Potent HIV-1 protease inhibitors incorporating *meso*-bicyclic urethanes as P2-ligands: structure-based design, synthesis, biological evaluation and protein–ligand X-ray studies†

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Recently, we designed a series of novel HIV-1 protease inhibitors incorporating a stereochemically defined bicyclic fused cyclopentyl (Cp-THF) urethane as the high affinity P2-ligand. Inhibitor **1** with this P2-ligand has shown very impressive potency against multi-drug-resistant clinical isolates. Based upon the **1**-bound HIV-1 protease X-ray structure, we have now designed and synthesized a number of *meso*-bicyclic ligands which can conceivably interact similarly to the Cp-THF ligand. The design of *meso*-ligands is quite attractive as they do not contain any stereocenters. Inhibitors incorporating urethanes of bicyclic-1,3-dioxolane and bicyclic-1,4-dioxane have shown potent enzyme inhibitory and antiviral activities. Inhibitor **2** ($K_i = 0.11$ nM; $IC_{50} = 3.8$ nM) displayed very potent antiviral activity in this series. While inhibitor **3** showed comparable enzyme inhibitory activity ($K_i = 0.18$ nM) its antiviral activity ($IC_{50} = 170$ nM) was significantly weaker than inhibitor **2**. Inhibitor **2** maintained an antiviral potency against a series of multi-drug resistant clinical isolates comparable to amprenavir. A protein–ligand X-ray structure of **3**-bound HIV-1 protease revealed a number of key hydrogen bonding interactions at the S2-subsite. We have created an active model of inhibitor **2** based upon this X-ray structure.

Introduction

The proteolytic enzyme HIV-1 protease is essential for viral assembly and maturation.[1] As a consequence, the design of specific inhibitors for HIV-1 protease has become the subject of immense interest. In 1996, protease inhibitors (PIs) were introduced in combination with reverse transcriptase inhibitors to become a highly active antiretroviral therapy (HAART).[2] This treatment regimen significantly increased life expectancy, improved quality of life and decreased mortality and morbidity among HIV/AIDS patients. Despite these notable advances, the emergence of drug-resistant HIV-1 variants is severely limiting the efficacy of HAART treatment regimens. Therefore, the development of new broad-spectrum antiretroviral drugs that produce minimal adverse effects remains an important therapeutic objective for the treatment of HIV/AIDS.[3] We have recently reported our structure-based design and development of a series of novel HIV-1 protease inhibitors including darunavir,[4,5] TMC-126,[6] and GRL-06579A (**1**, Fig. 1).[7] These inhibitors were designed with specific features to help combat drug resistance. They have exhibited marked potency in enzyme inhibitory and cell-culture assays. Furthermore, these inhibitors have shown impressive activity against a broad-spectrum of HIV isolates including a variety of multi-PI-resistant clinical strains. Darunavir has been recently approved for the therapy of HIV/AIDS patients who are harboring drug-resistant HIV and do not respond to other antiretroviral drugs.

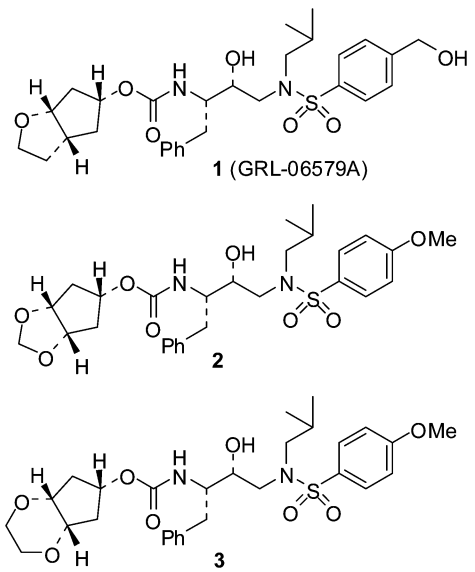

Fig. 1 Structure of inhibitors **1–3**.

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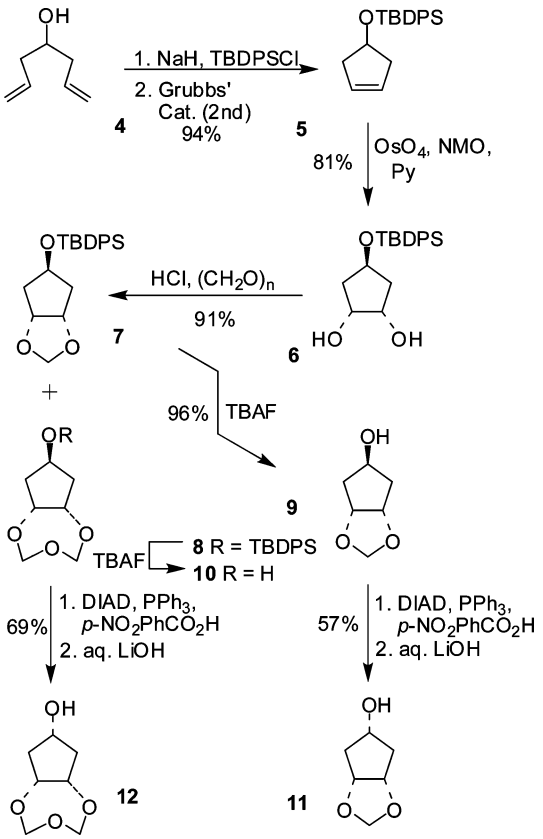
† Electronic supplementary information (ESI) available: HPLC and HRMS data of inhibitors **2–3** and **26–30**; crystallographic data collection and refinement statistics. See DOI: 10.1039/b809178a

One of our design principles to combat drug resistance is to maximize the ligand-binding interactions in the active site and particularly to promote extensive hydrogen bonding with the active site protein backbone. Indeed, inhibitor **1** incorporates a stereochemically defined bicyclic cyclopentanyltetrahydrofuran (Cp-THF) as the P2-ligand in the hydroxylethylsulfonamide isostere. The protein–ligand X-ray structure of inhibitor **1** revealed extensive hydrogen bonding interactions with the backbone atoms throughout the enzyme active site.[8] The cyclic ether oxygen is involved in hydrogen bonding with the backbone NH of Asp29. The presence of this oxygen is critical for its superb antiviral properties, especially against drug resistant HIV strains. Based upon further examination of the protein–ligand X-ray structure of **1**-bound HIV-1 protease, we subsequently speculated that a simplified *meso*-hexahydrocyclopenta-1,3-dioxolane ligand could conceivably maintain similar interactions with respect to the Cp-THF ligand in inhibitor **1**. Particularly, it appears that one of the oxygens of this *meso* ligand can hydrogen bond with the Asp29 NH. Since the Cp-THF ligand in inhibitor **1** contains three chiral centers, incorporation of a *meso* ligand as shown in inhibitor **2** would remarkably simplify the synthesis compared to the bicyclic Cp-THF ligand. Furthermore, we speculated that the second oxygen atom in the *meso*-P2-ligand could conceivably engage in further interactions at the S2-subsite. Herein, we report the design, synthesis and biological investigation of a series of protease inhibitors that incorporate structure-based designed symmetrical *meso*-bicyclic 1,3-dioxolane and 1,3-dioxane derivatives as the P2-ligands. Inhibitors (**2** and **3**) incorporating these ligands have shown exceedingly potent enzyme inhibitory potency as well as antiviral activity. Furthermore, we evaluated the drug-resistance profile of inhibitor **2** against multi-drug-resistant clinical isolates and it was shown to maintain tremendous potency. The protein–ligand X-ray structure of **3**-bound HIV-1 protease has been determined and this structure has provided molecular insight into the ligand-binding site interactions.

Chemistry

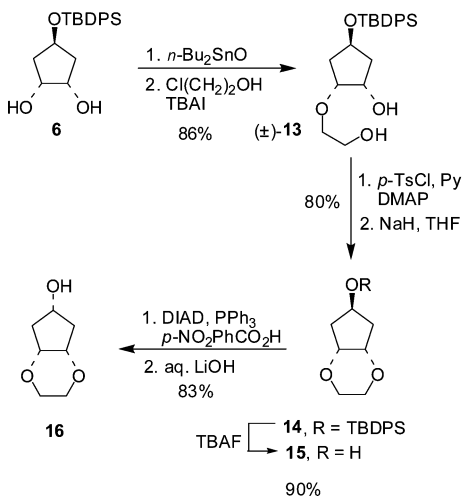
The hexahydrocyclopenta-1,3-dioxolan-5-ol (**11**), required for the synthesis of **2**, was prepared as described in Scheme 1. Commercially available 1,6-heptadien-4-ol **4** was protected as the corresponding *t*-butyldiphenylsilyl ether using sodium hydride as the base in THF. The resulting diene was subjected to a ring closing metathesis reaction using second generation Grubbs' catalyst to afford the protected cyclopenten-1-ol **5** in 94% overall yield. Osmium tetroxide-promoted dihydroxylation of olefin **5** was accomplished using a catalytic amount of osmium tetroxide and NMO and pyridine to afford diol **6** as a 6 : 1 mixture of *anti*- and *syn*-isomers which were easily separated by column chromatography. The *anti*- isomer **6** was subsequently treated with paraformaldehyde, preliminarily cracked with aqueous hydrochloric acid in chloroform under reflux,[9] affording the cyclic acetal **7** in good yield. Along with the desired compound **7**, the trioxepane **8** was also isolated from the reaction mixture in a 1 : 1 ratio. We therefore decided to incorporate the tetrahydro-5a*H*-cyclopenta[*f*][1,3,5]trioxepan-7-yl-moiety as a P2-ligand (resulting in inhibitors **27**–**28**, Table 1) because the higher flexibility of the trioxepane ring could allow an improved adaptability to enzyme amino acid mutations, leading to better activity against HIV-



Scheme 1 Synthesis of alcohols **9**–**12**.

resistant strains. Accordingly, both intermediates **7** and **8** were deprotected using tetrabutylammonium fluoride (TBAF) in THF to provide the *anti*-alcohols **9** and **10**. Compounds **9** and **10** were subsequently subjected to Mitsunobu inversion to afford the corresponding *syn*-alcohols **11** and **12**.

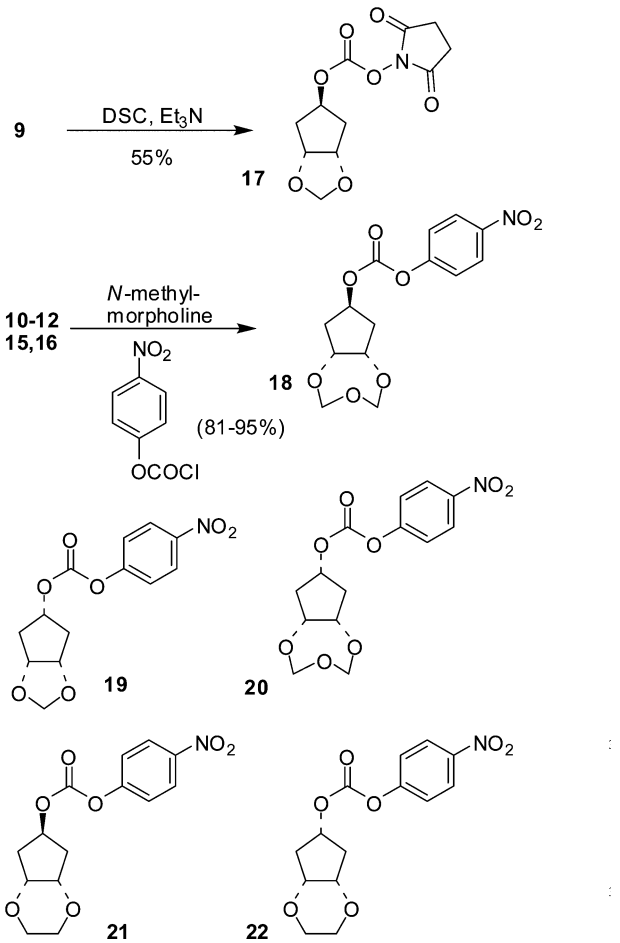
For the preparation of inhibitors **3** and **29**, alcohols **15** and **16** were synthesized as described in Scheme 2. Diol **6** was heated under reflux in toluene in the presence of dibutyltin oxide with azeotropic removal of water. The resulting stannylene acetal intermediate was treated with chloroethanol to obtain the monoalkylated derivative **13** in 68% overall yield.[10] Subsequently, the primary



Scheme 2 Synthesis of alcohols **15** and **16**.

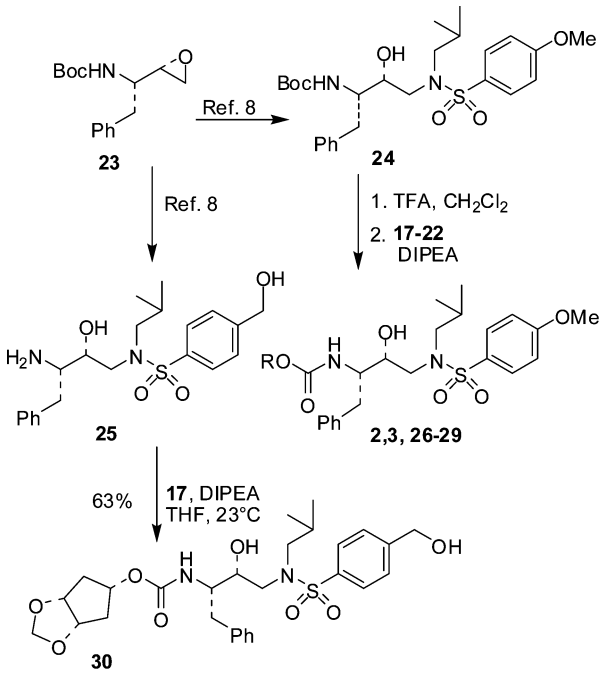
alcohol was selectively tosylated with *p*-toluenesulfonyl chloride in the presence of pyridine. Exposure of the resulting compound to sodium hydride resulted in an intramolecular substitution reaction leading to the corresponding cyclization compound **14**. TBAF-mediated deprotection furnished the target *anti*-alcohol **15** in good overall yield. The *syn*-alcohol **16** was then obtained after Mitsunobu inversion of **15** as described above.

The synthesis of the active carbonates required for the synthesis of the various inhibitors is shown in Scheme 3. Alcohol **9** was converted to the succinimidyl-derivative **17** by treatment with *N*,*N*′-succinimidylcarbonate in the presence of Et_3N as described previously.[11] Alcohols **10–12**, **15** and **16** were activated by conversion to the corresponding *p*-nitrophenylcarbamates **18–22** (81–95% yield) by using *p*-nitrophenylchloroformate and *N*-methylmorpholine in THF. The general procedure for the synthesis of inhibitors **2**, **3** and **26–30** is outlined in Scheme 4. Epoxide **23**[12] was converted into intermediate **24** following our previously reported procedure.[8] Deprotection of **24** by using trifluoroacetic acid followed by reaction with the activated alcohols **17–22** furnished inhibitors **2**, **3** and **26–29** in 43–85% yields.



Scheme 3 Synthesis of activated alcohols **17–22**.

Finally, inhibitor **30** was synthesized from the known[8] amine **25**. This amine was reacted with the activated carbonate **17** in the presence of diisopropylethylamine in THF at 23 °C to provide **30**. Inhibitor **30** was obtained in 63% yield.



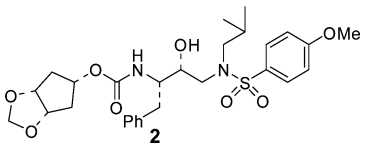
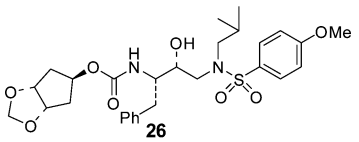
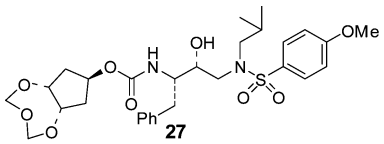
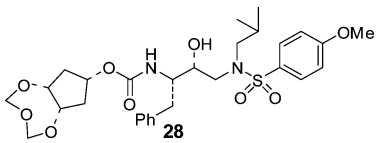
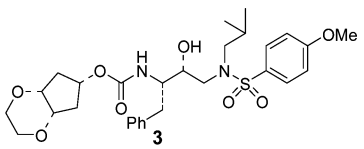
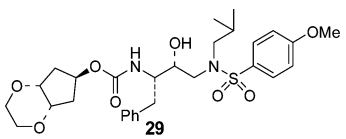
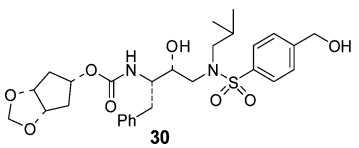
Scheme 4 Synthesis of inhibitors **2**, **3** and **26–30**.

Results and discussion

The inhibitory potencies of the synthetic inhibitors were evaluated using the assay protocol of Toth and Marshall,[13] and the results are shown in Table 1. As can be seen, inhibitor **2** has shown an enzyme inhibitory potency of 0.11 nM. It appears that the bicyclic 1,3-dioxolane ring can be accommodated by the S2-subsite of HIV-1 protease. Inhibitor **26** with a *meso* ligand containing a *trans*-bicyclic-1,3-dioxolane ring is 2.5-fold less potent than the *syn*-isomer **2**. We have examined the effect of both *syn* and *anti*-trioxepane rings as P2-ligands in inhibitors **27** and **28**. The *syn*-isomer **28** is significantly more potent ($K_i = 0.51$ nM) than the *anti*-isomer **27**. Considering the acid sensitivity of 1,3-dioxolane rings, we not only speculated that the stable 1,4-dioxane ring may fill the hydrophobic S2-site, but also that the oxygens on the dioxane ring may interact with backbone atoms or residues in the active site. As shown, the *meso* ligand in inhibitor **3** with a *syn*-bicyclic-1,4-dioxane ring has shown an enzyme inhibitory potency of 0.18 nM (K_i value). Consistent with previous results, the corresponding *anti*-isomer **29** is significantly less potent. As reported previously, the P2-ligand Cp-THF with a P2′-hydroxymethyl sulfonamide (inhibitor **1**) is significantly more potent than the corresponding P2′-methoxybenzene sulfonamide derivative. We have, therefore, compared the inhibitory potency of inhibitor **30**, containing a P2′-hydroxymethyl benzene sulfonamide derivative, with inhibitor **2**. However, inhibitor **30** did not exhibit this potency enhancing effect.

We have examined selected compounds for their activity against HIV-1 using a human CD4+ T-cell line (MT-2 cells). The activity of inhibitor **2** against a variety of multi-drug-resistant HIV-1 variants was also examined in detail using human peripheral blood mononuclear cells (PBMCs) as target cells. We employed two endpoints for the activity against HIV-1: (i) the inhibition of the HIV-1-elicited cytopathic effect for MT-2 cells and (ii) the inhibition of HIV-1 p24 production for PBMCs.[5]

Table 1 Enzymatic inhibitory activity of compounds **2**, **3**, **26–30** and antiviral activity of selected inhibitors against HIV-1_{LAI}

Entry	Inhibitor	K_i/nM[a]	IC_{50}/μM[b]
1	 **2**	0.11 ± 0.01	0.0038 ± 0.0001
2	 **26**	0.40 ± 0.04	nd
3	 **27**	5.4 ± 0.22	>1
4	 **28**	0.51 ± 0.01	0.38 ± 0.02
5	 **3**	0.18 ± 0.03	0.21 ± 0.04
6	 **29**	0.50 ± 0.04	nd
7	 **30**	0.34 ± 0.07	0.0077 ± 0.003

[a] Values are means of at least two experiments. [b] MT-2 human T-lymphoid cells exposed to HIV-1_{LAI}; antiviral activity of amprenavir (APV), saquinavir (SQV) and indinavir (IDV) were 0.03 μM, 0.02 μM and 0.03 μM respectively in this assay. nd: not determined.

When examined in MT-2 cells as the target cells, inhibitor **2** displayed an impressive antiviral IC_{50} of 3.8 nM (Table 1). Inhibitor **3** showed an antiviral IC_{50} value in the high nanomolar range (IC_{50} = 210 nM, Table 1), while it exhibited a similar K_i to inhibitor **2**. We subsequently examined inhibitor **2** for its activity against a clinical wild-type X4-HIV-1 isolate (HIV-$1_{ERS104pre}$) along with various multi-drug-resistant clinical X4- and R5-HIV-1 isolates (Table 2) using PBMCs as the target cells.[5] The activity of inhibitor **2** against HIV-$1_{ERS104pre}$ (IC_{50} = 29 nM) was comparable to those of currently available protease inhibitors, SQV, APV, and IDV, which display IC_{50} values of 12, 33, and 26 nM, respectively. Of particular note, the IC_{50} value of inhibitor **2** in PBMCs (IC_{50}= 29 nM) was nearly 8-fold greater than the IC_{50} value in MT-2 cells (IC_{50} = 3.8 nM). With regard to this difference, considering that **2** is highly potent as examined in human T-cells (MT-2 cells) but its activity is slightly less in PBMCs, it is possible that relatively higher concentrations of **2** are required to suppress HIV-1 production in chronically infected macrophages.[14] IDV was not capable of efficiently suppressing the replication of most of the multi-drug-resistant clinical isolates examined (HIV-$1_{MDR/MM}$, HIV-$1_{MDR/JSL}$, HIV-$1_{MDR/C}$, and HIV-$1_{MDR/A}$), with IC_{50} values of >1.0 μM. The potency of inhibitor **2** against most of the multi-drug-resistant variants was generally comparable to that of SQV and APV, although DRV was found to be the most potent among those tested, including inhibitor **2**, against HIV-$1_{ERS104pre}$ as well as all the multi-drug-resistant variants.

Table 2 Antiviral activity of inhibitor **2** against clinical HIV-1 isolates in PBMC cells

	IC_{50} values[a](nM)				
Virus[b]	**2**	DRV[c]	SQV[d]	APV[e]	IDV[f]
$HIV\text{-}1_{ERS104pre}$ (wild-type: X4)	29	3.5	12	33	26
$HIV\text{-}1_{MDR/MM}$(R5)	150 (5)	17 (5)	190 (16)	300 (9)	>1000 (>38)
$HIV\text{-}1_{MDR/JSL}$ (R5)	550 (19)	26 (7)	330 (28)	430 (13)	>1000 (>38)
$HIV\text{-}1_{MDR/C}$ (X4)	300 (10)	7 (2)	36 (3)	230 (7)	>1000 (>38)
$HIV\text{-}1_{MDR/G}$ (X4)	340 (12)	7 (2)	29 (2)	340 (10)	290 (11)
$HIV\text{-}1_{MDR/A}$ (X4)	21 (1)	3 (1)	81 (7)	100 (3)	>1000 (>38)

[a] Amino acid substitutions identified in the protease-encoding region compared to the consensus type B sequence cited from the Los Alamos database include L63P in $HIV\text{-}1_{ERS104pre}$; L10I, K43T, M46L, I54V, L63P, A71V, V82A, L90M, and Q92K in $HIV\text{-}1_{MDR/MM}$; L10I, L24I, I33F, E35D, M36I, N37S, M46L, I54V, R57K, I62V, L63P, A71V, G73S, and V82A in $HIV\text{-}1_{MDR/JSL}$; L10I, I15V, K20R, L24I, M36I, M46L, I54V, I62V, L63P, K70Q, V82A, and L89M in $HIV\text{-}1_{MDR/C}$; L10I, V11I, T12E, I15V, L19I, R41K, M46L, L63P, A71T, V82A, and L90M in $HIV\text{-}1_{MDR/G}$; and L10I, I15V, E35D, N37E, K45R, I54V, L63P, A71V, V82T, L90M, I93L, and C95F in $HIV\text{-}1_{MDR/A}$. $HIV\text{-}1_{ERS104pre}$ served as a source of wild-type HIV-1. The IC_{50} values were determined by employing PHA-PBMC (phytohemagglutinin-activated peripheral blood mononuclear cells) as target cells and the inhibition of p24Gag protein production as the endpoint. All values were determined in triplicate. [b] X4 denotes CXCR4-tropic HIV-1 while R5 CCR5-tropic HIV-1. [c] DRV (darunavir). [d] SQV (saquinavir). [e] APV (amprenavir). [f] IDV (indinavir).

X-Ray crystallography

To obtain molecular insight into the ligand-binding site interactions responsible for the impressive enzyme inhibitory potency of compound **3**, we determined the X-ray structure of **3**-bound HIV-1 protease. The crystal structure was solved and refined to an *R* factor of 15.2% at a 1.07 Å resolution. The inhibitor binds with extensive interactions from P2 to P2′ with the protease atoms, and most notable are the favorable polar interactions including hydrogen bonds, as shown in Fig. 2. The transition-state hydroxyl group forms hydrogen bonds to the side chain carboxylate oxygen atoms of the catalytic Asp25 and Asp25′. Of particular interest, the *meso*-bicyclic 1,4-dioxane ligand appears to be involved in hydrogen bonding interactions with the backbone atoms and residues at the S2-site. One of the dioxane oxygens hydrogen bonds with the backbone NH of Asp29. The other oxygen makes a water-mediated hydrogen bond with the carbonyl oxygen of Gly48. These interactions are described in several peptide substrate analogs.[15] However, the design of high affinity ligands incorporating this interaction with Gly48 has not been previously demonstrated. The inhibitor also hydrogen bonds with the protease main chain amide carbonyl oxygen of Gly27, and there are water-mediated interactions with the amides of Ile50 and Ile50′ that are conserved in the majority of protease complexes with inhibitors[16] and substrate analogs.[15] The weaker polar interactions such as C–H···O and water-π interactions can be analyzed accurately in atomic resolution structures.[17,18] Inhibitor **3** also shows a water-mediated interaction of the π system of the P2′ aromatic ring with the amide of Asp29′, which was also observed for darunavir and inhibitor **1**.[19] Furthermore, the P2′ methoxy group forms a hydrogen bond to the backbone NH of Asp30′. Importantly, the P2 group forms a hydrogen bond interaction with the carbonyl oxygen of Gly48 and a water-mediated interaction with the amide of Gly48, similar to the interactions described for several peptide substrate analogs.[15] These interactions of the P2 group confirm the design strategy of incorporating new polar interactions with conserved backbone regions of the protease.

In an effort to understand the binding interactions of the corresponding *meso*-1,3-dioxolane ligand in the S2-subsite, we have created an active model of inhibitor **2** (Fig. 3) based upon the X-ray structure of **3**-bound HIV-1 protease. The model suggests that both dioxolane oxygens may interact with both active site residues Asp29 and Asp30, as well as Gly48 through the structural water molecule. In comparison, it appears that the dioxane oxygens of inhibitor **3** are not within hydrogen bonding distance of the backbone NH of Asp30. This may explain the marked difference in antiviral activity of inhibitor **2** compared with inhibitor **3**.

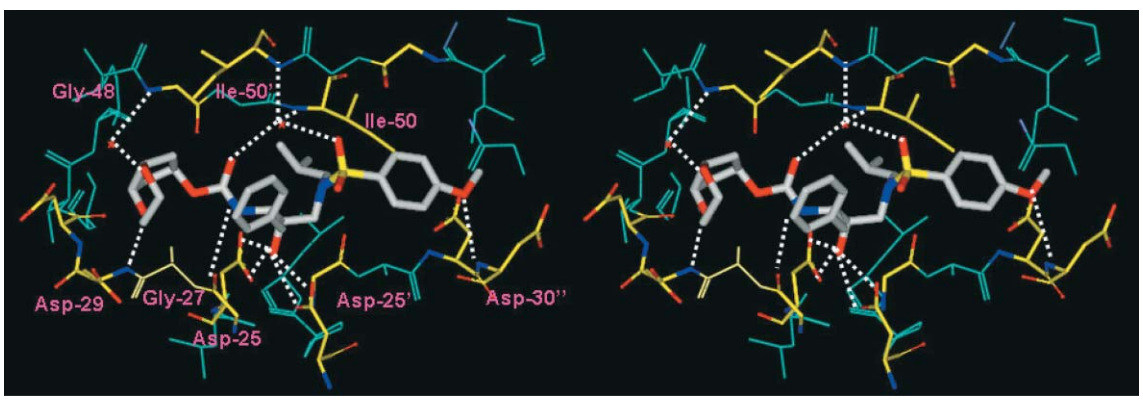


Fig. 2 Stereoview of the X-ray structure of inhibitor **3** bound to the active site of wild-type HIV-1 protease.

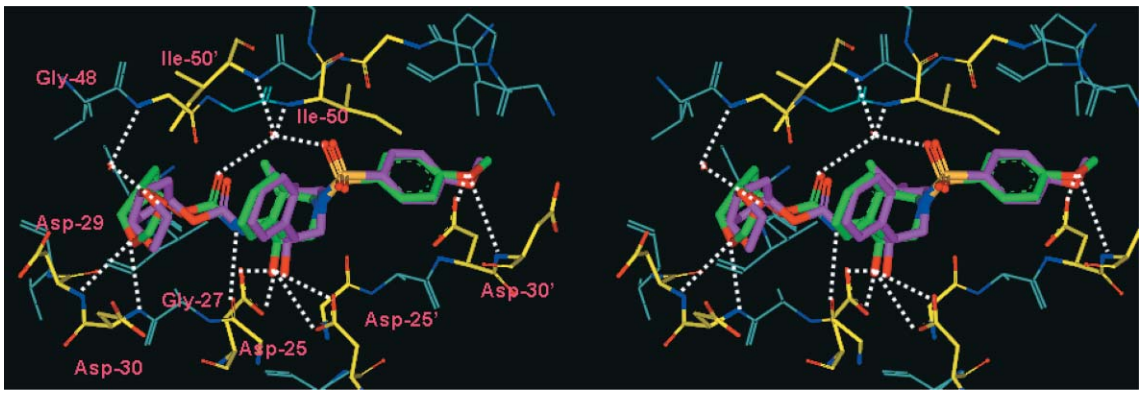


Fig. 3 A stereoview of an active model of inhibitor **2** (green) with the X-ray structure of inhibitor **3** (magenta)-bound HIV-1 protease.

Conclusions

In summary, a series of novel HIV-1 protease inhibitors were designed and synthesized by incorporating bicyclic *meso*-1,3-dioxolane and 1,4-dioxane derivatives as the P2-ligands. A number of inhibitors have shown very impressive enzyme inhibitory and antiviral potency, similar to inhibitor **1** with a stereochemically defined Cp-THF ligand. The design of *meso*-1,3-dioxolane and 1,4-dioxane P2-ligands as exemplified in inhibitors **2** and **3**, respectively, has remarkably simplified the stereochemical complexity as well as chemical synthesis over the Cp-THF ligand in inhibitor **1**. We have developed efficient synthetic routes to these ligands. Inhibitor **2** has shown potent antiviral activity in both MT-2 cells and PBMCs. Inhibitor **2** was profiled against a series of multi-drug-resistant clinical isolates. While inhibitor **2** is less potent than darunavir, it is significantly more potent than IDV and comparable to APV and SQV in suppressing the replication of multi-drug-resistant isolates MDR_{MM} and MDR_{JSL}. A protein–ligand X-ray structure of **3**-bound HIV-1 protease revealed extensive interactions of the inhibitor with the active site of HIV-1 protease. Most notably, both oxygens of the *meso*-P2-ligand are involved in hydrogen bonding interactions with the protein backbone atoms. In particular, a water-mediated hydrogen bond to the Gly48 carbonyl is very unique. An active model of inhibitor **2** indicates similar ligand binding site interactions. Our design principle of increasing 'backbone binding' appears to maintain key interactions in the enzyme active site leading to retained potency against multi-drug-resistant variants. Further design and ligand optimization involving these interactions is in progress.

Experimental

General. All moisture sensitive reactions were carried out under a nitrogen or argon atmosphere. Anhydrous solvents were obtained as follows: THF, diethyl ether, and benzene, distilled from sodium and benzophenone; dichloromethane, pyridine, triethylamine, and diisopropylethylamine, distilled from CaH_2. All other solvents were HPLC grade. Column chromatography was performed with Whatman 240–400 mesh silica gel under low pressure (5–10 psi). TLC was carried out with E. Merck silica gel 60 F_{254} plates. ^1H and ^{13}C NMR spectra were recorded on Varian Mercury 300 and Bruker Avance 400 and 500 spectrometers. Optical rotations were measured using a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Mattason Genesis II FT-IR spectrometer.

4-(*tert*-Butyldiphenylsilyloxy)-4*H*-cyclopentene (5)

To a suspension of sodium hydride (60% in mineral oil, 0.92 g, 23 mmol) in THF (10 mL), cooled to 0 °C, 1,6-heptadien-4-ol **4** (1 mL, 7.7 mmol) was added dropwise over 10 min. The resulting suspension was stirred at 0 °C for 30 min and then *tert*-butyldiphenylchlorosilane (2 mL, 7.9 mmol) was added. The reaction mixture was stirred at 23 °C for 4 h and then quenched with a saturated solution of ammonium chloride. The solvent was removed *in vacuo* and the aqueous phase was extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4), the solvent removed and the residue purified by flash-chromatography (1 : 10, EtOAc–Hex) to afford 4-(*tert*-butyldiphenylsilyloxy)hepta-1,6-diene (2.6 g, 96%) as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 3066, 2951, 1421, 1103 and 696; δ_H (300 MHz, $CDCl_3$) 7.70 (4 H, dd, *J* 1.6, 7.6 Hz, ArH), 7.47–7.38 (6 H, m, ArH), 5.83–5.69 (2 H, m, 2 × C*H*=CH_2), 5.02–4.91 (4 H, m, 2 × CH=CH_2), 3.87–3.80 (1 H, m, CHOSi), 2.31–2.12 (4 H, m, 3-H_2, 5-H_2) and 1.08 [9 H, s, $C(CH_3)_3$]; δ_C (75 MHz, $CDCl_3$) 135.9, 134.7, 134.3, 129.5, 127.5, 117.1, 72.4, 40.5, 27.0 and 19.4; *m*/*z* (CI) 351 (M + H, 100); HRMS (M + H)$^+$ calcd for $C_{23}H_{31}OSi$, 351.2144; found, 351.2146.

To a solution of the above compound (2.0 g, 5.7 mmol) in dry CH_2Cl_2 (20 mL), second generation Grubbs' catalyst (48 mg) was added and the resulting mixture was heated under reflux for 2 h. Subsequently, the reaction mixture was cooled to 23 °C, the solvent removed under reduced pressure and the residue purified by flash-chromatography (1 : 10 EtOAc–Hex) to afford **5** (1.8 g, 98%) as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 3067, 2853, 2736, 1428, 1109 and 702; δ_H (300 MHz, $CDCl_3$) 7.67 (4 H, dd, *J* 1.8, 7.8, ArH), 7.45–7.34 (6 H, m, ArH), 5.61 (2 H, s, 1-H, 2-H), 4.57–4.51 (1 H, m, 4-H), 2.47–2.33 (4 H, m, 3-H_2, 5-H_2) and 1.05 [9 H, s, $C(CH_3)_3$]; δ_C (100 MHz, $CDCl_3$) 135.7, 134.5, 129.5, 128.3, 127.5, 73.5, 42.4, 26.9 and 19.1; *m*/*z* (CI) 323 (M + H, 100); HRMS (M + H)$^+$ calcd for $C_{21}H_{27}OSi$, 323.1831; found, 323.1834.

(1α,2α,4β)-4-(*tert*-Butyldiphenylsilyloxy)-1,2-cyclopentanediol (6)

A mixture of **5** (5.1 g, 15.8 mmol), osmium tetroxide (2.5 wt% solution in *tert*-butanol, 4 mL), *N*-methylmorpholine-*N*-oxide (2.6 g, 22.2 mmol), and pyridine (1.3 mL, 15.8 mmol) in a

3 : 2 : 1 mixture of *tert*-butanol, THF, and water (80 mL) was heated under reflux for 4 h. The reaction mixture was cooled to 23 °C and treated with a 20% aqueous solution of sodium bisulfite (10 mL). The organic solvents were removed under reduced pressure and the aqueous phase was extracted with EtOAc. The organic extracts were washed with 1 N hydrochloric acid, water and brine, and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was purified by flash-chromatography (1 : 1 EtOAc–Hex) to yield diol **6** (5.3 g, 94%) as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 3006, 2676, 1427, 1112 and 702; δ_H (300 MHz, $CDCl_3$) 7.62 (4 H, dd, *J* 1.8, 7.5, ArH), 7.45–7.33 (6 H, m, ArH), 4.84–4.42 (1 H, m, 4-H), 4.30–4.29 (2 H, m, 1-H, 2-H), 2.22 (2 H, br. s, 2 × OH), 1.99–1.80 (4 H, m, 3-H_2, 5-H_2) and 1.04 [9 H, s, $C(CH_3)_3$]; δ_C (100 MHz, $CDCl_3$) 135.6, 134.0, 129.6, 127.6, 72.4, 71.2, 41.9, 26.8 and 19.0; *m/z* (EI) 356 (M, 100); HRMS $(M)^+$ calcd for $C_{21}H_{28}O_3Si$, 356.1808; found, 356.1803.

(1β,2β,4α)-4-(*tert*-Butyldiphenylsilyloxy)-1,2-(methylenedioxy)-cyclopentane (7) and (5aα,7β,8aα)-7-(*tert*-butyldiphenylsilyloxy)-tetrahydrocyclopenta[*f*]-1,3,5-trioxepane (8)

A mixture of paraformaldehyde (0.77 g, 25.7 mmol) and concentrated hydrochloric acid (2 mL) in $CHCl_3$ (2 mL) was stirred at 23 °C until a clear solution was formed (6 h) and then a solution of **6** (0.2 g, 0.54 mmol) in $CHCl_3$ (2 mL) was added. The resulting mixture was heated under reflux overnight and the aqueous phase was extracted with $CHCl_3$. The organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to yield **7** (0.18 g, 86%) after flash-chromatography (1 : 10, EtOAc–Hex): IR ν_{max} (NaCl; cm^{-1}) 2791, 1589, 1471, 1428, 822 and 699; δ_H (300 MHz, $CDCl_3$) 7.64 (4 H, d, *J* 6.3, ArH), 7.45–7.35 (6 H, m, ArH), 4.78 (1 H, s), 4.60 (1 H, s), 4.51 (2 H, d, *J* 5.4, 1-H, 2-H), 4.47–4.39 (1 H, m, 4-H), 1.99 (2 H, dd, *J* 6.0, 13.8, 3-H′, 5-H′), 1.77–1.68 (2 H, m, 3-H″, 5-H″) and 1.04 [9 H, s, $C(CH_3)_3$]; δ_C (75 MHz, $CDCl_3$) 135.6, 134.0, 129.7, 127.6, 94.0, 78.8, 72.7, 41.0, 26.9 and 19.1; *m/z* (EI) 368 (M, 100). After further elution of the column **8** (0.5 g, 5%) was obtained: IR ν_{max} (NaCl; cm^{-1}) 2827, 2726, 1427, 1113 and 703; δ_H (300 MHz, $CDCl_3$) 7.66–7.62 (4 H, m, ArH), 7.46–7.35 (6 H, m, ArH), 5.17 (2 H, d, *J* 7.8, 2-H′, 4-H′), 4.70 (2 H, d, *J* 7.8, 2-H″, 4-H″), 4.52–4.43 (3 H, m, 5a-H, 7-H, 8a-H), 2.15–2.08 (2 H, m, 6-H′, 8-H′), 1.93–1.85 (2 H, m, 6-H″, 8-H″) and 1.06 [9 H, s, $C(CH_3)_3$]; δ_C (75 MHz, $CDCl_3$) 135.6, 133.8, 129.7, 127.7, 96.1, 82.5, 71.7, 41.1, 26.9 and 19.1; *m/z* (CI) 397 (M − H, 100); HRMS $(M - H)^+$ calcd for $C_{23}H_{29}O_4Si$, 397.1832; found, 397.1832.

(4α,1β,2β)-4-Hydroxy-1,2-(methylenedioxy)cyclopentane (9)

A mixture of **7** (0.47 g, 1.3 mmol) and *n*-$Bu_4N^+F^-$ (1.0 M solution in THF, 1.4 mL, 1.4 mmol) in dry THF (10 mL) was stirred at 23 °C for 16 h. To the reaction mixture was added a saturated solution of $NaHCO_3$, the solvent was removed *in vacuo* and the aqueous phase extracted with Et_2O. The organic extracts were dried (Na_2SO_4) and evaporated and the residue was purified by flash-chromatography (1 : 1 EtOAc–Hex) to yield **9** (0.16 g, 96%) as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 3044, 2792, 2602, 1065, 821 and 602; δ_H (300 MHz, $CDCl_3$) 4.89 (1 H, s, OC*H*HO), 4.59 (1 H, s, OCH*H*O), 4.50 (2 H, d, *J* 6.0, 1-H, 2-H), 4.41–4.32 (1 H, m, 4-H), 3.13 (1 H, br. s, OH), 2.09 (2 H, dd, *J* 5.6, 14.0, 3-H′, 5-H′) and 1.61–1.51 (2 H, m, 3-H″, 5-H″); δ_C (75 MHz, $CDCl_3$) 94.1, 78.9, 70.8 and 40.6; *m/z* (CI) 129 (M − H, 100); HRMS $(M - H)^+$ calcd for $C_6H_9O_3$, 129.0552; found, 129.0556.

(5aα,7β,8aα)-7-Hydroxytetrahydrocyclopenta[*f*]-1,3,5-trioxepane (10)

The title compound was obtained as described for **9** in 83% yield. Flash-chromatography was performed using EtOAc: IR ν_{max} (NaCl; cm^{-1}) 3036, 2649, 1424, 1118 and 930; δ_H (300 MHz, $CDCl_3$) 5.15 (2 H, d, *J* 7.2, 2-H′, 4-H′), 4.67 (2 H, d, *J* 7.2, 2-H″, 4-H″), 4.47–4.40 (3 H, m, 5a-H, 7-H, 8a-H), 2.07–2.02 (4 H, m, 6-H_2, 8-H_2) and 1.86 (1 H, br. s, OH); δ_C (75 MHz, $CDCl_3$) 96.1, 82.3, 70.0 and 40.8; *m/z* (EI) 160 (M, 100); HRMS $(M)^+$ calcd for $C_7H_{12}O_4$,160.0736; found, 160.0738.

(1β,2β,4β)-4-Hydroxy-1,2-(methylenedioxy)cyclopentane (11)

To a mixture of **9** (100 mg, 0.77 mmol), *p*-nitrobenzoic acid (250 mg, 1.5 mmol), and triphenylphosphine (450 mg, 1.5 mmol), was added diisopropylazodicarboxylate (300 μL, 1.5 mmol) dropwise and the resulting mixture was stirred at 23 °C. After 16 h, the solvent was removed under reduced pressure and the residue purified by flash-chromatography (1 : 2 EtOAc–Hex). The resulting ester was dissolved in a 3 : 2 : 1 mixture of THF, methanol, and water (10 mL) and $LiOH \cdot H_2O$ (162 mg, 3.8 mmol) was added. The yellow mixture was stirred at 23 °C for 5 h and then the solvent was removed *in vacuo.* The residue was diluted with water and the aqueous phase extracted with Et_2O. The organic extracts were dried (Na_2SO_4) and the solvent was evaporated. Purification of the residue by flash-chromatography (1 : 1 EtOAc–Hex) afforded **11** (57 mg, 57%) as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 3052, 2804, 2577, 1164, 1096, 1011 and 924; δ_H (300 MHz, $CDCl_3$) 5.17 (1 H, s, OC*H*HO), 4.68 (1 H, s, OCH*H*O), 4.61 (2 H, d, *J* 4.8, 1-H, 2-H), 4.27 (1 H, t, *J* 4.7, 4-H), 2.33 (1 H, br. s, OH), 2.21 (2 H, d, *J* 15.3, 3-H′, 5-H′) and 1.85–1.77 (2 H, m, 3-H″, 5-H″); δ_C (75 MHz, $CDCl_3$) 94.7, 81.5, 74.0 and 41.0; *m/z* (EI) 129 (M − H, 100); HRMS $(M - H)^+$ calcd for $C_6H_9O_3$, 129.0611; found, 129.1012.

(5aα,7α,8aα)-7-Hydroxytetrahydrocyclopenta[*f*]-1,3,5-trioxepane (12)

The title compound **12** was obtained as described for **11** in 69% yield. Flash-chromatography was performed using EtOAc: IR ν_{max} (NaCl; cm^{-1}) 3044, 2832, 2633, 1481, 1116 and 928; δ_H (300 MHz, $CDCl_3$) 5.18 (2 H, d, *J* 7.2, 2-H′, 4-H′), 4.67 (2 H, d, *J* 7.2, 2-H″, 4-H″), 4.31–4.25 (2 H, m, 5a-H, 8a-H), 4.18–4.13 (1 H, m, 7-H), 2.40 (1 H, br. s, OH), 2.17–2.08 (2 H, m, 6-H′, 8-H′) and 2.03–1.96 (2 H, m, 6-H″, 8-H″); δ_C (75 MHz, $CDCl_3$) 95.3, 82.8, 71.0 and 41.1; *m/z* (CI) 161 (M + H, 100); HRMS $(M + H)^+$ calcd for $C_7H_{12}O_4$, 161.0814; found, 161.0814.

(±)-(1β,2β,4α)-2-(2′-Hydroxyethoxy)-4-(*tert*-butyldiphenylsilyloxy)cyclopentane-1-ol (13)

A mixture of **6** (1.4 g, 3.9 mmol) and dibutyltin oxide (0.94 g, 3.9 mmol) in dry toluene (130 mL) was heated under reflux with azeotropic removal of water. After 5 h, the reaction mixture was concentrated to half the initial volume and chloroethanol (2.5 mL,

39 mmol) and *n*-$Bu_4N^+I^-$ (1.4 g, 3.9 mmol) were added. The resulting mixture was heated under reflux for 19 h. Afterwards the solvent was evaporated and the residue was purified by flash-chromatography (10 : 1 EtOAc–MeOH) to afford **13** (1.3 g, 86%) as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 3102, 2604, 1589, 1471, 1062, 823 and 612; δ_H (300 MHz, $CDCl_3$) 7.62 (4 H, d, *J* 8.7, ArH), 7.44–7.33 (6 H, m, ArH), 4.45–4.40 (1 H, m, 4-H), 4.33–4.28 (1 H, m, 2-H), 4.04–3.98 (1 H, m, 1-H), 3.76–3.71 (2 H, m, CH_2O), 3.66–3.55 (2H, m, CH_2O), 3.01 (2 H, br. s, 2 × OH), 1.97–1.80 (4 H, m, 3-H_2, 5-H_2) and 1.04 (s, 9H); δ_C (75 MHz, $CDCl_3$) 135.6, 134.1, 129.6, 127.6, 80.7, 71.1, 71.0, 70.8, 61.7, 42.3, 39.0, 26.9 and 14.2; *m*/*z* (ESI) 423 (M + Na, 100).

(1β,2β,4α)-4-(*tert*-butyldiphenylsilyloxy)-1,2-(ethylenedioxy)-cyclopentane (14)

A mixture of **13** (1.2 g, 3.0 mmol), *p*-toluenesulfonyl chloride (1.3 mg, 6.6 mmol), pyridine (1.2 mL, 15 mmol) and a catalytic amount of *N*,*N*-dimethylaminopyridine in CH_2Cl_2 (40 mL) was stirred at 23 °C for 24 h. The reaction mixture was treated with 1 N HCl and the aqueous phase was extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and the solvent removed. Purification of the residue by flash-chromatography (1 : 1 EtOAc–Hex) afforded the tosylated alcohol (990 mg, 60%) as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 3104, 2992, 2691, 1598, 1359, 1177, 923 and 705; δ_H (300 MHz, $CDCl_3$) 7.76 (2 H, d, *J* 8.4, ArH), 7.61 (4 H, d, *J* 7.8, ArH), 7.42–7.26 (8 H, m, ArH), 4.53–4.25 (1 H, m, CHO), 4.15–4.09 (3 H, m, CHO, CH_2O), 3.96–3.91 (1 H, m, CHO), 3.68–3.62 (2 H, m, CH_2O), 2.41 (3 H, s, CH_3), 1.89–1.75 (4 H, m, 3-H_2, 5-H_2) and 1.03 [9 H, s, $C(CH_3)_3$]; δ_C (100 MHz, $CDCl_3$) 135.6, 134.0, 132.8, 129.8, 129.6, 127.9, 127.8, 127.6, 80.7, 71.3, 71.0, 69.0, 68.6, 67.0, 42.1, 38.7, 26.8, 21.6 and 18.9. To a solution of the above product (150 mg, 0.27 mmol) in dry THF (12 mL), NaH (60% in mineral oil, 22 mg, 0.54 mmol) was added and the resulting suspension was heated under reflux for 30 min. After cooling to 23 °C, the reaction mixture was quenched with a saturated solution of NH_4Cl, the solvent was removed and the aqueous phase was extracted with EtOAc. The organic extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue was purified by flash-chromatography (1 : 3 EtOAc–Hex) to afford **14** (82 mg, 80%) as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 2803, 1427, 1136, 957 and 703; δ_H (300 MHz, $CDCl_3$) 7.65 (4 H, d, *J* 7.8, ArH), 7.46–7.36 (6 H, m, ArH), 4.55–4.48 (1 H, m, 4-H), 4.18 (2 H, t, *J* 5.1, 1-H, 2-H), 3.70–3.62 (2 H, m, CH_2O), 3.53–3.46 (2 H, m, CH_2O), 2.16–2.07 (2 H, m, 3-H′, 5-H′), 1.82–1.74 (2 H, m, 3-H″, 5-H″) and 1.06 [9 H, s, $C(CH_3)_3$]; δ_C (75 MHz, $CDCl_3$) 135.6, 134.1, 129.5, 127.6, 75.2, 71.1, 62.2, 37.5, 27.0 and 19.1; *m*/*z* (CI): 383.25 (M + H, 100).

(1β,2β,4α)-4-Hydroxy-1,2-(ethylendioxy)cyclopentane (15)

The above compound was deprotected as described for **9** to afford **15** in 90% yield as a colorless oil: IR ν_{max} (NaCl; cm^{-1}) 3013, 2797, 2550, 1129 and 664; δ_H (300 MHz, $CDCl_3$) 4.58–4.51 (1 H, m, 4-H), 4.17 (2 H, t, *J* 4.8, 1-H, 2-H), 3.78–3.71 (2 H, m, CH_2O), 3.58–3.51 (2 H, m, CH_2O), 2.34–2.25 (2 H, m, 3-H′, 5-H′) and 1.72–1.66 (3 H, m, 3-H″, 5-H″, OH); δ_C (75 MHz, $CDCl_3$) 75.1, 69.6, 62.3 and 37.2; *m*/*z* (EI) 144 (M, 100).

(1β,2β,4β)-4-Hydroxy-1,2-(ethylendioxy)cyclopentane (16)

Starting from **15** the title compound **16** was obtained as described for **11** in 83% yield as a colorless oil. Flash-chromatography was performed using EtOAc: IR ν_{max} (NaCl; cm^{-1}) 3014, 2571, 1135, 1081 and 875; δ_H (300 MHz, $CDCl_3$) 4.22–4.16 (1 H, m, 4-H), 4.01 (2 H, t, *J* 4.2, 1-H, 2-H), 3.88–3.80 (2 H, m, CH_2O), 3.63–3.55 (2 H, m, CH_2O), 2.57 (1 H, br. s, OH) and 2.10–1.93 (4 H, m, 3-H_2, 5-H_2); δ_C (75 MHz, $CDCl_3$) 76.0, 71.4, 62.3 and 37.5; *m*/*z* (EI) 144 (M, 100).

(1β,2β,4β)-1,2-(Methylenedioxy)cyclopent-4-yl succinimidylcarbonate (17)

To a solution of **9** (67 mg, 0.52 mmol) in dry acetonitrile (2 mL), *N*,*N*′-disuccinimidyl carbonate (198 mg, 0.77 mmol) and triethylamine (145 μL, 1.0 mmol) were added and the resulting mixture was stirred at 23 °C. After 8 h the solvent was removed, the residue was taken up in a saturated solution of $NaHCO_3$ and the aqueous phase was extracted with EtOAc. The organic extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo*. Purification of the residue by flash-chromatography (10 : 1 $CHCl_3$–MeOH) yielded **17** (58 mg, 55%): IR ν_{max} (NaCl; cm^{-1}) 2759, 1787, 1740, 1210, 1090; δ_H (300 MHz, $CDCl_3$) 5.27 (1 H, t, *J* 7.2, 4-H), 4.97 (1 H, s, OC*H*HO), 4.69 (1 H, s, OCH*H*O), 4.61–4.59 (2 H, m, 1-H, 2-H), 2.82 (4 H, s, CH_2CH_2), 2.38 (2 H, dd, *J* 6.2, 14.2, 3-H′, 5-H′) and 1.99–1.89 (2 H, m, 3-H″, 5-H″); δ_C (100 MHz, $CDCl_3$) 168.6, 150.8, 94.5, 81.2, 78.1, 37.3 and 25.4; *m*/*z* (CI) 270 (M – H, 100); HRMS $(M - H)^+$ calcd for $C_{11}H_{12}NO_7$, 270.0614; found, 270.0607.

(5aα,7β,8aα)-7-(4-nitrophenoxycarbonyloxy)-tetrahydrocyclopenta[*f*]-1,3,5-trioxepane (18)

To a solution of **10** (15 mg, 0.094 mmol) and *N*-methylmorpholine (31 μL, 0.28 mmol) in dry THF (3 mL), *p*-nitrophenylchloroformate (57 mg, 0.28 mmol) was added and the resulting mixture was stirred at 23 °C. After 1 h, water was added, the solvent was removed under reduced pressure and the aqueous phase was extracted with $CHCl_3$. The organic extracts were dried (Na_2SO_4) and the solvent evaporated. The residue was purified by flash-chromatography (1 : 4 EtOAc–$CHCl_3$) to afford **18** (31 mg, 95%) as a pale yellow viscous oil: IR ν_{max} (NaCl; cm^{-1}) 2831, 2598, 1766, 1529, 1350, 1116 and 859; δ_H (300 MHz, $CDCl_3$) 8.27 (2 H, d, *J* 8.7, ArH), 7.38 (2 H, d, *J* 8.7, ArH), 5.34–5.31 (1 H, m, 7-H), 5.19 (2 H, d, *J* 6.9, 2-H′, 4-H′), 4.77 (2 H, d, *J* 6.9, 2-H″, 4-H″), 4.51–4.47 (2 H, m, 5a-H, 8a-H) and 2.38–2.26 (4 H, m, 6-H_2, 8-H_2); δ_C (75 MHz, $CDCl_3$) 155.3, 126.2, 125.3, 121.7, 115.6, 95.5, 81.2, 78.5 and 37.6; *m*/*z* (EI) 325 (M, 100).

(1β,2β,4β)-4-(4-Nitrophenoxycarbonyloxy)-1,2-(methylenedioxy)cyclopentane (19)

The title compound **19** was obtained from **11** as described for **18** in 81% yield. Flash-chromatography was performed using 1 : 1 EtOAc–Hex: IR ν_{max} (NaCl; cm^{-1}) 2739, 1764, 1527, 1348 and 1204; δ_H (300 MHz, $CDCl_3$) 8.27 (2 H, d, *J* 5.1, ArH), 7.38 (2 H, d, *J* 5.1, ArH), 5.20–5.16 (2 H, m, OCH_2O), 4.83–4.81 (1 H, m, 4-H), 4.68 (2 H, d, *J* 5.7, 1-H, 2-H), 2.38 (2 H, d, *J* 14.7, 3-H′, 5-H′) and 2.11–2.02 (2 H, m, 3-H″, 5-H″); δ_C (100 MHz, $CDCl_3$)

155.4, 126.1, 125.2, 121.8, 115.5, 95.0, 80.7, 80.4 and 38.4; *m/z* (CI) 296 (M + H, 100); HRMS (M + H)$^+$ calcd for $C_{13}H_{14}NO_7$, 296.0770; found, 296.0769.

(5aα,7α,8aα)-7-(4-Nitrophenoxycarbonyloxy)-tetrahydrocyclopenta[*f*]-1,3,5-trioxepane (20)

The title compound was obtained from **12** as described for **18** in 94% yield. Flash-chromatography was performed using 1 : 6 EtOAc–$CHCl_3$: IR ν_{max} (NaCl; cm^{-1}) 2587, 1765, 1594, 1528, 1349 and 858; δ_H (300 MHz, $CDCl_3$) 8.25 (2 H, d, *J* 8.0, ArH), 7.39 (2 H, d, *J* 8.0, ArH), 5.20 (2 H, d, *J* 7.5, 2-H′, 4-H′), 5.10–5.02 (1 H, m, 7-H), 4.75 (2 H, d, *J* 7.5, 2-H″, 4-H″), 4.29–4.24 (2 H, m, 5a-H, 8a-H), 2.51–2.41 (2 H, m, 6-H′, 8-H′) and 2.25–2.17 (2 H, m, 6-H″, 8-H″); δ_C (75 MHz, $CDCl_3$) 155.2, 126.2, 125.2, 121.7, 115.6, 94.6, 80.8, 76.6 and 36.9; *m/z* (CI) 324 (M – H, 100).

(1β,2β,4α)-4-(4-Nitrophenoxycarbonyloxy)-1,2-(ethylenedioxy)cyclopentane (21)

The title compound was obtained from **15** as described for **18** in 81% yield. Flash-chromatography was performed using 1 : 4 EtOAc–$CHCl_3$: IR ν_{max} (NaCl; cm^{-1}) 2655, 1757, 1592, 1503, 1337, 852 and 754; δ_H (300 MHz, $CDCl_3$) 8.29 (2 H, d, *J* 7.3, ArH), 7.36 (2 H, d, *J* 7.3, ArH), 5.22–5.18 (1 H, m, 4-H), 3.86–384 (2 H, m, 1-H, 2-H), 3.78–3.63 (4 H, m, CH_2O), 2.38–2.24 (4 H, m, 3-H_2, 5-H_2); δ_C (100 MHz, $CDCl_3$) 161.8, 126.2, 125.3, 121.7, 115.6, 78.1, 74.3, 62.1 and 33.9; *m/z* (CI) 310 (M + H, 100).

(1β,2β,4β)-4-(4-Nitrophenoxycarbonyloxy)-1,2-(ethylenedioxy)cyclopentane (22)

The title compound was obtained from **16** as described for **18** in 95% yield. Flash-chromatography was performed using 1 : 4 EtOAc–$CHCl_3$: IR ν_{max} (NaCl; cm^{-1}) 2588, 1725, 1594, 1222, 1109 and 773; δ_H (400 MHz, $CDCl_3$) 8.27 (2 H, d, *J* 7.0, ArH), 7.38 (2 H, d, *J* 7.0, ArH), 5.14–5.10 (1 H, m, 4-H), 3.99 (2 H, t, *J* 4.6, 1-H, 2-H), 3.91–3.86 (2 H, m, CH_2O), 3.64–3.59 (2 H, m, CH_2O) and 2.31–2.18 (4 H, m, 3-H_2, 5-H_2); δ_C (100 MHz, $CDCl_3$) 162.5, 126.1, 125.2, 121.7, 115.5, 81.4, 74.3, 62.3 and 32.5; *m/z* (CI) 310 (M + H, 100).

(1′*S*,2′*R*)-{1′-Benzyl-2′-hydroxy-3′-[*iso*butyl(4-methoxybenzenesulfonyl)amino]propyl} carbamic acid (1β,2β,4β)-1,2-(methylenedioxy)cyclopent-4-yl ester (2)

A solution of **24** (25 mg, 0.05 mmol) in 30% trifluoroacetic acid in CH_2Cl_2 (4 mL) was stirred at 23 °C for 40 min and then the solvent was removed under reduced pressure. The residue was dissolved in THF (3 mL), a solution of **19** (18 mg, 0.059 mmol) in THF (1 mL) and diisopropylethylamine (100 μL, 0.6 mmol) were added. After 24 h the organic phase was diluted with $CHCl_3$, washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by flash-chromatography eluting with a 1 : 1 mixture of EtOAc and hexanes to afford **2** (20 mg, 74%) as a white solid: $[\alpha]_{DP}^{20}$ +4.5 (*c* 1.2 in CH_2Cl_2), mp 68 °C (from EtOAc–Hex); IR ν_{max} (NaCl; cm^{-1}) 3129, 2801, 2660, 1711, 1597, 1497, 1155 and 761; δ_H (300 MHz, $CDCl_3$) 7.71 (2 H, d, *J* 8.8, ArH), 7.32–7.19 (5 H, m, ArH), 6.98 (2 H, d, *J* 8.8, ArH), 5.01 (1 H, s, OC*H*HO), 4.92 (1 H, br. s, NH), 4.80 (2 H, m, 4-H, OCH*H*O), 4.57 (2 H, d, *J* 5.4, 1-H, 2-H), 3.87 (3 H, s, OCH_3), 3.79 (2 H, m, CHN, C*H*OH), 3.10–2.76 (6 H, m, CH_2N, CH_2Ph), 2.11–1.80 [5 H, m, 3-H_2, 5-H_2, C*H*(CH_3)$_2$], 0.90 (3 H, d, *J* 6.6, CHC*H*$_3$) and 0.86 (3 H, d, *J* 6.6, CHC*H*$_3$); δ_C (75 MHz, $CDCl_3$) 162.9, 155.3, 137.5, 129.9, 129.6, 129.3, 128.5, 126.4, 114.3, 94.7, 80.5, 74.2, 72.3, 58.8, 55.6, 54.9, 53.8, 38.5, 35.4, 27.3, 20.2 and 19.9; *m/z* (ES) 563 (M + H, 100); HRMS (M + H)$^+$ calcd For $C_{28}H_{39}N_2O_8S$, 563.2427; found, 563.2406.

(1′*S*,2′*R*)-{1′-Benzyl-2′-hydroxy-3′-[*iso*butyl(4-methoxybenzenesulfonyl)amino]propyl} carbamic acid (1β,2β,4α)-1,2-(methylenedioxy)cyclopent-4-yl ester (26)

A solution of **24** (40 mg, 0.079 mmol) in 30% trifluoroacetic acid in CH_2Cl_2 (6 mL) was stirred at 23 °C for 40 min and then the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (4 mL), a solution of **17** (23 mg, 0.1 mmol) in CH_2Cl_2 (2 mL) and diisopropylethylamine (140 μL, 0.8 mmol) were added. After 2 h the organic phase was washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by flash-chromatography (1 : 1 EtOAc–Hex) to afford **26** (34 mg, 76%) as a white foam: $[\alpha]_{DP}^{20}$ +3.6 (*c* 1.3 in CH_2Cl_2); IR ν_{max} (NaCl; cm^{-1}) 3216, 2801, 2670, 1712, 1597, 1497, 1154 and 755; δ_H (300 MHz, $CDCl_3$) 7.70 (2 H, d, *J* 8.7, ArH), 7.32–7.21 (5 H, m, ArH), 7.00 (2 H, d, *J* 8.7, ArH), 5.06 (1 H, t, *J* 7.0, 4-H), 4.93 (1 H, s, OC*H*HO), 4.76 (1 H, d, *J* 8.4, NH), 4.71 (1 H, s, OCH*H*O), 4.52 (2 H, m, 1-H, 2-H), 3.87 (3 H, s, OCH_3), 3.84 (2 H, m, CHN, C*H*OH), 3.11 (1 H, dd, *J* 8.0, 14.8, C*H*HN), 3.04–2.91 (4 H, m, CH*H*N, CH_2N, C*H*HPh), 2.78 (1 H, dd, *J* 6.7, 13.1, CH*H*Ph), 2.17–2.10 (2 H, m, 3-H′, 5-H′), 1.86–1.58 [3 H, m, 3-H″, 5-H″, C*H*(CH_3)$_2$], 0.91 (3 H, d, *J* 6.6, CHC*H*$_3$) and 0.87 (3 H, d, *J* 6.9, CHC*H*$_3$); δ_C (75 MHz, $CDCl_3$) 162.9, 155.8, 137.6, 129.9, 129.7, 129.4, 128.4, 126.5, 114.3, 94.3, 78.5, 74.5, 72.6, 58.8, 55.6, 54.9, 53.7, 37.8, 35.3, 27.3, 20.2 and 19.9; *m/z* (ES) 585 (M + Na, 100); HRMS (M + Na)$^+$ calcd for $C_{28}H_{38}N_2NaO_8S$, 585.2247; found, 585.2228.

(1*S*,2*R*)-{1′-Benzyl-2′-hydroxy-3′-[*iso*butyl(4-methoxybenzenesulfonyl)amino]propyl} carbamic acid (5aα,7β,8aα)-tetrahydrocyclopenta[*f*]-1,3,5-trioxaepan-7-yl ester (27)

The title compound was obtained from **24** and **18** as described for **2** in 43% yield. Flash-chromatography was performed with 1 : 4 EtOAc–$CHCl_3$: $[\alpha]_{DP}^{20}$ +5.2 (*c* 1.7 in CH_2Cl_2); IR ν_{max} (NaCl; cm^{-1}) 3118, 2825, 2656, 1712, 1596, 1012 and 771; δ_H (300 MHz, $CDCl_3$) 7.70 (2 H, d, *J* 9.0, ArH), 7.32–7.21 (5 H, m, ArH), 6.98 (2 H, d, *J* 9.0, ArH), 5.15 (2 H, d, *J* 7.2, 2-H′, 4-H′), 5.05 (1 H, br. s, NH), 4.76 (1 H, d, *J* 8.4, 7-H), 4.68 (2 H, d, *J* 7.2, 2-H″, 4-H″), 4.32–4.23 (2 H, m, 5a-H, 8a-H), 3.87 (3 H, s, OCH_3), 3.83–3.80 (2 H, m, CHN, C*H*OH), 3.10 (1 H, dd, *J* 8.4, 15.3, C*H*HN), 3.04–2.88 (4 H, m, CH*H*N, CH_2N, C*H*HPh), 2.78 (1 H, dd, *J* 6.9, 13.5, CH*H*Ph), 2.09–1.94 (4 H, m, 6-H_2, 8-H_2), 1.86–1.77 [1 H, m, C*H*(CH_3)$_2$], 0.91 (3 H, d, *J* 6.9, CHC*H*$_3$) and 0.87 (3 H, d, *J* 6.3, CHC*H*$_3$); δ_C (75 MHz, $CDCl_3$) 163.0, 155.7, 137.6, 129.8, 129.7, 129.4, 128.4, 126.5, 114.3, 95.4, 81.5, 73.6, 72.7, 58.8, 55.7, 54.9, 53.7, 37.8, 35.4, 27.3, 20.2 and 19.9; *m/z* (ES) 615 (M + Na, 100); HRMS (M + Na)$^+$ calcd for $C_{29}H_{40}N_2NaO_9S$, 615.2353; found, 615.2361.

(1′*S*,2′*R*)-{1′-Benzyl-2′-hydroxy-3′-[*iso*butyl(4-methoxybenzenesulfonyl)amino]propyl} carbamic acid (5aα,7α,8aα)-tetrahydrocyclopenta[*f*]-1,3,5-trioxaepan-7-yl ester (28)

The title compound was obtained from **24** and **20** as described for **2** in 42% yield. Flash-chromatography was performed with 1 : 1 EtOAc–Hex: $[\alpha]_{DP}^{20}$ +7.3 (*c* 1.7 in CH_2Cl_2); IR ν_{max} (NaCl; cm^{-1}) 3117, 2801, 2707, 1711, 1596, 1260 and 1153; δ_H (300 MHz, $CDCl_3$) 7.70 (2 H, d, *J* 8.7, ArH), 7.31–7.21 (5 H, m, ArH), 6.97 (2 H, d, *J* 8.7, ArH), 5.14 (2 H, d, *J* 6.9, 2-H′, 4-H′), 4.91 (1 H, d, *J* 7.8, NH), 4.83–4.78 (1 H, m, 7-H), 4.68 (2 H, d, *J* 6.9, 2-H″, 4-H″), 4.15–4.10 (2 H, m, 5a-H, 8a-H), 3.87 (3 H, s, OCH_3), 3.81–3.83 (2 H, m, CHN, C*H*OH), 3.12–2.85 (5 H, m, 2 × CH_2N, C*H*HPh), 2.77 (1 H, dd, *J* 6.9, 13.5, CH*H*Ph), 2.34–2.21 (2 H, m, 6-H′, 8-H′), 1.94–1.76 [3 H, m, 6-H″, 8-H″, C*H*$(CH_3)_2$], 0.90 (3 H, d, *J* 6.6, CHCH_3) and 0.86 (3 H, d, *J* 6.6, CHCH_3); δ_C (100 MHz, $CDCl_3$) 162.9, 156.1, 137.5, 129.8, 129.5, 129.4, 128.4, 126.4, 114.3, 94.8, 81.0, 72.3, 71.3, 58.6, 55.6, 55.0, 53.6, 37.1, 35.5, 27.1, 20.1 and 19.8; *m*/*z* (ES) 615 (M + Na, 100); HRMS (M + Na)$^+$ calcd for $C_{29}H_{40}N_2NaO_9S$, 615.2353; found, 615.2349.

(1′*S*,2′*R*)-{1′-Benzyl-2′-hydroxy-3′-[*iso*butyl(4-methoxybenzenesulfonyl)amino]propyl} carbamic acid (1β,2β,4β)-1,2-(ethylenedioxy)cyclopent-4-yl ester (3)

The title compound was obtained from **24** and **22** as described for **2** in 40% yield. Flash-chromatography was performed with 1 : 1 EtOAc–Hex: $[\alpha]_{DP}^{20}$ +6.9 (*c* 0.7 in CH_2Cl_2); IR ν_{max} (NaCl; cm^{-1}) 3120, 2788, 2656, 2542, 1712, 1596, 1259, 1154 and 755; δ_H (500 MHz, $CDCl_3$) 7.70 (2 H, d, *J* 9.0, ArH), 7.31–7.22 (5 H, m, ArH), 6.97 (2 H, d, *J* 9.0, ArH), 4.90–4.86 (2 H, m, NH, 4-H), 3.87 (3 H, s, OCH_3), 3.85–3.79 (7 H, m, 2 × CH_2O, 1-H, 2-H, OH), 3.57–3.54 (2 H, m, CHN, C*H*OH), 3.11 (1 H, dd, *J* 8.2, 14.7, C*H*HN), 3.03–2.88 (4 H, m, CH*H*N, CH_2N, C*H*HPh), 2.78 (1 H, dd, *J* 6.7, 13.2, CH*H*Ph), 2.17–2.08 (2 H, m, 3-H′, 5-H′), 1.98–1.95 (2 H, m, 3-H″, 5-H″), 1.90 [1 H, dt, *J* 5.2, 15.0, C*H*$(CH_3)_2$], 0.91 (3 H, d, *J* 6.5, CHCH_3) and 0.86 (3 H, d, *J* 6.5, CHCH_3); δ_C (75 MHz, $CDCl_3$) 163.0, 156.2, 137.6, 129.8, 129.6, 129.5, 128.5, 126.5, 114.3, 74.5, 73.2, 72.5, 71.8, 62.5, 62.3, 58.8, 55.6, 55.0, 53.8, 35.5, 33.8, 33.5, 27.3, 20.2 and 19.9; *m*/*z* (ES) 599 (M + Na, 100); HRMS (M + Na)$^+$ calcd for $C_{29}H_{40}N_2NaO_8S$, 599.2403; found, 599.2394.

(1′*S*,2′*R*)-{1′-Benzyl-2′-hydroxy-3′-[*iso*butyl(4-methoxybenzenesulfonyl)amino]propyl} carbamic acid (1β,2β,4α)-1,2-(ethylenedioxy)cyclopent-4-yl ester (29)

The title compound was obtained from **24** and **21** as described for **2** in 40% yield. Flash-chromatography was performed with 1 : 1 EtOAc–Hex: $[\alpha]_{DP}^{20}$ +8.2 (*c* 1.0 in CH_2Cl_2); IR ν_{max} (NaCl; cm^{-1}) 3121, 2706, 1711, 1596, 1260, 1154 and 757; δ_H (500 MHz, $CDCl_3$) 7.70 (2 H, d, *J* 8.7, ArH), 7.31–7.28 (2 H, m, ArH), 7.24–7.22 (3 H, m, ArH), 6.98 (2 H, d, *J* 8.7, ArH), 5.09 (1 H, br. s, NH), 4.74 (1 H, d, *J* 8.0, 4-H), 4.06–4.01 (2 H, m, 1-H, 2-H), 3.87 (3 H, s, OCH_3), 3.82–3.81 (2 H, m, CH_2O), 3.75–3.71 (2 H, m, CH_2O), 3.55–3.51 (2 H, m, CHN, C*H*OH), 3.10 (1 H, dd, *J* 15.0, 8.5, C*H*HN), 3.03–2.86 88 (4 H, m, CH*H*N, CH_2N, C*H*HPh), 2.78 (1 H, dd, *J* 13.5, 6.5, CH*H*Ph), 2.32–2.23 (2 H, m, 3-H′, 5-H′), 1.81 (1 H, q, *J* = 6.5, 3-H″), 1.79–1.68 (1 H, m, 5-H″), 1.62–1.53 [1 H, m, C*H*$(CH_3)_2$], 0.91 (3 H, d, *J* 6.6, CHCH_3) and 0.86 (3 H, d, *J* 6.6, CHCH_3); δ_C (75 MHz, $CDCl_3$) 163.1, 156.1, 137.6, 129.8, 129.6, 129.5, 128.5, 126.6, 114.4, 74.6, 73.2, 72.7, 62.2, 58.8, 55.7, 54.9, 53.8, 35.4, 34.3, 34.2, 27.3, 20.2 and 19.9; *m*/*z* (ES) 599 (M + Na, 100); HRMS (M + H)$^+$ calcd for $C_{29}H_{40}N_2NaO_8S$, 599.2403; found, 599.2421.

(1′*S*,2′*R*)-{1′-Benzyl-2′-hydroxy-3′-[*iso*butyl(4-(hydroxymethyl)benzenesulfonyl)amino]propyl} carbamic acid (1β,2β,4β)-1,2-(methylenedioxy)cyclopent-4-yl ester (30)

To a solution of **25**[8] (40 mg, 0.1 mmol) and diisopropylethylamine (150 μL, 0.9 mmol) in THF (3 mL), a solution of **17** (30 mg, 0.11 mmol) was added and the resulting mixture was stirred at 23 °C. After 48 h, the organic phase was diluted with $CHCl_3$, washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by flash-chromatography (2 : 1 EtOAc–Hex) to afford **30** (35 mg, 63%) as an amorphous solid: $[\alpha]_{DP}^{20}$ +7.8 (*c* 1.3 in $CHCl_3$); IR ν_{max} (NaCl; cm^{-1}) 3042, 2996, 2707, 1710, 1530, 1334, 1156 and 755; δ_H (400 MHz, $CDCl_3$) 7.77 (2 H, d, *J* 8.1, ArH), 7.52 (2 H, d, *J* 8.1, ArH), 7.32–7.21 (5 H, m, ArH), 5.00 (1 H, s, NH), 4.92 (1 H, m, 4-H), 4.82–4.80 (4 H, m, OCH_2O, CH_2OH), 4.58–4.57 (2 H, m, l-H, 2-H), 3.81–3.79 (2 H, m, CHN, C*H*OH), 3.11–2.83 (6 H, m, 2 × CH_2N, CH_2Ph), 6H), 2.10–1.82 [5 H, m, 3-H_2, 5-H_2, C*H*$(CH_3)_2$], 0.91 (3 H, d, *J* 6.6, CHCH_3) and 0.83 (3 H, d, *J* 6.6, CHCH_3); δ_C (100 MHz, $CDCl_3$) 156.2, 146.2, 137.5, 137.1, 129.5, 128.5, 127.5, 127.1, 126.5, 94.6, 80.5, 75.8, 72.2, 64.0, 58.5, 55.1, 53.5, 38.4, 35.4, 27.1, 20.0 and 19.8; *m*/*z* (ES) 585 (M + Na, 100); HRMS (M +Na)$^+$ calcd for $C_{28}H_{38}N_2NaO_8S$, 585.2247; found, 585.2246.

X-Ray crystallography. The HIV-1 protease construct with the substitutions Q7K, L33I, L63I, C67A, and C95A to optimize protein stability,[20] was expressed and purified as described.[21] Crystals were grown by the hanging drop vapor diffusion method using a 1 : 15 molar ratio of protease at 2.0 mg mL^{-1} and the inhibitor dissolved in dimethylsulfoxide. The reservoir contained 0.1 M sodium acetate buffer (pH = 4.2) and 1.5 M NaCl. Crystals were transferred into a cryoprotectant solution containing the reservoir solution and 20–30% (v/v) glycerol, mounted on a nylon loop and flash-frozen in liquid nitrogen. X-ray diffraction data were collected on the SER-CAT beamline of the Advanced Photon Source, Argonne National Laboratory. Diffraction data were processed using HKL2000[22] resulting in a R_{merge} value of 7.0% (41.8%) for 90 315 unique reflections between 50 and 1.07 Å resolution with a completeness of 88.1% (51.3%), where the values in parentheses are for the final highest resolution shell. Data were reduced in space group $P2_12_12$ with unit cell dimensions of $a = 58.00$ Å, $b = 86.34$ Å and $c = 45.83$ Å with one dimer in the asymmetric unit. The structure was solved by molecular replacement using the CPP4i suite of programs,[23,24] with the structure of the D30N mutant of HIV protease in complex with GRL-98065 (2QCI)[19] as the starting model. The structure was refined using SHELX97[25] and refitted manually using the molecular graphics programs O[26] and COOT.[27] Alternate conformations were modeled for the protease residues when obvious in the electron density maps. Anisotropic atomic displacement parameters (*B*-factors) were refined for all atoms including solvent molecules. Hydrogen atoms were added at the final stages of the refinement. The identity of ions and other solvent molecules from the crystallization conditions was

deduced from the shape and peak height of the $2F_o–F_c$ and $F_o–F_c$ electron density, the hydrogen bond interactions and interatomic distances. The solvent structure was refined with one sodium ion, three chloride ions, and 203 water molecules including partial occupancy sites. The final R_{work} was 15.2% and RB_{free} was 17.7% for all data between 10 and 1.07 Å resolution. The rmsd values from ideal bonds and angle distances were 0.015 Å and 0.034 Å, respectively. The average *B*-factor was 13.1 and 18.2 $Å^2$ for protease main chain and side chain atoms, respectively, 12.5 $Å^2$ for inhibitor atoms and 24.0 $Å^2$ for solvent atoms. The X-ray crystal structure of the inhibitor **3** complex with the HIV-1 protease has been deposited in the Protein Databank (PDB)[28] with an access code of 3DKJ.

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