7.26 (m, 5H), 5.94 (d, 1H, *J* = 7.6 Hz), 5.12 (s, 2H), 4.49 (m, 1H), 3.50 (m, 2H), 3.30 (s, 3H). 2.25–1.91 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$,) $\delta$ 176.2, 156.4, 136.2, 128.6, 128.2, 128.2, 69.2, 67.1, 58.8, 52.3, 31.4.

*4.5.4. General method for preparation of compounds **16a–c***

The acid (1 equiv) was dissolved in dry $CH_2Cl_2$ (2 mL) and pyBOP (1 equiv) was added followed by DIEA (1 equiv). After 45 min of stirring in room temperature, methylamine (1.1 equiv, 2 M in THF) was added to the mixture, followed by DIEA (1 equiv). The reaction mixture was stirred for another 2 h, and was then diluted with $CH_2Cl_2$ (5 mL), washed with $NaHCO_3$ (aq., sat.) (5 × 5 mL), and $NH_4Cl$ (1 × 5 mL). The aqueous layers were extracted with $CH_2Cl_2$ (3×), and the pooled organic phases were washed with brine, dried ($MgSO_4$), concentrated and purified on a silica gel column ($CH_2Cl_2$) to yield compounds **19a–c**.

*4.5.4.1. Benzyl (**2*S*,3*R***)-4-methoxy-3-methyl-1-(methylamino)-1-oxo butan-2-ylcarbamate (**16a**).* The compound **16a** was prepared from **15a**, according to general procedure, vide supra, in 66% yield (110 mg, 0.37 mmol). $[\alpha]^{20}_D$ + 0.7 (*c* 1.0, MeOH); $^1$HNMR (400 MHz, $CD_3OD$,) $\delta$ 7.39–7.26 (m, 5H), 5.08 (dd, 2H, *J* = 10.0, 12.4 Hz) 4.06 (d, 1H, *J* = 6.7 Hz), 3.34 (d, 1H, *J* = 4.8 Hz), 3.28 (s, 3H), 2.73 (s, 3H). 2.67 (bs, 1H), 2.17 (m, 1H), 0.98 (d, 3H, *J* = 7.0 Hz) $^{13}$C NMR (100 MHz, $CDCl_3$,) $\delta$ 174.5, 158.4, 138.1, 129.5, 129.1, 129.0, 75.5, 67.8, 59.5, 59.3, 36.8, 26.2, 14.7; MS (ESI) *m*/*z* 317.1 ($[M + Na]^+$ calcd for $C_{15}H_{22}N2NaO_4^+$ 317.1).

*4.5.4.2. Benzyl (**2*S*,3*S***)-4-methoxy-3-methyl-1-(methylamino)-1-oxo butan-2-ylcarbamate (**16b**).* The compound **16b** was prepared from **15b**, according to general procedure, vide supra, in 62% yield (40 mg, 0.14 mmol). $[\alpha]^{20}_D$ + 12.0 (*c* 0.10, $CHCl_3$); $^1$H NMR (400 MHz, $CDOD_3$,) $\delta$ 7.39–7.27 (m, 5H), 5.09 (dd, 2H, *J* = 12.4, 13.6 Hz), 4.23 (d, 1H, *J* = 5.4 Hz), 3.29–3.19 (m, 5H), 2.73 (s, 3H), 2.69 (s, 1H), 2.25 (m, 1H), 0.89 (d, 3H, *J* = 7.0); $^{13}$C NMR (100 MHz, $CDOD_3$,) $\delta$ 174.6, 158.6, 138.2, 129.4, 129.1, 128.9, 75.6, 67.8, 59.1, 58.1, 37.1, 26.3, 12.6; MS (ESI) *m*/*z* 317.1 ($[M + Na]^+$ calcd for $C_{15}H_{22}N2NaO_4^+$ 317.1).

*4.5.4.3. (**S**)-benzyl 4-methoxy-1-(methylamino)-1-oxobutan-2-ylcarbamate (**16c**).* The compound **16c** was prepared from **15c**, according to general procedure, vide supra, in 88% yield (115 mg, 0.41 mmol). $[\alpha]^{20}_D$ − 14.1 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, $CD_3OD$,) $\delta$ 7.38–7.23 (m, 5H), 5.09 (m, 2H), 4.86 (s, 1H), 4.19 (dd, 1H, *J* = 4.9, 8.9 Hz), 3.42 (dd, 1H, *J* = 5.2, 6.8 Hz), 3.29 (s, 3H), 2.73 (s, 3H). 2.02 (m, 1H), 1.83 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$,) $\delta$ 172.8, 156.5, 136.0, 128.4, 128.1, 127.9, 69.1, 66.9, 58.6, 32.1, 25.9; MS (ESI) *m*/*z* 303.1 ($[M + Na]^+$ calcd for $C_{14}H_{20}N_2NaO_4^+$ 303.1).

*4.5.4.4. (**R**)-Methyl 2-(benzyloxycarbonylamino)-2-(oxetan-3-yl acetate) (**19a**).* $[\alpha]^{20}_D$ + 14.4 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, $CDCl_3$,) $\delta$ 7.40–7.29 (m, 5H), 5.50 (d, 1H, *J* = 7.5 Hz), 5.13 (s, 2H), 4.78–4.51 (m, 5H), 3.76 (s, 3H), 3.36 (q, 1 H, *J* = 6.9 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$,) $\delta$ 171.4, 156.4, 136.1, 128.7, 128.4, 128.3, 73.7, 73.4, 67.5, 55.1, 52.8. 37.7; MS (ESI) *m*/*z* 302.1 ($[M + Na]^+$ calcd for $C_{14}H_{17}NNaO_5^+$ 302.1).

*4.5.4.5. (**S**)-methyl 2-(benzyloxycarbonylamino)-2-(oxetan-3-yl)acetate (**19b**).* $[\alpha]^{20}_D$ − 19.5 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, $CDCl_3$,) $\delta$ 7.39–7.29 (m, 5H), 5.50 (d, 1H, *J* = 8.2 Hz), 5.13 (s, 2H), 4.78–4.52 (m, 5H), 3.73 (s, 3H), 3.36 (q, 1 H, *J* = 7.3 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$,) $\delta$ 171.4, 156.4, 136.1, 128.7, 128.4, 128.3, 73.7, 73.4, 67.5, 55.1, 52.8. 37.7; MS (ESI) *m*/*z* 302.1 ($[M + Na]^+$ calcd for $C_{14}H_{17}NNaO_5^+$ 302.1).

*4.5.5. General method for preparation of compounds **17a** and **17b***

To a stirred solution of the ester (1 equiv) in EtOH (1 mL) was added $MeNH_2$ (33% in EtOH) (7.5 equiv). The reaction mixture was left stirring in room temperature overnight before the solvent was removed. The product was recrystallised from EtOH and diethyl ether to obtain the title compounds.

*4.5.5.1. (**R**)-benzyl 2-(methylamino)-1-(oxetan-3-yl)-2-oxoethylcarbamate (**17a**).* The title compound was prepared from **19a** according to general procedure, vide supra, in 86% yield (86 mg, 0.31 mmol) and obtained as a white solid. $[\alpha]^{20}_D$ + 8.0 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, $CDCl_3$,) $\delta$ 7.36–7.30 (m, 5H), 6.61 (s, 1H), 5.76 (d, 1H, *J* = 8.3 Hz), 5.10 (s, 2H), 4.75 (m, 2H), 4.49 (m, 3H), 3.36 (m, 1H), 2.75 (d, 3 H, *J* = 4.6 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$,) $\delta$ 170.9, 157.0, 135.9, 128.7, 128.5, 128.1, 73.9, 67.5, 56.5, 37.3, 26.3; MS (ESI) *m*/*z* 301.1 ($[M + Na]^+$ calcd for $C_{14}H_{18}N_2NaO_4^+$ 301.1).

*4.5.5.2. (**S**)-benzyl 2-(methylamino)-1-(oxetan-3-yl)-2-oxoethylcarbamate (**17b**).* The title compound was prepared from **19b** according to general procedure, vide supra, in 49% yield (49 mg, 0.18 mmol) and obtained as a white solid. $[\alpha]^{20}_D$ − 8.5 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 7.39–7.27 (m, 5H), 5.11 (dd, 2H, *J* = 12.5, 11.0 Hz), 4.57 (t, 1H, *J* = 6.4 Hz), 4.50 (t, 1H, *J* = 6.3 Hz), 4.45 (d, 1H, *J* = 9.5 Hz), 2.70 (s, 3 H), 2.63 (bs, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$,) $\delta$ 170.9, 157.0, 135.9, 128.7, 128.5, 128.2, 73.9, 67.6, 56.5, 37.3, 26.3; MS (ESI) *m*/*z* 279.1 ($[M + H]^+$ calcd for $C_{14}H_{19}N_2O_4^+$ 279.1).

*4.5.6. General method for preparation of compounds **4a–d** and **4f***

The benzyl carbamate (1 equiv.) was dissolved in MeOH (3 mL) under an argon atmosphere and palladium on charcoal (~5%) was added followed by pressure reduction. The reaction mixture was left stirring under an $H_2$-atmosphere at 1 atm overnight. The Pd/C was filtered off and the filtrate concentrated to yield the free amine which was used in the next step without further purification. The amine (1.5 equiv) was dissolved in 2–5 mL 1,2-dichloroethane and lactone **21** (1.0 equiv) was added. The reaction mixture was stirred at reflux for 16 h. The solvent was removed and the residue purified by silica gel chromatography ($CH_2Cl_2$–MeOH 40:1) to yield compounds **4a–d** and **4f**. Compounds **4d** and **4f** were purified on LC–MS and **4a–c** were re-crystallized from *n*-hexane and ethyl acetate.

*4.5.6.1. (**2R**,**3R**,**4R**,**5R**)-2,5-bis(benzyloxy)-N1-((**S**)-1-(2-fluoroethyl amino)-3-methyl-1-oxobutan-2-yl)-3,4-dihydroxy-N$^6$-((**1S**,**2R**)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)hexanediamide (**4a**).* The title compound was prepared from **5a** according to general procedure, vide supra, in 28% yield (23 mg, 34 μmol). $[\alpha]^{20}_D$ − 11.6 (*c* 0.88, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$,) δ 7.43–7.16 (m, 15H), 5.34 (dd, 1H, *J* = 4.9, 8.5 Hz), 4.73–4.62 (m, 5H), 4.44 (m, 1H), 4.31 (m, 2H), 4.19 (m, 2H), 4.14 (m, 2H), 3.49 (m, 1H), 3.42 (m, 1H), 3.11 (dd, 1 H, *J* = 5.7, 16.6 Hz), 2.93 (dd, 1 H, *J* = 1.8, 16.6 Hz). 2.41 (m, 1H), 0.93 (d, 3H, *J* = 6.9 Hz), 0.84 (d, 3H, *J* = 6.9 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$,) δ 172.1, 171.9, 170.8, 141.0, 139.8, 136.7, 136.6, 128.9, 128.8, 128.8, 128.6, 128.5, 128.5, 128.3, 128.2, 127.2, 125.5, 124.1, 83.6, 81.6, 81.4, 81.3, 73.5, 73.4, 72.9, 72.6, 72.4, 58.4, 58.1, 40.2, 39.9, 39.4, 29.2 19.7 17.2; HRMS (ESI) *m*/*z* 666.3173 ($[M + H]^+$ calcd for $C_{36}H_{45}FN_3O_8^+$ 666.3185).

*4.5.6.2. (**2R**,**3R**,**4R**,**5R**)-2,5-bis(benzyloxy)-3,4-dihydroxy-N1-((**1S**,**2R**)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)-N$^6$-((**S**)-3-methyl-1-oxo-1-(2,2,2-trifluoroethylamino)butan-2-yl)hexanediamide (**4b**).* The title compound was prepared from **5b** according to general procedure, vide supra, in 15% yield (12 mg, 17 μmol). $[\alpha]^{20}_D$ − 12.3 (*c* 0.22, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.95 (m, 1H), 7.38–7.13 (m, 14H), 5.31 (dd, 1H, *J* = 5.3, 8.8 Hz), 4.71–4.55 (m, 5H), 4.24 (dd, 1H, *J* = 5.4, 9.0 Hz), 4.16–4.03 (m, 4H), 3.77 (m, 2H), 3.11 (dd, 1H, *J* = 5.3, 16.5 Hz), 2.91 (dd, 1 H, *J* = 1.5, 16.5 Hz), 2.28 (m, 1H), 0.90 (d, 3H, *J* = 6.9 Hz), 0.82 (d, 3H, *J* = 6.9 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$,) δ 172.7, 172.2, 170.8, 141.0, 139.5, 136.4, 136.4, 129.0, 128.9, 128.8, 128.7, 128.7, 128.4, 128.2, 127.3, 125.6, 124.1, 82.0, 81.3, 77.4, 73.7, 73.5, 73.3, 72.7, 72.5, 58.5, 58.4, 58.2, 40.8, 40.3, 39.3, 29.0, 19.6, 17.0; HRMS (ESI) *m*/*z* 724.2810 ($[M + Na]^+$ calcd for $C_{36}H_{42}F_3N_3NaO_8^+$ 724.2816).

*4.5.6.3. (**2R**,**3R**,**4R**,**5R**)-2,5-bis(benzyloxy)-3,4-dihydroxy-N1-((**1S**,**2R**)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)-N$^6$-((**S**)-1-(2-methoxy ethylamino)-3-methyl-1-oxobutan-2-yl)hexanediamide (**4c**).* The title compound was prepared from **5c** according to general procedure, vide supra, in 18% yield (51 mg, 75 μmol). $[\alpha]^{20}_D$ − 11.8 (*c* 0.38, $CDOD_3$); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.48–7.13 (m, 14H), 5.36 (d, 1H, *J* = 5.1 Hz), 4.69–4.55 (m, 6H), 4.23 (m, 2H), 4.14 (m, 3H), 3.45–3.32 (m, 4H), 3.29 (s, 3H), 3.16 (dd, 1H, *J* = 5.4, 16.4 Hz), 2.92 (dd, 1 H, *J* = 1.4, 16.4 Hz), 2.13 (m, 1H), 0.95 (d, 3H, *J* = 6.8 Hz), 0.91 (d, 3H, *J* = 6.8 Hz); $^{13}$C NMR (75 MHz, $CDOD_3$,) δ 173.8, 173.7, 173.5, 142.0, 141.8, 138.8, 138.7 129.5, 129.4, 129.3, 129.3, 129.0, 129.0, 129.0, 127.9, 126.2, 125.4, 81.6, 81.4, 74.0, 73.8, 73.6, 72.1, 72.1, 71.8, 59.9, 58.9, 58.6, 40.7, 40.2, 31.7, 19.8, 18.4; HRMS (ESI) *m*/*z* 678.3372 ($[M + H]^+$ calcd for $C_{37}H_{48}N_3O_9^+$ 678.3385).

*4.5.6.4. (**2R**,**3R**,**4R**,**5R**)-2,5-bis(benzyloxy)-3,4-dihydroxy-N1-((**1S**,**2R**)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)-N$^6$-((**2S**,**3S**)-4-methoxy-3-methyl-1-(methylamino)-1-oxobutan-2-yl)hexanediamide (**4d**).* The title compound was prepared from **16a** according to general procedure, vide supra, in 15% yield (6 mg, 9 μmol). $[\alpha]^{20}_D$ + 5.7 (*c* 0.35, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$,) δ 8.50 (d, 1H, *J* = 8.4 Hz), 7.44–7.12 (m, 14 H), 5.34 (dd, 1H, *J* = 5.1, 8.8 Hz), 5.27 (s, 1H), 5.09 (bs, 1H), 4.71–4.57 (m 4H), 4.46 (m, 1H), 4.20 (s, 1H), 4.16 (s, 2H), 4.03 (s, 1H), 3.55 (dd, 1H, *J* = 2.6, 9.8 Hz), 3.30 (dd, 1 H, *J* = 2.8, 9.8 Hz), 3.21–3.09 (m, 4H), 2.97 (d, 1H, *J* = 16.5 Hz), 2.76 (d, 3H, *J* = 4.7 Hz), 2.63 (m, 1H), 1.11 (d, 3H, *J* = 7.3 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$,) δ 172.6, 172.6, 170.6, 141.2, 139.5, 136.6, 136.5, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 127.2, 125.7, 124.0, 82.8, 82.5, 77.4, 75.2, 74.1, 73.7, 73.5, 73.3, 72.4, 59.4, 58.6, 58.2, 39.3, 33.4, 26.3, 15.3; HRMS (ESI) *m*/*z* 664.3213 ($[M + H]^+$ calcd for $C_{36}H_{46}N_3O_9^+$ 664.3229).

*4.5.6.5. (**2R**,**3R**,**4R**,**5R**)-2,5-bis(benzyloxy)-3,4-dihydroxy-N1-((1S,2R)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)-N$^6$-((**S**)-4-methoxy-1-(methyl-amino)-1-oxobutan-2-yl)hexanediamide (**4f**).* The title compound was prepared from **16c** according to general procedure, vide supra, in 28% yield (23 mg, 35 μmol). $[\alpha]^{20}_D$ − 3.0 (*c* 0.15, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$,) δ 8.28 (d, 1H, *J* = 7.9 Hz), 7.37–7.10 (m, 14 H), 5.34 (m 1H), 5.17 (bs, 1H), 5.01 (bs, 1H), 4.71–4.55 (m 5H), 4.18 (s, 1H), 4.15 (s, 2H), 4.03 (d, 1H, *J* = 2.3 Hz), 3.34 (m, 2H), 3.19–3.09 (m, 4H) 2.97 (d, 1H, *J* = 16.7 Hz), 2.75 (d, 3H, *J* = 4.7 Hz), 2.10 (m, 1H), 2.02 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$,) δ 172.5, 172.0, 170.7, 141.1, 139.6, 136.7, 136.7, 128.9, 128.8, 128.6, 128.5, 128.3, 128.3, 127.2, 125.6, 124.0, 82.6, 82.2, 77.4, 73.6, 73.6, 73.5, 73.0, 72.4, 70.6, 59.0, 58.5, 52.9, 39.4, 30.5, 26.4; HRMS (ESI) *m*/*z* 650.3054 ($[M + H]^+$ calcd for $C_{35}H_{44}N_3O_9^+$ 650.3072).

*4.5.7. General method for preparation of compounds **4e**, **4g** and **4h***

The benzyl carbamate (1 equiv) was dissolved in MeOH (3 mL) under an argon atmosphere and palladium on charcoal (~5%) was added followed by pressure reduction. The reaction mixture was left stirring under an $H_2$-atmosphere at 1 atm overnight. The Pd/C was filtered off and the filtrate concentrated to yield the free amine which was used in the next step without further purification. The amine (2 equiv) was solved in diisopropyl ethyl amine (DIPEA) (1 mL). The lactone **21** (1 equiv) and 2-hydroxypyridine (2 equiv) was added. The round bottom flask was mounted with a cooler and the reaction mixture was warmed (70 °C) and left stirring for 16 h. The solvent was removed and the residue purified by silica gel chromatography ($CH_2Cl_2$–MeOH 40:1) to yield compounds **4e**, **4g** and **4h** which were further purified on LC–MS.

*4.5.7.1. (**2R**,**3R**,**4R**,**5R**)-2,5-bis(benzyloxy)-3,4-dihydroxy-N1-((**1S**,**2R**)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)-N$^6$-((**2S**,**3R**)-4-methoxy-3-methyl-1-(methylamino)-1-oxobutan-2-yl)hexanediamide (**4e**).* The title compound was prepared from **16b** according to general procedure, vide supra, in 32% yield (9 mg, 13 μmol) and obtained as a white solid. $[\alpha]^{20}_D$ + 40.3 (*c* 0.3, MeOH); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, 1H, *J* = 8.6 Hz), 7.39–7.18 (m, 14H), 7.14 (m, 1H), 6.92 (m, 1H), 5.34 (dd, 1H, *J* = 4.9, 8.6 Hz), 4.70–4.63 (m 5H), 4.50 (dd, 1H, *J* = 3.6, 8.7 Hz), 4.18–4.12 (m, 3H), 4.06 (d, 1H, *J* = 3.0 Hz), 3.31 (d, 2H, *J* = 6.4 Hz), 3.22 (s, 3H), 3.12 (dd, 1H, *J* = 5.4, 16.5 Hz), 2.95 (dd, 1 H, *J* = 1.3, 16.5 Hz), 2.71 (d, 3H, *J* = 4.7 Hz), 2.37 (m, 1H) 1.80 (bs, 2H), 0.98 (d, 3H, *J* = 7.1 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.8, 170.5, 141.1, 139.7, 136.7, 136.6, 128.9, 128.8, 128.6, 128.5, 128.3, 128.3, 127.2, 125.6, 124.1, 82.3, 81.9, 77.4, 75.6, 73.6, 73.6, 73.3, 72.7, 72.5, 59.1, 58.3, 55.8, 39.4, 35.5, 26.3, 13.5; HRMS (ESI) *m*/*z* 686.3036 ($[M + Na]^+$ calcd for $C_{36}H_{45}N_3NaO_9^+$ 686.3048).

*4.5.7.2. N1-[(**1R**,**2S**)-2-Hydroxy-2,3-dihydro-1H-indenyl]-N6-[N(methyl)-2-oxetan-amide]-(**2R**,**3R**,**4R**,**5R**)-2,5-dibenzyloxy-3,4-O-dihydroxyhexanediamide (**4g**).* The title compound was prepared from **17a** according to general procedure, vide supra, in 61% yield (33 mg, 50 μmol) and obtained as white solid. $[\alpha]^{20}_D$ + 25.5 (*c* 0.1, MeOH); $^1$H NMR (400 MHz, $CD_3OD$,) δ 7.40–7.15 (m, 14H), 5.37 (d, 1H, *J* = 5.1 Hz), 4.78 (m, 2H), 4.71–4.53 (m, 7H), 4.49 (m, 1H), 4.21 (m, 2H), 4.14 (m, 3H) 3.65 (m, 1H), 3.39 (m, 1H), 3.17 (dd, 1H, *J* = 5.2, 16.5 Hz), 2.94 (dd, 1 H, *J* = 1.4, 16.4 Hz), 2.69 (s, 3H); $^{13}$C NMR (100 MHz, $CD_3OD$), δ 174.5, 173.9, 172.7, 142.0, 141.8, 138.6, 129.5, 129.5, 129.4, 129.3, 129.1, 129.0, 127.9, 126.2, 125.4, 82.0, 80.7, 75.7, 75.4, 73.9, 73.5, 72.7, 71.6, 58.6, 55.9, 40.8, 38.2, 26.3; HRMS (ESI) *m*/*z* 670.2721 ($[M + Na]^+$ calcd for $C_{35}H_{41}N_3NaO_9^+$ 670.2735).

*4.5.7.3. N1-[(**1R**,**2S**)-2-Hydroxy-2,3-dihydro-1H-indenyl]-N6-[N(methyl)-2-oxetan-amide]-(**2R**,**3R**,**4R**,**5R**)-2,5-dibenzyloxy-3,4-O-dihydroxyhexanediamide (**4h**).* The title compound was prepared from **17b** according to general procedure, vide supra, in 35% yield

(10 mg, 15 μmol) and obtained as an oil. $[\alpha]^{20}_D + 3.7$ (*c* 0.2, MeOH); $^1$H NMR (400 MHz, $CD_3OD$,) $\delta$ 7.40–7.16 (m, 14H), 5.37 (d, 1H, $J = 5.1$ Hz), 4.81–4.75 m (2H), 4.72–4.54 (m, 7H), 4.48 (t, 1H, $J = 6.4$ Hz), 4.21 (m, 1H), 4.13 (m, 3H) 3.65 (m, 1H), 3.41 (m, 1H), 3.17 (dd, 1H, $J = 5.1$, 16.4 Hz), 2.93 (dd, 1 H, $J = 1.4$, 16.4 Hz), 2.70 (s, 3H), 1.61 (bs, 1H); $^{13}$C NMR (125 MHz, $CD_3OD$,) $\delta$ 174.3, 173.8, 172.7, 142.0, 141.8, 138.8, 138.7, 129.5, 129.4, 129.3, 129.1, 129.0, 127.9, 126.2, 125.4, 81.5, 81.4, 75.7, 75.5, 74.0, 73.8, 73.6, 72.5, 72.1, 58.6, 56.1, 40.8, 38.2, 26.3; HRMS (ESI) *m/z* 648.2921 ($[M + H]^+$ calcd for $C_{35}H_{42}N_3O_9^+$ 648.2916).

*4.5.7.4. (**R**)-2-(benzyloxycarbonylamino)-2-(oxetan-3-yl)acetic acid (**20a**).* To a stirred mixture of **19a** (80 mg, 0.30 mmol) in MeOH (2 mL) was added NaOH (1 M, 0.40 mL). After stirring for 2 h in room temperature the mixture was concentrated to approx. 1 mL thereafter diluted with $H_2O$ (4 mL). The aqueous solution was extracted with $Et_2O$ (2 × 5 mL) before being transferred to a round bottom flask and $Et_2O$ (5 mL) was added. During vigorous stirring of the mixture, the pH was set to ~2 using HCl (1 M.). The phases were separated and the aqueous phase was extracted with $Et_2O$ (5 mL). The pooled organic phases were washed with brine, dried ($Na_2SO_4$), concentrated and purified on a Sep-Pak C18 column ($H_2O$–MeOH, gradient 20:1–1:1). The product **20a** was obtained in 33% yield (26 mg, 0.098 mmol) as a white solid. $[\alpha]^{20}_D + 30.4$ (*c* 1.0, MeOH); $^1$H NMR (400 MHz, $CD_3OD$,) $\delta$ 7.38–7.27 (m, 5H), 5.10 (s, 2H), 4.46 (t, 1H, $J = 8.7$ Hz), 4.30 (d, 1H, $J = 11.0$ Hz), 4.14 (t, 1H, $J = 9.4$ Hz), 3.75–3.62 (m, 2H), 2.76 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 177.2, 158.5, 137.9, 129.5, 129.4, 129.1, 128.9, 128.7, 69.4, 67.9, 60.6, 52.9, 44.9.

*4.5.7.5. (+)-3-Oxetanylglycine.* The benzyl carbamate **20a** (26 mg, 0.098 mmol) was solved in MeOH (5 mL) under an argon atmosphere and palladium on charcoal (~5%) was added followed by pressure reduction. The reaction mixture was left stirring under an $H_2$-atmosphere at 1 atm overnight. The Pd/C was filtered off and the filtrate concentrated to obtain the title compound as a white solid in 78% yield (10 mg, 0.076 mmol). $[\alpha]^{20}_D + 18.2$ (*c* 1.0, $H_2O$) this is in accordance with published data [36].

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