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

Arun K. Ghosh,*[a] Sandra Gemma,[a] Jun Takayama,[a] Abigail Baldridge,[a] Sofiya Leshchenko-Yashchuk,[a] Heather B. Miller,[a] Yuan-Fang Wang,[b] Andrey Y. Kovalevsky,[b] Yashiro Koh,[c] Irene T. Weber[b] and Hiroaki Mitsuya[c,d]

Received 30th May 2008, Accepted 4th July 2008
First published as an Advance Article on the web 11th August 2008
DOI: 10.1039/b809178a

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.

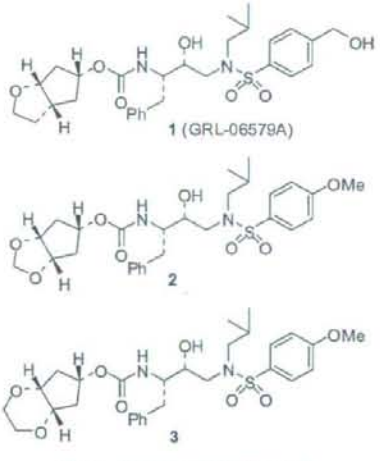


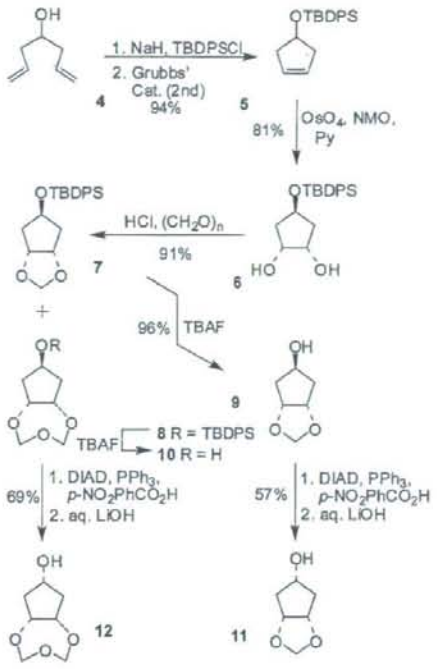
Fig. 1 Structure of inhibitors **1–3**.

[a]*Departments of Chemistry and Medicinal Chemistry, Purdue University, West Lafayette, IN 47907, USA. E-mail: akghosh@purdue.edu; Fax: +1 765 4961612; Tel: +1 765 4945323*
[b]*Department of Biology, Molecular Basis of Disease, Georgia State University, Atlanta, Georgia 30303, USA*
[c]*Kumamoto University School of Medicine, Kumamoto 860-8556, Japan and Department of Hematology and Infectious Diseases*
[d]*Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, Bethesda, Maryland 20892, USA*
† 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

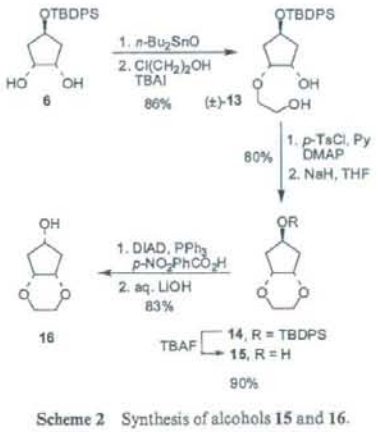
The hexahydrocyclopenta-1,3-dioxolan-5-ol (**11**), required for the synthesis of **2**, was prepared as described in Scheme 1. Commercially available 1,6-heptadien-4-ol **4** was protected as the corresponding *t*-butyldiphenylsilyl ether using sodium hydride as the base in THF. The resulting diene 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

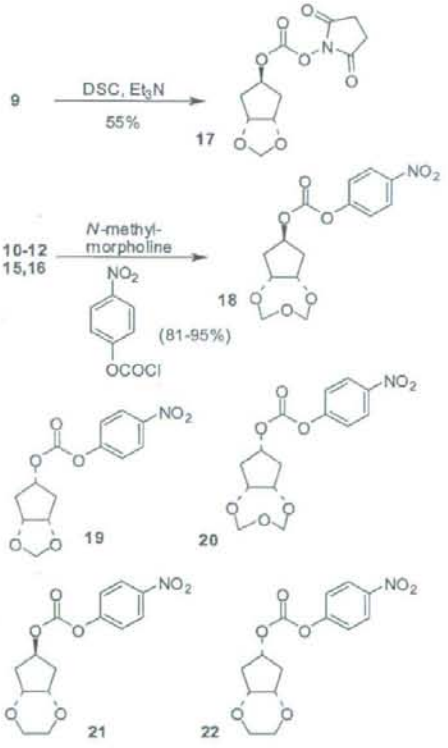


Scheme 2 Synthesis of alcohols **15** and **16**.

This journal is © The Royal Society of Chemistry 2008

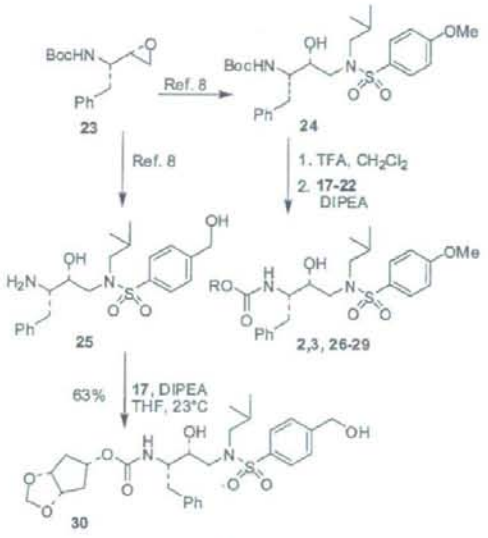
alcohol was selectively tosylated with *p*-toluenesulfonyl chloride in the presence of pyridine. Exposure of the resulting compound to sodium hydride resulted in an intramolecular substitution reaction leading to the corresponding cyclization compound **14**. TBAF-mediated deprotection furnished the 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.



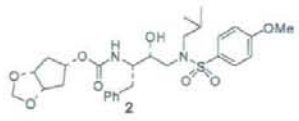
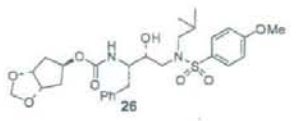
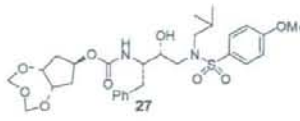
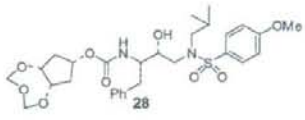
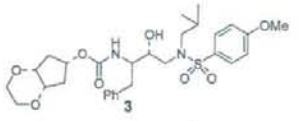
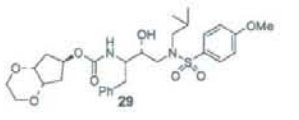
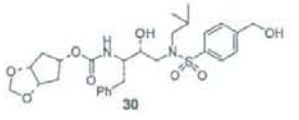
Scheme 4 Synthesis of inhibitors **2**, **3** and **26–30**.

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_{95}/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.

This Journal is © The Royal Society of Chemistry 2008

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

Virus[b]	IC_{50} values[a](nM)				
	2	DRV[e]	SQV[d]	APV[c]	IDV[f]
HIV-$1_{ERS104pre}$ (wild-type: X4)	29	3.5	12	33	26
HIV-$1_{MDR/MM}$(R5)	150 (5)	17 (5)	190 (16)	300 (9)	>1000 (>38)
HIV-$1_{MDR/HL}$ (R5)	550 (19)	26 (7)	330 (28)	430 (13)	>1000 (>38)
HIV-$1_{MDR/C}$ (X4)	300 (10)	7 (2)	36 (3)	230 (7)	>1000 (>38)
HIV-$1_{MDR/G}$ (X4)	340 (12)	7 (2)	29 (2)	340 (10)	290 (11)
HIV-$1_{MDR/A}$ (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-$1_{ERS104pre}$; L10I, K43T, M46L, I54V, L63P, A71V, V82A, L90M, and Q92K in HIV-$1_{MDR/MM}$; L10I, L24I, I33F, E35D, M36I, N37S, M46L, I54V, R57K, I62V, L63P, A71V, G73S, and V82A in HIV-$1_{MDR/HL}$; L10I, I15V, K20R, L24I, M36I, M46L, I54V, I62V, L63P, K70Q, V82A, and L89M in HIV-$1_{MDR/C}$; L10I, V11I, T12E, I15V, L19I, R41K, M46L, L63P, A71T, V82A, and L90M in HIV-$1_{MDR/G}$; and L10I, I15V, E35D, N37E, K45R, I54V, L63P, A71V, V82T, L90M, I93L, and C95F in HIV-$1_{MDR/A}$. HIV-$1_{ERS104pre}$ 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**.

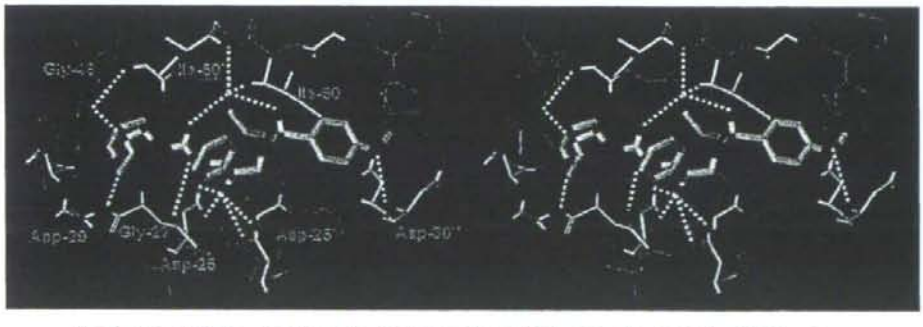


Fig. 2 Stereoview of the X-ray structure of inhibitor **3** bound to the active site of wild-type HIV-1 protease.

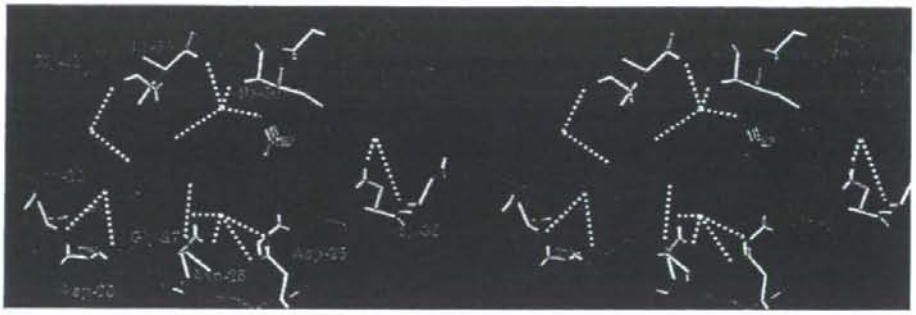


Fig. 3 A stereoview of an active model of inhibitor **2** (green) with the X-ray structure of inhibitor **3** (magenta)-bound HIV-1 protease.

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

This journal is © The Royal Society of Chemistry 2008

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, $3\text{-}H_2$, $5\text{-}H_2$) 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 $n\text{-}Bu_4N^+F^-$ (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, $6\text{-}H_2$, $8\text{-}H_2$) 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 $LiOH\text{-}H_2O$ (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, $CDCl_3$)

This journal is © The Royal Society of Chemistry 2008

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, CHCH_3) and 0.86 (3 H, d, *J* 6.6, CHCH_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″, CH(CH_3)$_2$], 0.91 (3 H, d, *J* 6.6, CHCH_3) and 0.87 (3 H, d, *J* 6.9, CHCH_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, CHCH_3) and 0.87 (3 H, d, *J* 6.3, CHCH_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, *CH*OH), 3.12–2.85 (5 H, m, 2 × CH_2N, *CH*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″, *CH*$(CH_3)_2$], 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 (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, *CH*OH), 3.11 (1 H, dd, *J* 8.2, 14.7, *CH*HN), 3.03–2.88 (4 H, m, CH*H*N, CH_2N, *CH*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, *CH*$(CH_3)_2$], 0.91 (3 H, d, *J* 6.5, CHC*H*$_3$) and 0.86 (3 H, d, *J* 6.5, CHC*H*$_3$); 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, *CH*OH), 3.10 (1 H, dd, *J* 15.0, 8.5, *CH*HN), 3.03–2.86 88 (4 H, m, CH*H*N, CH_2N, *CH*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, *CH*$(CH_3)_2$], 0.91 (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, $CDCl_3$) 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, *CH*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, *CH*$(CH_3)_2$], 0.91 (3 H, d, *J* 6.6, CHC*H*$_3$) and 0.83 (3 H, d, *J* 6.6, CHC*H*$_3$); 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

This Journal is © The Royal Society of Chemistry 2008

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.

Acknowledgements

The research was supported by grants from the National Institutes of Health (GM53386, AKG, and GM62920, IW). This work was also supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health and in part by a Grant-in-aid for Scientific Research (Priority Areas) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Monbu Kagakusho), a Grant for Promotion of AIDS Research from the Ministry of Health, Welfare, and Labor of Japan (Kosei Rohdosho: H15-AIDS-001), and a Grant to the Cooperative Research Project on Clinical and Epidemiological Studies of Emerging and Re-emerging Infectious Diseases (Renkei Jigyo: No. 78, Kumamoto University) of Monbu-Kagakusho.

Notes and references

1 (*a*) N. A. Roberts, J. A. Martin, D. Kinchington, A. V. Broadhurst, J. C. Craig, I. B. Duncan, S. A. Galpin, B. K. Handa, J. Kay and A. Krohn, *Science*, 1990, **248**, 358; (*b*) T. D. Meek, D. M. Lambert, G. B. Dreyer, T. J. Carr, T. A. Tomaszek, M. L. Moore, J. E. Strickler, C. Debouck, L. J. Hyland, T. J. Matthews, B. W. Metcalf and S. R. Petteway, *Nature*, 1990, **343**, 90; (*c*) T. J. McQuade, A. G. Tomasselli, L. Liu, V. Karacostas, B. Moss, T. K. Sawyer, R. L. Heinrikson and W. G. Tarpley, *Science*, 1990, **247**, 454.

2 (*a*) C. Flexner, *N. Engl. J. Med.*, 1998, **338**, 1281; (*b*) T. Cihlar and N. Bischofberger, *Annu. Rep. Med. Chem.*, 2000, **35**, 177.

3 (*a*) M. A. Wainberg and G. Friedland, *JAMA, J. Am. Med. Assoc.*, 1998, **279**, 1977; (*b*) S. Grabar, C. Pradier, E. Le Corfec, R. Lancar, C. Allavena, M. Bentata, P. Berlureau, C. Dupont, P. Fabbro-Peray, I. Poizot-Martin and D. Costagliola, *AIDS*, 2000, **14**, 141; (*c*) K. Hertogs, S. Bloor, S. D. Kemp, C. Van den Eynde, T. M. Alcorn, R. Pauwels, M. Van Houtte, S. Staszewski, V. Miller and B. A. Larder, *AIDS*, 2000, **14**, 1203.

4 On June 23, 2006, the FDA approved a new HIV treatment for patients who do not respond to existing drugs. http://www.fda.gov/bbs/topics/NEWS/2006/NEW01395.html.

5 (*a*) D. L. N. G. Surleraux, A. Tahri, W. G. Verschueren, G. M. E. Pille, H. A. de Kock, T. H. M. Jonckers, A. Peeters, S. De Meyer, H. Azijn, R. Pauwels, M.-P. de Bethune, N. M. King, M. Prabu-Jeyabalan, C. A. Schiffer and P. B. T. P. Wigerinck, *J. Med. Chem.*, 2005, **48**, 1813; (*b*) Y. Koh, H. Nakata, K. Maeda, H. Ogata, G. Bilcer, T. Devasamudram, J. F. Kincaid, P. Boross, Y.-F. Wang, Y. Tie, P. Volarath, L. Gaddis, R. W. Harrison, I. T. Weber, A. K. Ghosh and H. Mitsuya, *Antimicrob. Agents Chemother.*, 2003, **47**, 3123; (*c*) A. K. Ghosh, E. Pretzer, H. Cho, K. A. Hussain and N. Duzgunes, *Antiviral Res.*, 2002, **54**, 29.

6 (*a*) A. K. Ghosh, J. F. Kincaid, W. Cho, D. E. Walters, K. Krishnan, K. A. Hussain, Y. Koo, H. Cho, C. Rudall, L. Holland and J. Buthod, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 687; (*b*) K. Yoshimura, R. Kato, M. F. Kavlick, A. Nguyen, V. Maroun, K. Maeda, K. A. Hussain, A. K. Ghosh, S. V. Gulnik, J. W. Erickson and H. Mitsuya, *J. Virol.*, 2002, **76**, 1349.

7 (*a*) A. K. Ghosh, J. F. Kincaid, W. Cho, D. E. Walters, K. Krishnan, K. A. Hussain, Y. Koo, H. Cho, C. Rudall, L. Holland and J. Buthod, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 687; (*b*) K. Yoshimura, R. Kato, M. F. Kavlick, A. Nguyen, V. Maroun, K. Maeda, K. A. Hussain, A. K. Ghosh, S. V. Gulnik, J. W. Erickson and H. Mitsuya, *J. Virol.*, 2002, **76**, 1349.

8 A. K. Ghosh, P. R. Sridhar, S. Leshchenko, A. K. Hussain, J. Li, A. Y. Kovalevsky, D. E. Walters, J. E. Wedekind, V. Grum-Tokars, D. Das, Y. Koh, K. Maeda, H. Gatanaga, I. T. Weber and H. Mitsuya, *J. Med. Chem.*, 2006, **49**, 5252.

9 P. Ashkenazi, J. Kalo, A. Rüttimann and D. Ginsburgh, *Tetrahedron*, 1978, 2161.

10 G. Godjoian, V. R. Wang, A. M. Ayala, R. V. Martínez, R. Martinez-Bernhardt and C. G. Gutiérrez, *Tetrahedron Lett.*, 1996, **37**, 433.

11 A. K. Ghosh, T. T. Duong, S. P. McKee and W. J. Thompson, *Tetrahedron Lett.*, 1992, **33**, 2781.

12 A. K. Ghosh and S. Fidanze, *J. Org. Chem.*, 1998, **63**, 6146.

13 M. V. Toth and G. R. Marshall, *Int. J. Pept. Protein Res.*, 1990, **36**, 544.

14 C. F. Perno, R. Yarchoan, D. A. Cooney, N. R. Hartman, D. S. A. Webb, Z. Hao, H. Mitsuya, D. G. Johns and S. Broder, *J. Exp. Med.*, 1989, **169**, 933.

15 Y. Tie, P. I. Boross, Y. F. Wang, L. Gaddis, F. Liu, X. Chen, J. Tozser, R. W. Harrison and I. T. Weber, *FEBS J.*, 2005, **272**, 5265.

16 A. Gustchina, C. Sansom, M. Prevost, J. Richelle, S. Wodak, A. Wlodawer and I. Weber, *Protein Eng.*, 1994, **7**, 309.

17 S. Panigrahi and G. Desiraju, *Proteins: Struct., Funct., Bioinf.*, 2007, **67**, 128.

18 T. Steiner, *Biophys. Chem.*, 2002, **95**, 195.

19 Y. F. Wang, Y. Tie, P. I. Boross, J. Tozser, A. K. Ghosh, R. W. Harrison and I. T. Weber, *J. Med. Chem.*, 2007, **50**, 4509.

20 J. M. Louis, G. M. Clore and A. M. Gronenborn, *Nat. Struct. Biol.*, 1999, **6**, 868.

21 B. Mahalingam, J. M. Louis, J. Hung, R. W. Harrison and I. T. Weber, *Proteins: Struct., Funct., Genet.*, 2001, **43**, 455–464.

22 Z. Otwinowski and W. Minor, *Methods Enzymol.*, 1997, **276**, 307.

23 Collaborative Computational Project, Number 4. The CCP4 Suite: Programs for Protein Crystallography, *Acta Crystallogr.*, 1994, **D50**, 760.

24 E. Potterton, P. Briggs, M. Turkenburg and E. Dodson, *Acta Crystallogr.*, 2003, **D59**, 1131.

25 G. M. Sheldrick and T. R. Schneider, *Methods Enzymol.*, 1997, **277**, 319.

26 T. A. Jones, J. Y. Zou, S. W. Cowan and M. Kjeldgaard, *Acta Crystallogr.*, 1991, **A47**, 110.

27 P. Emsley and K. Cowtan, *Acta Crystallogr.*, 2004, **D60**, 2126.

28 H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, *Nucleic Acids Res.*, 2000, **28**, 235.

Journal of
Medicinal Chemistry

Subscriber access provided by KUMAMOTO UNIV

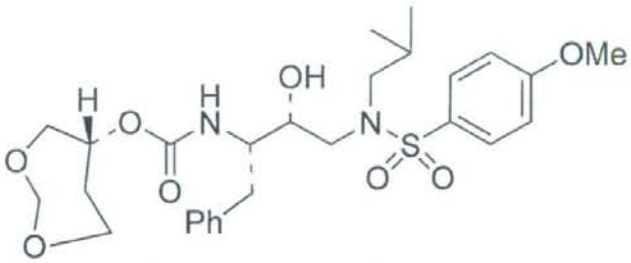
Article

Flexible Cyclic Ethers/Polyethers as Novel P2-Ligands for HIV-1 Protease Inhibitors: Design, Synthesis, Biological Evaluation, and Protein#Ligand X-ray Studies

Arun K. Ghosh, Sandra Gemma, Abigail Baldridge, Yuan-Fang Wang, Andrey Yu. Kovalevsky, Yashiro Koh, Irene T. Weber, and Hiroaki Mitsuya

J. Med. Chem., **2008**, 51 (19), 6021-6033 • DOI: 10.1021/jm8004543 • Publication Date (Web): 11 September 2008

Downloaded from http://pubs.acs.org on February 5, 2009



3d, K_i = 26 pM; IC_{50} = 4.9 nM

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of Medicinal Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Flexible Cyclic Ethers/Polyethers as Novel P2-Ligands for HIV-1 Protease Inhibitors: Design, Synthesis, Biological Evaluation, and Protein−Ligand X-ray Studies†

Arun K. Ghosh,*,‡ Sandra Gemma,‡ Abigail Baldridge,‡ Yuan-Fang Wang,§ Andrey Yu. Kovalevsky,§ Yashiro Koh,|| Irene T. Weber,§ and Hiroaki Mitsuya||,⊥

Departments of Chemistry and Medicinal Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, Department of Biology, Molecular Basis of Disease, Georgia State University, Atlanta, Georgia 30303, Kumamoto University School of Medicine, Kumamoto 860-8556, Japan, Department of Hematology and Infectious Diseases, Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, Bethesda, Maryland 20892

Received April 21, 2008

We report the design, synthesis, and biological evaluation of a series of novel HIV-1 protease inhibitors. The inhibitors incorporate stereochemically defined flexible cyclic ethers/polyethers as high affinity P2-ligands. Inhibitors containing small ring 1,3-dioxacycloalkanes have shown potent enzyme inhibitory and antiviral activity. Inhibitors **3d** and **3h** are the most active inhibitors. Inhibitor **3d** maintains excellent potency against a variety of multi-PI-resistant clinical strains. Our structure−activity studies indicate that the ring size, stereochemistry, and position of oxygens are important for the observed activity. Optically active synthesis of 1,3-dioxepan-5-ol along with the syntheses of various cyclic ether and polyether ligands have been described. A protein−ligand X-ray crystal structure of **3d**-bound HIV-1 protease was determined. The structure revealed that the P2-ligand makes extensive interactions including hydrogen bonding with the protease backbone in the S2-site. In addition, the P2-ligand in **3d** forms a unique water-mediated interaction with the NH of Gly-48.

Introduction

The introduction of protease inhibitors (PIs) into highly active antiretroviral therapy (HAART), a combination therapy based on coadministration of PIs with reverse-transcriptase inhibitors, marked the beginning of a new era in HIV/AIDS chemotherapy. HAART treatment regimens have led to a significant decline in the number of deaths due to HIV infection in the developed world.[1] Unfortunately, there are a number of factors that severely limit current HAART treatment regimens. High frequency of dosing, heavy pill burden, and issues of tolerability and toxicity can lead to poor adherence to treatment.[2] The need for more potent, less toxic drug regimens is quite apparent.

It is the rapid emergence of drug resistance, however, that is proving to be the most formidable problem. Mutations causing drug resistance are thought to occur spontaneously, through the recombination of mixed viral populations, and also due to drug pressure, particularly when administered at substandard doses.[3−6] A growing number of patients are developing multidrug-resistant HIV-1 variants.[7,8] There is ample evidence that these viral strains can be transmitted. Thus, the development of antiretroviral agents able to maintain potency against resistant HIV strains has become an urgent priority.

Darunavir (TMC-114, **1**, Figure 1) is a new nonpeptidic PI recently approved by the FDA for the treatment of antiretroviral therapy-experienced patients.[9] Inhibitor **1**, and its related analogue **2**, are exceedingly active against both wild-type and multidrug resistant HIV strains. Both PIs demonstrated potent

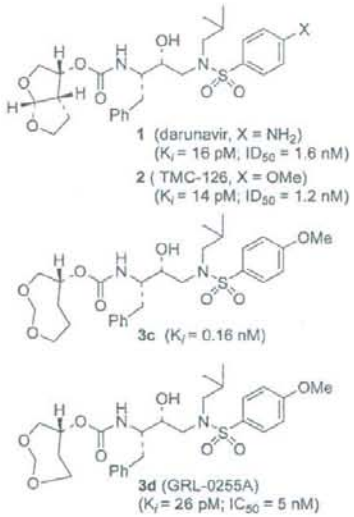


Figure 1. Structure of inhibitors **1**, **2**, and **3c,d**.

in vitro activity against viral isolates resistant to currently licensed PIs.[10−12] Our structure-based design strategies for these PIs are based on the presumption that maximizing active site interactions with the inhibitor, particularly hydrogen bonding with the protein backbone, would give rise to potent inhibitors retaining activity against mutant strains.[13,14] Indeed, side chain amino acid mutations cannot easily disrupt inhibitor−backbone interactions because the active site backbone conformation of mutant proteases is only minimally distorted compared to the wild-type HIV-1 protease.[15−17] In this context, the fused bis-tetrahydrofuran (bis-THF) urethane of compounds **1** and **2** was demonstrated to be a privileged P2-ligand, being able to engage

† The PDB accession code for **3d**-bound HIV-1 protease X-ray structure is 3DJK.

* To whom correspondence should be addressed. Phone: (765)-494-5323. Fax: (765)-496-1612. E-mail: akghosh@purdue.edu.

‡ Departments of Chemistry and Medicinal Chemistry, Purdue University.

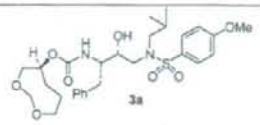
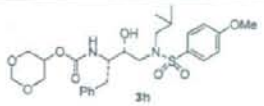
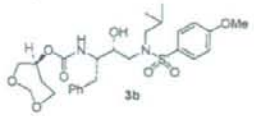
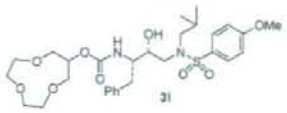
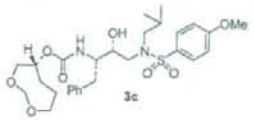
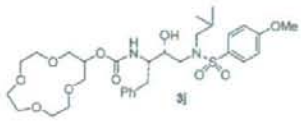
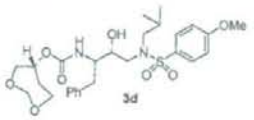
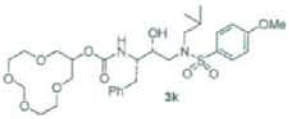
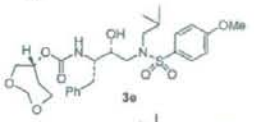
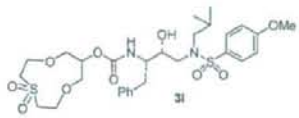
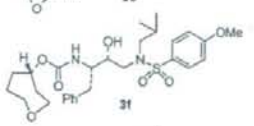
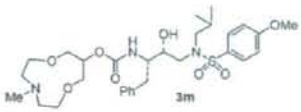
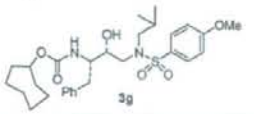
§ Department of Biology, Molecular Basis of Disease, Georgia State University.

|| Kumamoto University School of Medicine.

⊥ HIV and AIDS Malignancy Branch, National Cancer Institute.

10.1021/jm8004543 CCC: $40.75 © 2008 American Chemical Society
Published on Web 09/11/2008

Table 1. Enzyme Inhibitory and Antiviral Activity of Inhibitors **3a**–**m**

entry	inhibitor	K_i (nM)	IC_{50} (nM)[a]	entry	inhibitor	K_i (nM)	IC_{50} (nM)[a]
1	 **3a**	0.15 ± 0.019	nd[b]	8	 **3h**	0.041 ± 0.002	3.4 ± 0.7
2	 **3b**	0.16 ± 0.04	30 ± 1	9	 **3i**	16 ± 2.2	nd
3	 **3c**	0.16 ± 0.011	nd	10	 **3j**	33 ± 1.9	nd
4	 **3d**	0.026 ± 0.012	4.9 ± 0.3	11	 **3k**	6.3 ± 0.57	>1000
5	 **3e**	0.81 ± 0.12	nd	12	 **3l**	1.9 ± 0.2	>1000
6	 **3f**	0.74 ± 0.15	nd	13	 **3m**	19 ± 0.76	>1000
7	 **3g**	27 ± 0.81	nd	SQV[c]	-	-	16 ± 3
				APV[d]	-	-	27 ± 6

[a] MT-2 human T-lymphoid cells exposed to HIV-1_{LAI}. [b] nd = not determined. [c] SQV = saquinavir. [d] APV = amprenavir.

in a number of hydrogen bonding interactions with the backbone atoms of amino acids at the protease S2-site.

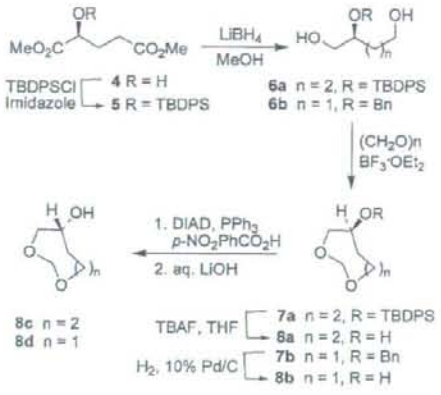
We are continuing our efforts toward the development of novel PIs characterized by a high activity against both wild-type HIV-1 and resistant strains. We further speculated that an inhibitor interacting strongly with the protein backbone, while being able to accommodate amino acid side chain variations by means of repacking with a flexible ring, would maintain significant affinity against both wild-type and mutant enzymes. With this goal in mind, we designed a series of PIs based on the (*R*)-(hydroxyethylamino)sulfonamide isostere and bearing flexible cyclic ethers and polyethers as P2-ligands (inhibitors **3a**–**m**, Table 1). Starting from compound **3c**, incorporating a (1*R*)-3,5-dioxacyclooctan-1-yl urethane, which can be considered as the flexible counterpart of the bis-THF moiety, we designed a series of structural variants of this inhibitor. These inhibitors contain polyether-based P2-ligands ranging from 6- to 13-membered rings coupled to a *p*-methoxyphenylsulfonamide as the P2′-ligand. Herein we report the structure-based design, synthesis, and preliminary biological evaluation of inhibitors **3a**–**m**. Among these inhibitors, **3d** (Figure 1) is the most potent, with an impressive enzyme inhibitory and antiviral activity (K_i = 26 pM, IC_{50} = 4.9 nM). Furthermore, a protein–ligand X-ray structure of **3d**-bound HIV-1 protease has revealed important molecular insight regarding ligand-binding site interactions.

Chemistry. The syntheses of seven- and eight-membered 1,3-dioxacycloalkanes **8a**–**d** for the corresponding inhibitors **3a**–**d** are shown in Scheme 1. Protected diol **6a** was prepared by a two-step procedure starting from (*S*)-hydroxyglutaric acid **4**, obtained by following a known protocol.[18] The hydroxyl group of **4** was protected as a *tert*-butyldiphenylsilylether **5** in quantitative yield. $LiBH_4$ reduction of both ester groups afforded **6a** in good yield.[19]

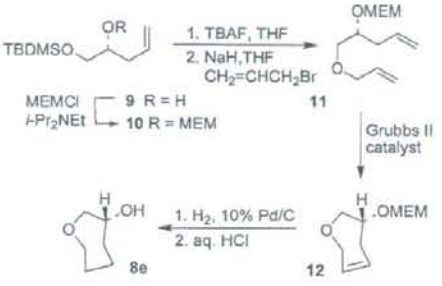
Compounds **6a** and **6b**[20] were converted to cyclic derivatives by exposure to paraformaldehyde and $BF_3 \cdot OEt_2$[21] to afford cyclic ethers **7a** and **7b** in 51% and 82% yield, respectively. Deprotection of compounds **7a** to **8a** was carried out by using n-$Bu_4N^+F^-$ in THF. Benzylether of **7b** was removed by a catalytic hydrogenation over 10% Pd–C to furnish **8b**. Mitsunobu inversion of the secondary hydroxyl groups of **8a**,**b** was accomplished by using *p*-nitrobenzoic acid, triphenylphosphine, and diisopropylazodicarboxylate in benzene at 23 °C. Saponification of the resulting esters provided **8c** and **8d**.

For the synthesis of compounds **8e** and **8f**, which represent the monoxygenated analogues of **8d**, a synthetic strategy based on a ring-closing metathesis reaction as the key step was planned (Schemes 2 and 3). Accordingly, secondary alcohol **9**[22] (Scheme

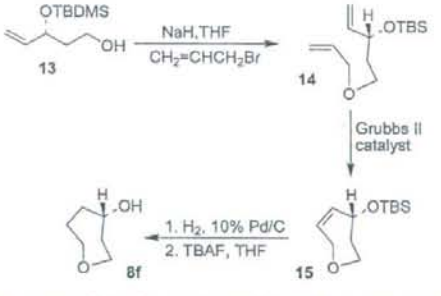
Scheme 1. Synthesis of Optically Active 1,3-Dioxacycloalkanes



Scheme 2. Synthesis of Cyclic Ether **8e**



Scheme 3. Synthesis of Cyclic Ether **8f**

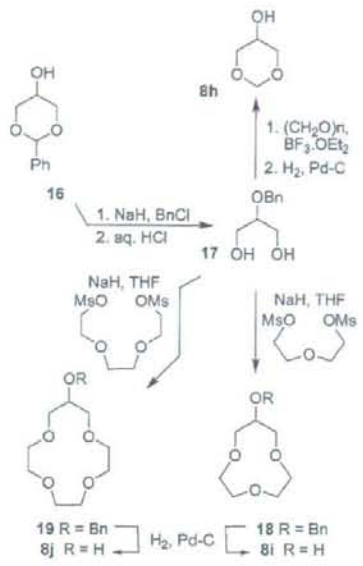


2) was protected as the corresponding methoxyethoxymethyl (MEM)-ether **10** in 90% yield using an excess of MEM-Cl in the presence of DIPEA in CH_2Cl_2.

Subsequent n-$Bu_4N^+F^-$-promoted deprotection of the TBDMS-group afforded the corresponding primary alcohol, which was treated with sodium hydride and alkylated with allyl bromide in the presence of a catalytic amount of n-$Bu_4N^+I^-$ to afford olefin **11** in 78% yield (2 steps). A 0.01 M solution of **11** in CH_2Cl_2 was then treated with a catalytic amount (5 mol%) of second generation Grubbs catalyst and heated to 45 °C to afford the cyclooxepane **12** in 94% yield. The double bond of **12** was finally reduced by catalytic hydrogenation using 10% Pd−C as the catalyst, and the MEM-ether was removed by acidic hydrolysis in a 1:1 THF/H_2O mixture to obtain the target alcohol **8e** in good overall yield.

For the synthesis of alcohol **8f** (Scheme 3), compound **13** was used as the starting material. It was in turn prepared

Scheme 4. Synthesis of Polyethers **8h**−**j**



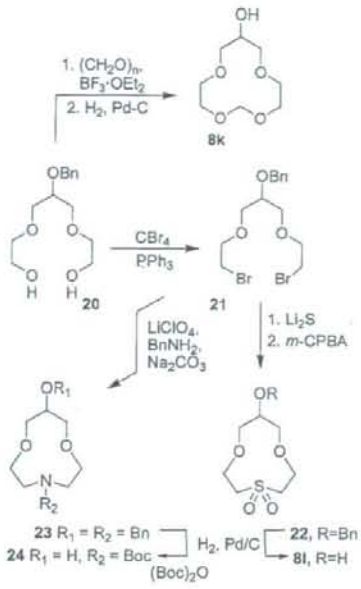
following a described procedure starting from acrolein and *tert*-butylacetate.[23] Alkylation of the primary hydroxyl group of **13** with allyl bromide and n-$Bu_4N^+I^-$ using sodium hydride as the base furnished the ring closing metathesis precursor **14**. The cyclization reaction was performed by using second generation Grubbs catalyst (5 mol%) in CH_2Cl_2 and afforded olefin **15** in good yield. Subsequent hydrogenation of the double bond and n-$Bu_4N^+F^-$-mediated removal of TBDMS-ether finally afforded the target alcohol **8f**.

Alcohols **8h**−**j** required for the preparation of inhibitors **3h**−**j** were synthesized by starting from the common intermediate 2-benzyloxypropane-1,3-diol **17** as shown in Scheme 4. Compound **17** was prepared by alkylation of commercially available benzylidene acetal **16** with benzyl chloride in the presence of sodium hydride and a catalytic amount of n-$Bu_4N^+I^-$ in THF at 23 °C. The benzylidene group was subsequently removed by hydrolysis with 6 N HCl in a mixture (1:1) of THF and water to give 2-benzyloxy-1,3-propanediol **17** in quantitative yield. Treatment of **17** with paraformaldehyde and $BF_3 \cdot OEt_2$ as described above, followed by hydrogenolysis of the resulting *O*-benzylether afforded **8h** in 78% overall yield.

Treatment of diol **17** with an excess of sodium hydride in refluxing THF followed by addition of di(ethyleneglycol)dimesylate or tri(ethyleneglycol)dimesylate afforded macrocycles **18** and **19** in 19% and 29% yield, respectively. Dilution of the reaction mixture to assist the intramolecular cyclization reaction did not result in a significant improvement of the reaction yields. Given the poor enzymatic inhibitory activity observed for the corresponding final compounds **3i** and **3j**, no further attempts were made to improve the cyclization yield for the preparation of these 10- and 13-membered polyether rings. Compounds **18** and **19** were subsequently deprotected by hydrogenolysis to obtain alcohols **8i** and **8j**.

We planned to investigate the effect of heteroatom functionalities in the polyether rings. In this context, we prepared the compounds **8k**, **8l**, and **24** from known diols **20**[24] as shown in Scheme 5. Thus, exposure of **20** to paraformaldehyde in the presence of $BF_3 \cdot OEt_2$ furnished the corresponding cyclic

Scheme 5. Synthesis of Alcohols **8k,l** and **24**



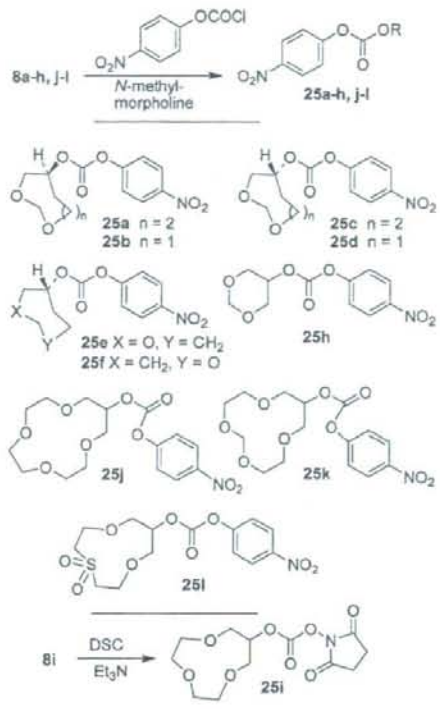
polyether product, which, upon hydrogenolysis, gave alcohol **8k**. Bromination of **20** using carbon tetrabromide and triphenylphosphine afforded dibromide **21**.[24] This dibromide was used for the synthesis of sulfone **8l** and protected amine **24**. Thus, compound **21** was reacted with one equivalent of benzylamine in refluxing MeCN in the presence of sodium carbonate, as reported by Calverley and Dale,[25] to provide **23** in 24% yield. Dimerization is the main side product in this reaction and one can reduce such dimerization by using an excess of $LiClO_4$.[26] Benzylamine **23** was hydrogenated over 10% Pd−C in the presence of di-*t*-butyl dicarbonate to provide *N*-Boc protected alcohol **24**. Sulfone **22** was obtained by cyclization of **21** with lithium sulfide, followed by oxidation of the corresponding sulfide with an excess of *m*-CPBA in CH_2Cl_2 at 23 °C. Benzyl derivative **22** was converted to **8l** by a catalytic hydrogenation over 10% Pd−C.

Scheme 6 depicts the conversion of various P2-ligands to the corresponding active carbonates for urethane formation. Accordingly, alcohols **8a−h,j−l** were reacted with *p*-nitrophenylchloroformate and *N*-methylmorpholine in THF at 23 °C to provide corresponding carbonates **25a−h,j−l** in 67−89% yields. Alcohol **8i** was converted to succinimidylcarbamate **25i** by treatment with *N,N'*-succinimidylcarbonate in the presence of Et_3N in MeCN in 37% isolated yield.

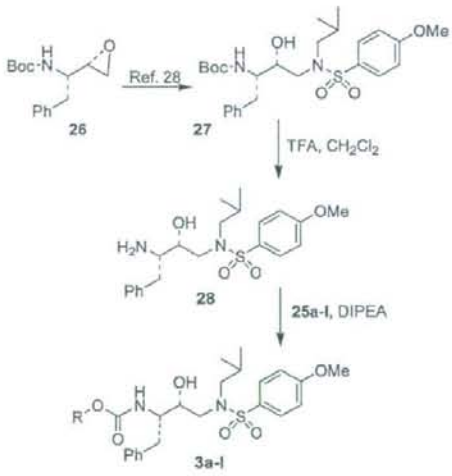
The synthesis of designed inhibitors **3a−l** is shown in Scheme 7. Methoxysulfonamide derivative **27** was prepared from commercially available epoxide **26** as described previously.[27] The Boc group in **27** was removed by exposure to a 30% solution of TFA in CH_2Cl_2 at 23 °C. The resulting amine **28** was reacted with the suitable mixed activated carbonates **25a−l** in THF at 23 °C for 2−4 days to furnish inhibitors **3a−l** in 36−89% yield.

The synthesis of inhibitor **3m** is shown in Scheme 8. Alcohol **24** was converted to active carbonate **29** as described above in Scheme 6. Reaction of **29** with amine **28** provided urethane **30** in good yield. Removal of Boc group of **30** by exposure to 30% TFA in CH_2Cl_2 furnished amine **31**. The resulting secondary

Scheme 6. Synthesis of Various Active Carbonates



Scheme 7. Synthesis of Inhibitors **3a−l**

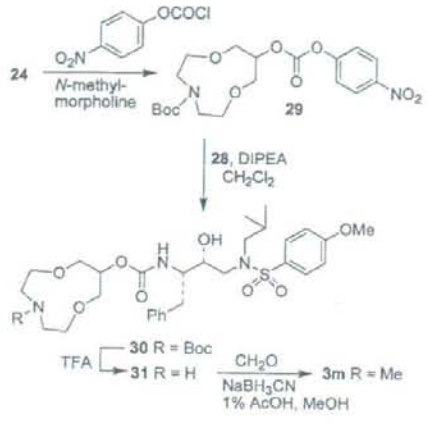


amine was subjected to a reductive amination reaction using 37% aqueous formaldehyde and sodium cyanoborohydride in 1% acetic acid in MeOH to furnish *N*-methyl derivative **3m** in 87% yield.

Results and Discussion

All inhibitors contain a (*R*)-hydroxyethylamine sulfonamide isostere with a *p*-methoxysulfonamide as the P2'-ligand and various designed cyclic ethers and polyethers as the P2-ligands.

Scheme 8. Synthesis of Inhibitor **3m**



These inhibitors were first evaluated in an enzyme inhibitory assay utilizing a protocol described by Toth and Marshall.[28] Compounds that showed potent enzymatic K_i values were then further evaluated in an antiviral assay. The results are shown in Table 1. The K_i-values denote the mean values of at least four determinations.

As it can be seen, introduction of the 8-membered (*S*)- or (*R*)-1,3-dioxacyclooctan-5-yl urethanes as P2-ligands (inhibitors **3a** and **3c**) resulted in subnanomolar inhibitors. However, these inhibitors are significantly less potent than inhibitor **2** that contains the bis-THF ligand. Interestingly, incorporation of a (5*R*)-1,3-dioxacycloheptan-5-yl urethane as the P2-ligand resulted in the most potent inhibitor **3d** in this series with a K_i value of 26 pM. We speculated that the 7-membered 1,3-dioxepanyl-ligand with *R*-configuration may bind to residues in the S2-site similar to bis-THF ligand of inhibitor **2**. Inhibitor **3d** exhibited more than 6-fold potency increase relative to epimeric (5*S*)-1,3-dioxacycloheptan-5-yl urethane **3b**, suggesting an important role for the ring stereochemistry. Inhibitors **3e**−**g** were prepared to assess the role played by both oxygen atoms of **3d** on the binding mode of this latter compound. As shown in Table 1, a dramatic drop in enzymatic inhibitory activity was observed when the cycloheptanol was introduced as the P2-ligand (**3g**). Moreover, nearly 30-fold reduction in enzymatic inhibitory potency of both **3e** and **3f** with respect to **3d** clearly demonstrated that both oxygen atoms are crucial for the interaction with the enzyme at the S2-subsite. It appears that both oxygen atoms engage in strong hydrogen bonding, which equally contribute to the binding affinity for the enzyme. This result was further confirmed by the determination of the X-ray crystal structure of **3d**-bound HIV-1 protease.

Further reduction of the ring size of the P2-ligand resulted in the design of inhibitor **3h**, bearing a 6-membered 1,3-dioxan-5-yl urethane. This inhibitor also showed an impressive enzymatic K_i value of 41 pM. This result suggested that the 1,3-dioxane ring could be nicely accommodated by the S2-site. Furthermore, both oxygens may be involved in specific interactions with the amino acid residues in this region.

Subsequently, we tested compounds **3i**−**m**, presenting larger polyether rings, but all compounds showed K_i values in the high nM range (K_is ranging from 6.3 to 33 nM), proving that large rings could not be easily accommodated at the S2-site.

However, subtle differences in the activity among these compounds suggested that not only the ring size, but also the position of the oxygen atoms within the polyether structure, could be important for inhibitory activity. In fact, compound **3k**, presenting a 12-membered ring bearing a methylenedioxy unit instead of the ethylenedioxy of **3j**, exhibited 5-fold potency enhancement compared to inhibitor **3j**. It is also more than 2-fold more potent compared to **3i**, which contains a smaller 10-membered ring. Substitution of a ring oxygen in **3i** by a *N*-Me group provided inhibitor **3m** with no change in inhibitory activity. However, replacement of ring oxygen with a SO_2 moiety provided inhibitor **3l** with a 9-fold improvement in potency. The sulfone oxygens may be involved in specific interactions with the amino acid residues at the S2 site.

In MT-2 human T-lymphoid cells exposed to HIV-1_{LAI}, inhibitors **3d** and **3h** have shown antiviral IC_{50} values of 4.9 nM and 3.4 nM, respectively (Table 1). Consistent with its enzymatic potency, compound **3b** showed an antiviral activity of 30 nM in the same assay system, while compounds **3k**−**m** did not exhibit appreciable antiviral properties at doses up to 1 μM. We have examined two selected compounds, **3d** and **3h**, for their activity against HIV-1 using a human CD4+ T-cell line (MT-2 cells) and human peripheral blood mononuclear cells (PBMCs) as target cells. We employed two end points 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.[14a] As examined in MT-2 cells as target cells, the two compounds **3d** and **3h** exerted extremely potent antiviral activity against an X4-HIV-1 isolate (HIV-1_{LAI}) with IC_{50} values of 4.9 and 3.4 nM, respectively (Table 1). Such anti-HIV-1 potency was generally parallel to the potency in enzymatic inhibition of the compounds. We further examined the two compounds in PBMCs against a clinical wild-type X4-HIV-1 isolate (HIV-$1_{ERS104pre}$) along with various multidrug-resistant clinical X4- and R5-HIV-1 isolates (Table 2).[14] The activity of **3d** and **3h** against HIV-$1_{ERS104pre}$ was more potent or at least comparable as compared to those of currently available protease inhibitors, APV, IDV, and RTV. It is interesting to note that the values of **3d** were greater than those with MT-2 cells by factors of about 4. With regard to this difference, considering that **3d** was highly potent as examined in human T cells (MT-2 cells) but its activity was slightly less in PBMCs, it is possible that relatively higher concentrations of **3d** are required to suppress HIV-1 production in chronically infected macrophages.[42] Two currently available protease inhibitors (IDV and RTV) were not capable of efficiently suppressing the replication of most of the multidrug-resistant clinical isolates examined (HIV-1_{MDR-B}, HIV-1_{MDR-G}, HIV-1_{MDR-TM}, HIV-$1_{MDR-JSL}$, and HIV-1_{MDR-MM}) with IC_{50} values of >1.0 μM. Although the two selected compounds were also less potent against the multidrug-resistant clinical isolates examined, their IC_{50} values were quite low with 0.22−0.54 μM (Table 2). During testing of the anti-HIV-1 activity of compounds **3b**, **3d**, **3h**, and **3k**−**m**, we examined four concentrations (1, 0.1, 0.01, and 0.001 μM) in the antiretroviral assay, conducted on three independent occasions (each assay was performed in duplicate). As noted, no cytotoxicity was observed for any of the compounds examined. Thus, it was deemed that the CC_{50} values were greater than the highest concentration, 1 μM.

X-ray Crystallography. The mode of binding of the inhibitor was determined by analyzing the atomic resolution crystal structure of HIV-1 protease with **3d**. The crystal structure was solved and refined to an R factor of 14.9% at 1.00 Å resolution. The inhibitor binds with extensive interactions from P2 to P2′ with the protease atoms and, most notably, the favorable polar

Table 2. Antiviral Activity (IC_{50}) of Inhibitors **3d** and **3h** against Clinical HIV-1 Isolates in PBMC Cells (nM)

	IC_{50} (nM) values[a]					
virus	3d	3h	DRV	RTV	APV	IDV
ERS104pre(wild-type)	20	6	3.5	34	33	26
MDR/TM	220 (11)	64 (10)	4 (1)	>1000 (>29)	290 (9)	>1000 (>38)
MDR/MM	250 (13)	110 (5)	17 (5)	>1000 (>29)	300 (9)	>1000 (>38)
MDR/JSL	500 (25)	330 (55)	26 (7)	>1000 (>29)	430 (13)	>1000 (>38)
MDR/B	340 (17)	230 (38)	26 (7)	>1000 (>29)	320 (10)	>1000 (>38)
MDR/C	210 (11)	160 (27)	7 (2)	>1000 (>29)	230 (7)	>1000 (>38)
MDR/G	360 (18)	300 (50)	7 (2)	>1000 (>29)	340 (10)	290 (11)
MDR/A	20 (1)	13 (2)	3 (1)	>1000 (>29)	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-$1_{ERS104pre}$; L10I, K14R, L33I, M36I, M46I, F53I, K55R, I62V, L63P, A71V, G73S, V82A, L90M, and I93L in HIV-1_{MDR-B}; L10I, V11I, T12E, I15V, L19I, R41K, M46L, L63P, A71T, V82A, and L90 M in HIV-1_{MDR-G}; L10I, K14R, R41K, M46L, I54V, L63P, A71V, V82A, L90M, I93L in HIV-1_{MDR-TM}; L10I, L24I, I33F, E35D, M36I, N37S, M46L, I54V, R57K, I62V, L63P, A71V, G73S, and V82A in HIV-$1_{MDR-JSL}$; and L10I, K43T, M46L, I54V, L63P, A71V, V82A, L90M, and Q92K in HIV-1_{MDR-MM}. HIV-$1_{ERS104pre}$ served as a source of wild-type HIV-1.The IC_{50} values were determined by employing PHA-PBMC (phytohemaglutinin-activated peripheral blood mononuclear cells) as target cells and the inhibition of p24Gag protein production as the end point. All values were determined in triplicate. DRV (Darunavir), SQV (Saquinavir), APV (Amprenavir), IDV (Indinavir).

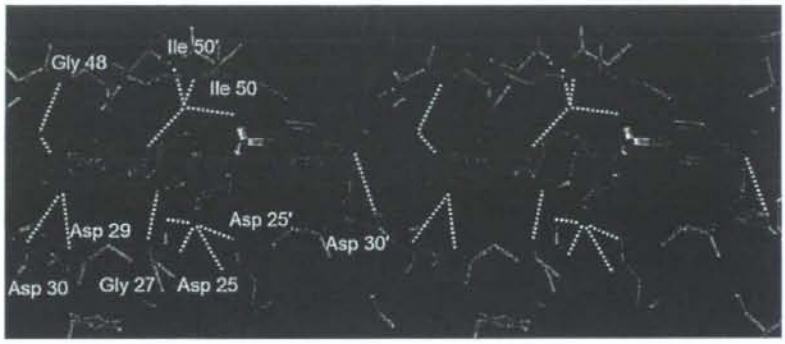


Figure 2. Stereoview of compound **3d** bound to the active site of wild-type HIV-1 protease.

interactions including hydrogen bonds, weaker C−H···O and C−H···π interactions, as shown in Figure 2. The central hydroxyl group forms hydrogen bonds to the side chain carboxylate oxygen atoms of the catalytic Asp25 and Asp25′ residues. The inhibitor hydrogen bonds with protease main chain atoms of the amides of Asp29 and Asp30, the carbonyl oxygen of Gly 27, and the water-mediated interactions with the amides of Ile50 and 50′, which is conserved in the majority of protease complexes with inhibitors[29] and substrate analogues.[30] Inhibitor **3d** has retained the water-mediated interaction with the π system of the P2′ aromatic ring, which was observed for darunavir (**1**) and GRL-98065.[31] The P2′ methoxy group forms a hydrogen bond with the amide of Asp30′. Interestingly, the P2 group forms a water-mediated interaction with the amide of Gly48, similar to the interactions described for several peptide substrate analogues.[30]

Conclusions. In summary, a series of novel and highly potent HIV-1 protease inhibitors were designed, synthesized, and evaluated. The inhibitors incorporate a variety of flexible cyclic ethers/polyethers as the P2-ligand. Inhibitors containing small size 1,3-dioxacycloakanes have shown potent inhibitory properties. In particular, inhibitors **3d** and **3h** have shown remarkable enzyme inhibitory and antiviral potency. Inhibitors incorporating medium-size cyclic polyethers or polyethers containing a sulfone or amine functionality were significantly less potent in antiviral assays. For inhibitor **3d**, we have carried out an optically active synthesis of (*R*)-1,3-dioxepan-5-ol using (*S*)-malic acid as the starting material. Syntheses of various cyclic ethers/polyethers were developed albeit in moderate yields. Inhibitor **3d** has shown excellent activity against multi-PI-resistant variants compared to other FDA approved inhibitors. A protein−ligand X-ray structure of **3d**-bound HIV-1 protease was determined at 1.0 Å resolution. One of the oxygens of the 1,3-dioxepane ligand is involved in hydrogen bonding with Asp29 and Asp30 NH's. The other oxygen is involved in a unique interaction with Gly-48 NH through a water molecule. One goal of our inhibitor design strategy is to combat drug-resistant HIV. The design of inhibitor using the concept of maximizing "backbone binding" has led to the development of PIs characterized by high potency against both wild-type and multidrug-resistant HIV-1 strains. Further design of inhibitors utilizing this molecular insight is in progress.

Experimental Section

General. All moisture sensitive reactions were carried out under 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 of 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.

(*S*)-2-(*tert*-Butyldiphenylsilyloxy)pentadioic Acid Dimethyl Ester (5). A mixture of (2*S*)-hydroxypentadioic acid dimethyl ester **4**[18] (0.39 g, 2.2 mmol), imidazole (0.45 g, 6.6 mmol), and *tert*-butyldiphenylsilyl chloride (1.2 mL, 4.4 mmol) in dry DMF (4 mL) was stirred at 23 °C for 4 h. Subsequently, the reaction mixture was poured into water and the aqueous phase was extracted with Et_2O, the organic extracts were washed with 1 N HCl and brine, dried (Na_2SO_4), and the solvent was removed. The residue was

purified by flash-chromatography (1:10 EtOAc/hex) to furnish 0.89 g (90%) of **5** as a colorless oil: $[\alpha]_D^{20}$ −21.1 (*c* 9.0, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.69−7.62 (m, 4H), 7.46−7.33 (m, 6H), 4.31 (t, *J* = 5.4 Hz, 1H), 3.64 (s, 3H), 3.45 (s, 3H), 2.57−2.34 (m, 2H), 2.14−2.04 (m, 2H), 1.11 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 173.4, 172.9, 135.9, 135.7, 133.0, 132.9, 129.9, 129.8, 127.7, 127.5, 71.4, 51.6, 51.5, 29.9, 28.9, 26.9, 19.4.

(*S*)-2-(*tert*-Butyldiphenylsilyloxy)pentan-1,5-diol (6a). Compound **5** (0.8 g, 1.8 mmol) was dissolved in dry Et_2O (8.5 mL) and the solution was cooled to 0 °C, afterward lithium borohydride (0.12 g, 5.4 mmol) and dry methanol (0.22 mL, 5.4 mmol) were sequentially added. The resulting suspension was stirred at 23 °C for 24 h, then a few drops of 6 N HCl were added and the salts were filtered off. The filtrate was concentrated under reduced pressure and the residue was purified by flash-chromatography (1:1 EtOAc/hex) to furnish 0.61 g (93%) of **6a** as a colorless oil: $[\alpha]_D^{20}$ −15.6 (*c* 3.1, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.70−7.65 (m, 4H), 7.44−7.32 (m, 6H), 3.82−3.77 (m, 1H), 3.53−3.48 (m, 2H), 3.45−3.41 (m, 2H), 1.65−1.47 (m, 4H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 135.9, 135.7, 133.8, 133.7, 130.1, 129.8, 127.7, 127.6, 73.6, 65.7, 62.7, 29.7, 28.0, 27.0, 19.3.

(*S*)-1-(*tert*-Butyldiphenylsilyloxy)-3,5-dioxacyclooctane (7a). To a mixture of **6a** (0.55 g, 1.5 mmol) and paraformaldehyde (46 mg, 1.5 mmol) in EtOAc (30 mL), boron trifluoride etherate (195 μL, 1.5 mmol) was added and the resulting mixture was stirred at 23 °C for 4 h. The organic phase was washed with a saturated solution of $NaHCO_3$, dried (Na_2SO_4), and the solvent was removed. The residue was purified by flash-chromatography (1:4 EtOAc/hex) to afford 0.29 g (51%) of **7a** as a colorless oil: $[\alpha]_D^{20}$ −8.7 (*c* 1.9, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.67−7.63 (m, 4H), 7.45−7.34 (m, 6H), 4.69 (d, *J* = 6.2 Hz, 1H), 4.45 (d, *J* = 6.2 Hz, 1H), 4.03−3.95 (m, 1H), 3.70−3.61 (m, 1H), 3.59−3.48 (m, 3H), 1.93−1.80 (m, 1H), 1.77−1.61 (m, 2H), 1.47−1.34 (m, 1H), 1.12 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 135.7, 134.2, 129.5, 127.5, 95.6, 72.2, 71.9, 69.0, 33.2, 27.0, 26.7, 19.2.

(*S*)-*O*-Benzyl-3,5-dioxacycloheptan-1-ol (7b). Compound **6b**[20] (50 mg, 0.26 mmol) was reacted as described for compound **6a** to afford 44 mg (82%) of **7b** after chromatographic purification (1:9 EtOAc/hex): $[\alpha]_D^{20}$ +64.6 (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.35−7.26 (m, 5H), 4.81−4.77 (m, 2H), 4.58 (s, 2H), 3.95−3.73 (m, 3H), 3.73−3.62 (m, 2H), 1.98−1.91 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 138.3, 128.3, 127.5, 126.2, 94.9, 75.8, 70.7, 68.8, 62.6, 35.0.

(*S*)-3,5-Dioxacyclooctan-1-ol (8a). Compound **7a** (0.27 g, 0.74 mmol) was dissolved in dry THF (5 mL) and TBAF (1.0 M solution in THF, 0.81 mL, 0.81 mmol) was added. The resulting mixture was stirred at 23 °C overnight, afterward a saturated solution of $NaHCO_3$ was added, the solvent was removed, and the aqueous phase was extracted with EtOAc. The organic extracts were dried and evaporated and the residue was purified by flash-chromatography (EtOAc) to afford 76 mg (77%) of **8a** as a colorless oil: $[\alpha]_D^{20}$ −12.6 (*c* 1.6, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 4.65 (d, *J* = 6.0 Hz, 1H), 4.57 (d, *J* = 6.0 Hz, 1H), 4.92−3.81 (m, 2H), 3.75−3.60 (m, 2H), 3.55 (dd, *J* = 3.4, 12.1 Hz, 1H), 2.96 (bs, 1H), 1.95−1.69 (m, 3H), 1.65−1.53 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 94.9, 73.7, 69.3, 68.2, 30.2, 24.7.

(*S*)-3,5-Dioxacycloheptan-1-ol (8b). To a solution of **7b** (38 mg, 0.18 mmol) in EtOAc (3 mL), 10% Pd/C was added and the resulting suspension was stirred at 23 °C under a hydrogen atmosphere. After 12 h, the catalyst was filtered off, the filtrate was evaporated in vacuo, and the residue (19 mg, 91%) was used in the next step without further purification: $[\alpha]_D^{20}$ +12.9 (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 4.78−4.74 (m 2H), 3.93−3.91 (m, 1H), 3.81−3.75 (m, 4H), 2.51 (bs, 1H), 1.93−1.83 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 94.4, 69.5, 68.4, 62.3, 37.8.

(*R*)-3,5-Dioxacyclooctan-1-ol (8c). To a mixture of (*S*)-**8a** (46 mg, 0.35 mmol), *p*-nitrobenzoic acid (86 mg, 0.52 mmol), and triphenylphosphine (181 mg, 0.69 mmol), diisopropylazodicarboxylate (135 μL, 0.69 mmol) was added dropwise and the resulting mixture was stirred at 23 °C overnight. The solvent was removed under reduced pressure, and the residue was purified by flash-chromatography (1:3 EtOAc/hex). The resulting ester was dissolved in a 3:2:1 mixture of THF, methanol, and water (4 mL) and $LiOH·H_2O$ (72 mg, 1.7 mmol) was added. The yellow mixture was stirred at 23 °C overnight and then the solvent was removed in vacuo, the residue was diluted with water, and the aqueous phase was extracted with ether. The organic extracts were dried (Na_2SO_4) and the solvent evaporated. Purification of the residue by flash-chromatography (EtOAc) afforded 20 mg (44%) of (*R*)-**8c** as a colorless liquid. $[\alpha]_D^{20}$ +12.1 (*c* 1.4, $CHCl_3$). ^1H and ^{13}C NMR are consistent with those reported for the (*S*)-enantiomer **8a**.

(*R*)-3,5-Dioxacycloheptan-1-ol (8d). The title compound was obtained from **8b** as described for (*S*)-**8c** in 73% yield. Flash-chromatography was performed using a 1:1 mixture of EtOAc and $CHCl_3$ as the eluant: $[\alpha]_D^{20}$ −12.6 (*c* 1.3, $CHCl_3$). ^1H and ^{13}C NMR are consistent with those reported for the (*S*)-enantiomer **8b**.

(*R*)-1-(*tert*-Butyldimethylsilyloxy)-2-[(2-methoxyethoxy) methoxy]pent-4-ene (10). To a mixture of **9** (350 mg, 1.6 mmol) and diisopropylethylamine (1.2 mL, 7.2 mmol) in CH_2Cl_2 (8 mL), cooled to 0 °C, MEM-Cl (550 μL, 4.8 mmol) was added and the resulting mixture was stirred at 23 °C for 56 h. The organic phase was washed with 0.1 N HCl, brine and dried (Na_2SO_4). The solvent was removed and the residue was purified by flash-chromatography (1:10 EtOAc/hex) to afford 440 mg (90%) of **10** as a colorless oil: $[\alpha]_D^{20}$ +12.0 (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 5.88−5.74 (m, 1H), 5.11−5.01 (m, 2H), 4.82 (d, *J* = 6.9 Hz, 1H), 4.74 (d, *J* = 6.9 Hz, 1H), 3.76−3.63 (m, 3H), 3.60−3.51 (m, 4H), 3.37 (s, 3H), 2.38−2.19 (m, 2H), 0.86 (s, 9H), 0.02 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 134.6, 117.0, 94.8, 77.4, 71.6, 66.7, 65.0, 58.9, 36.0, 25.7, 18.2, −5.5.

(*R*)-1-Allyloxy-2-[(2-methoxyethoxy)methoxy]pent-4-ene (11). A mixture of **10** (440 mg, 1.4 mmol) and TBAF (1.0 M solution in THF, 4.7 mL, 4.7 mmol) in THF (3 mL) was stirred at 23 °C for 3 h, afterward a saturated solution of $NaHCO_3$ was added, the solvent was removed, and the aqueous phase was extracted with $CHCl_3$. The organic extracts were dried (Na_2SO_4), and the solvent was removed. The residue was purified by flash-chromatography to afford 237 mg (87%) of (*R*)-2-[(2-methoxyethoxy)methoxy]pent-4-en-1-ol as a colorless oil: $[\alpha]_D^{20}$ −55.0 (*c* 1.3, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 5.85−5.71 (m, 1H), 5.11−5.02 (m, 2H), 4.81 (d, *J* = 7.5 Hz, 1H), 4.75 (d, *J* = 7.5 Hz, 1H), 3.87−3.80 (m, 1H), 3.71−3.61 (m, 3H), 3.59−3.46 (m, 3H), 3.37 (s, 3H), 3.22 (bs, 1H), 2.36−2.19 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 134.1, 117.3, 95.4, 81.0, 71.5, 67.3, 64.8, 58.9, 36.2. To a mixture of the above compound (240 mg, 1.25 mmol), allyl bromide (225 μL, 1.9 mmol) and a catalytic amount of TBAI in THF (12 mL), at 0 °C, sodium hydride (60% dispersion in oil, 102 mg, 2.5 mmol) was added in small portions. After 30 min, the reaction mixture was allowed to warm to 23 °C and was stirred at the same temperature for 18 h. Subsequently, the reaction was quenched with a saturated solution of NH_4Cl, the organic solvent was removed, and the aqueous phase was extracted with $CHCl_3$. The organic extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by flash-chromatography (10:1 $CHCl_3$/EtOAc) to afford 229 mg (80%) of **11** as a colorless oil. $[\alpha]_D^{20}$ −5.2 (*c* 3.1, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 5.87−5.79 (m, 2H), 5.34 (dd, *J* = 1.3, 19.1 Hz, 1H), 5.16−5.03 (m, 3H), 4.81 (d, *J* = 7.0 Hz, 1H), 4.77 (d, *J* = 7.0 Hz, 1H), 3.98−3.97 (m, 2H), 3.84−3.81 (m, 1H), 3.72 (t, *J* = 5.0 Hz, 2H), 3.54 (t, *J* = 5.0 Hz, 2H), 3.46−3.44 (m, 2H), 3.38 (s, 3H), 2.35−2.31 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 134.6, 134.3, 117.3, 116.7, 94.6, 75.3, 72.1, 71.9, 71.6, 66.7, 58.9, 36.3.

(*R,Z*)-3-[(2-Methoxyethoxy)methoxy]-2,3,4,7-tetrahydrooxepine (12). A mixture of **11** (100 mg, 0.43 mmol) and second generation Grubbs catalyst (18 mg, 0.02 mmol) in CH_2Cl_2 (10 mL) was heated to 45 °C for 1 h. After this time, the solvent was removed and the residue was purified by flash-chromatography (5:1 $CHCl_3$/EtOAc) to afford 83 mg (94%) of **12** as a colorless oil: ^1H NMR ($CDCl_3$) δ 5.87−5.66 (m, 2H), 4.77−4.18 (m, 2H), 4.18−4.14 (m, 2H), 4.01−3.89 (m, 2H), 3.75−3.68 (m, 3H), 3.56−3.53 (m, 2H), 3.38 (s, 3H), 2.54−2.51 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 130.6, 125.9, 94.3, 75.6, 75.1, 71.6, 70.3, 66.8, 58.9, 31.8.

(*R*)-Oxepan-3-ol (8e). A mixture of **12** (90 mg, 0.44 mmol) and a catalytic amount of 10% Pd/C in EtOAc (3 mL) was stirred at 23 °C under a hydrogen atmosphere for 3 h. After this time, the catalyst was filtered off through a pad of celite and the filtrate was concentrated under reduced pressure to afford (*R*)-3-[(2-methoxyethoxy)methoxy]oxepane (83 mg, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.70 (d, J = 7.2 Hz, 1H), 4.67 (d, J = 7.2 Hz, 1H), 3.83−3.58 (m, 7H), 3.50 (t, J = 4.6 Hz, 2H), 3.34 (s, 3H), 1.72−1.67 (m, 1H), 1.46−1.44 (m, 4H), 1.22−1.19 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 93.9, 76.5, 73.7, 71.8, 71.6, 66.7, 58.8, 32.6, 30.7, 20.9. A mixture of the above compound (50 mg, 0.24 mmol) and 6 N HCl (0.5 mL) in THF (2 mL) was stirred at 23 °C for 16 h. The solvent was removed, and the aqueous phase was extracted with $CHCl_3$. The organic extracts were washed with a saturated solution of $NaHCO_3$, dried (Na_2SO_4), and the solvent was removed. The residue was purified by flash-chromatography (1:4 EtOAc/$CHCl_3$) to afford **8e** (24 mg, 84%) as a colorless oil: $[\alpha]_D^{20}$ −4.2 (c 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 3.87−3.85 (m, 1H), 3.76−3.62 (m, 4H), 2.37 (bs, 1H), 1.78−1.65 (m, 5H), 1.54−1.52 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 73.2, 70.7, 70.4, 36.4, 30.0, 20.2.

(*R*)-3-(*tert*-Butyldimethylsilyloxy)-5-(allyloxy)pent-1-ene (14). A mixture of **13** (50 mg, 0.23 mmol), allyl bromide (30 μL, 0.35 mmol), and a catalytic amount of TBAI was cooled to 0 °C and sodium hydride (60% in mineral oil, 11 mg, 0.28 mmol) was added. The resulting mixture was allowed to warm to 23 °C and stirred for 18 h. The reaction was quenched by adding a saturated solution of NH_4Cl, the solvent was removed, and the aqueous phase was extracted with $CHCl_3$. The organic extracts were dried (Na_2SO_4), and the solvent was removed. The residue was purified by flash-chromatography (1:20 EtOAc/hex) to afford 57 mg (97%) of **14** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 5.96−5.87 (m, 1H), 5.85−5.78 (m, 1H), 5.29−5.24 (m, 1H), 5.19−5.13 (m, 2H), 5.04−5.00 (m, 1H), 4.31−4.26 (m, 1H), 3.96−3.94 (m, 2H), 3.55−3.42 (m, 2H), 1.84−1.67 (m, 2H), 0.90 (s, 9H), 0.06 (s, 3H), 0.02 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 141.5, 134.9, 116.6, 113.6, 71.8, 70.6, 66.5, 38.0, 25.8, 18.1, −4.5, −5.1.

(*R,Z*)-4-(*tert*-Butyldimethysilyloxy)-2,3,4,7-tetrahydrooxepine (15). The title compound was obtained from **14** as described for **12** in 80% yield. Flash-chromatography was performed using a 1:10 mixture of EtOAc and hex as the eluant. ^{1}H NMR ($CDCl_3$) δ 5.79−5.75 (m, 1H), 5.63−5.60 (m, 1H), 4.64−4.62 (m, 1H), 4.14−4.12 (m, 2H), 3.91−3.85 (m, 1H), 3.80−3.74 (m, 1H), 2.11−2.05 (m, 1H), 1.96−1.91 (m, 1H), 0.90 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 138.4, 127.8, 69.9, 68.2, 67.4, 38.8, 25.8, 18.3, −4.8.

(*S*)-Oxepan-4-ol (8f). Hydrogenolysis of **15** was carried out as described for **8e** to afford (*S*)-4-(*tert*-butyldimethylsilyloxy)oxepane in 95% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.03−3.96 (m, 1H), 3.79−3.57 (m, 4H), 1.98−1.69 (m, 5H), 1.64−1.51 (m, 1H), 0.88 (s, 9H), 0.044 (s, 3H), 0.038 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 70.2, 69.4, 64.2, 40.1, 34.5, 25.7, 23.7, 18.0, −4.9. Deprotection of the above compound was performed as described for compound **11** and afforded the title compound in 75% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.03−3.98 (m, 1H), 3.82−3.59 (m, 4H), 2.02−1.98 (m, 1H), 1.89−1.80 (m, 4H), 1.66−1.64 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 70.5, 69.6, 64.7, 38.9, 34.9, 24.3.

2-(Benzyloxy)propane-1,3-diol (17). To a solution of **16** (2.5 g, 13.8 mmol) in dry THF (20 mL), cooled to 0 °C, NaH (60% in mineral oil, 0.56 g, 14 mmol) was added portionwise. After 30 min, tetra-*n*-butylammonium iodide (51 mg, 0.14 mmol) and a solution of benzyl bromide (1.65 mL, 13.9 mmol) in THF (5 mL) were added. The reaction mixture was stirred at 23 °C for 3 h; afterward, it was poured into ice. The organic solvent was removed, and the aqueous phase was extracted with $CHCl_3$. The organic extracts were dried (Na_2SO_4), and the solvent was removed. The crude 5-(benzyloxy)-2-phenyl-1,3-dioxane thus obtained was dissolved in a 1:1 mixture of THF and H_2O (60 mL), and to the resulting solution, 6 N HCl was slowly added. After stirring at 23 °C, the reaction mixture was brought to pH 8 by addition of a saturated solution of $NaHCO_3$, the solvent was removed, and the aqueous phase was extracted with diethyl ether. The organic extracts were dried and evaporated, and the residue was purified by flash-column chromatography (2:1 EtOAc/hex) to afford the title compound as a colorless oil in quantitative yield. Physical and spectroscopic data are consistent with those reported in the literature.[32]

1,3-Dioxan-5-ol (8h). To a mixture of **17** (100 mg, 0.55 mmol) and paraformaldehyde (17 mg, 0.55 mmol) in EtOAc (10 mL), boron trifluoride etherate (70 μL, 0.55 mmol) was added and the reaction mixture was stirred at 23 °C for 4 h. The organic phase was washed with a saturated solution of $NaHCO_3$, dried, and the solvent was removed. The residue was purified by flash-chromatography eluting with a 1:4 mixture of EtOAc and hexanes to afford 84 mg (78%) of *O*-benzyl-1,3-dioxan-5-ol as a colorless oil. The above compound was dissolved in EtOAc (3 mL), Pd/C was added, and the resulting suspension was stirred at rt under a hydrogen atmosphere. After 12 h, the catalyst was filtered off, the filtrate was evaporated in vacuo, and the residue (39 mg, 100%) was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 4.93 (d, J = 6.3 Hz, 1H), 4.76 (d, J = 6.3 Hz, 1H), 3.94−3.84 (m, 4H), 3.64−3.61 (m, 1H), 2.78 (bs, 1H). ^{13}C NMR ($CDCl_3$) δ 94.0, 71.7, 64.1.

***O*-Benzyl-3,6,9-trioxacyclodecan-1-ol (18).** To a refluxing suspension of sodium hydride (60% in mineral oil, prewashed with hexane, 84 mg, 2.1 mmol) in dry THF (5 mL), a solution of **17** (182 mg, 1.0 mmol) and di(ethyleneglycol)dimethanesulfonate (260 mg, 1.0 mmol) in dry THF (5 mL) was added dropwise. The resulting mixture was heated under reflux for 20 h and afterward was cooled to 23 °C and H_2O (2 mL) was added. The solvent was removed, and the aqueous phase was extracted with $CHCl_3$. The organic extracts were washed several times with water, dried (Na_2SO_4), and evaporated. The residue was purified by flash-chromatography (2:3 CH_2Cl_2/EtOAc) to afford 49 mg (19%) of **18** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.36−7.26 (m, 5H), 4.66 (s, 2H), 3.75−3.57 (m, 13H). MS (ESI) m/z 275 $[M + Na]^+$.

***O*-Benzyl-3,6,9,12-tetraoxacyclotridecan-1-ol (19).** Compound **19** was obtained as described for **18** starting from **17** and tri(ethyleneglycol)dimethanesulfonate in 29% yield. ^{1}H NMR ($CDCl_3$) δ 7.39−7.26 (m, 5H), 4.72 (s, 2H), 3.83−3.58 (m, 17 H). MS (ESI) m/z 319 $[M + Na]^+$.

3,6,9-Trioxacyclodecan-1-ol (8i). A mixture of **18** (34 mg, 0.13 mmol) and a catalytic amount of 10% Pd/C in methanol (2 mL) was stirred at 23 °C under a hydrogen atmosphere. After 18 h, the catalyst was filtered off and the filtrate was evaporated to afford 22 mg (99%) of **8i** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 3.74−3.53 (m, 13H), 2.73 (bs, 1H).

3,6,9,12-Tetraoxacyclotridecan-1-ol (8j). Starting from **19**, compound **8j** was obtained as described for **8i** in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 3.81−3.60 (m, 17 H), 2.95 (bs, 1H).

3,6,8,11-Tetraoxa-1-cyclododecanol (8k). To a mixture of **20**[24] (78 mg, 0.29 mmol) and paraformaldehyde (8.7 mg, 0.29 mmol) in EtOAc (4 mL), boron trifluoride etherate (37 μL, 0.29 mmol) was added and the resulting mixture was stirred at 23 °C for 2 h. Subsequently, a saturated solution of $NaHCO_3$ was added and the aqueous phase was extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by flash-chromatography to afford 31 mg (37%) of *O*-benzyl-3,6,8,11-tetraoxacyclododecan-1-ol as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.35−7.27 (m, 5H), 4.67 (s, 2H), 3.88 (s, 2H), 3.86−3.81 (m, 2H), 3.77−3.61 (m, 11H). ^{13}C NMR ($CDCl_3$) δ 133.6, 128.3, 127.7, 126.2, 94.6, 75.8, 71.5, 69.6, 65.3, 64.6. MS (ESI) m/z 305 $[M + Na]^+$. A mixture of the above compound and a catalytic amount of 10% Pd/C in EtOAc (2 mL) was stirred at 23 °C under a hydrogen atmosphere. After 18 h, the catalyst was filtered off and the filtrate was evaporated to afford 21 mg (99%) of **8k** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.67 (s, 2H), 3.85−3.63 (m, 11H), 3.54 (dd, J = 6.4, 8.2 Hz, 2H), 2.22 (d, J = 8.7 Hz, 1H).

***O*-Benzyl-3,9-dioxa-6-thiacyclodecan-1-ol 6,6-dioxide (22).** A solution of lithium sulfide (11 mg, 0.23 mmol) in water (0.3 mL) was added dropwise within 30 min to a solution of **21**[24] (60 mg, 0.15 mmol) in refluxing ethanol (15 mL). The resulting mixture