Nonpeptidal P_2 Ligands for HIV Protease Inhibitors: Structure-Based Design, Synthesis, and Biological Evaluation

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Design and synthesis of nonpeptidal bis-tetrahydrofuran ligands based upon the X-ray crystal structure of the HIV-1 protease−inhibitor complex **1** led to replacement of two amide bonds and a 10π-aromatic system of Ro 31-8959 class of HIV protease inhibitors. Detailed structure−activity studies have now established that the position of ring oxygens, ring size, and stereochemistry are all crucial to potency. Of particular interest, compound **49** with (3*S*,3a*S*,-6a*S*)-bis-Thf is the most potent inhibitor (IC_{50} value 1.8 ± 0.2 nM; CIC_{95} value 46 ± 4 nM) in this series. The X-ray structure of protein−inhibitor complex **49** has provided insight into the ligand-binding site interactions. As it turned out, both oxygens in the bis-Thf ligands are involved in hydrogen-bonding interactions with Asp 29 and Asp 30 NH present in the S_2 subsite of HIV-1 protease. Stereoselective routes have been developed to obtain these novel ligands in optically pure form.

The understanding of protein−ligand interactions has been greatly advanced by the unprecedented advances in molecular biology and modern spectroscopic and X-ray crystallographic techniques. Concurrent to these remarkable achievements, structure-based design and synthesis of molecular probes for biologically important peptides and proteins has become a subject of great interest in contemporary bioorganic and medicinal chemistry.[1] Because of the therapeutic potential for the treatment of AIDS, the structure-based design and synthesis of HIV protease inhibitors perhaps has attracted the most attention.[2] An impressive number of X-ray crystal sturctures of the protein−ligand complexes of HIV protease have been resolved to obtain molecular insight into the ligand-binding site interaction.[3] A number of therapeutically promising HIV protease inhibitors have already resulted from structure-based design strategies.[4,5]

We recently reported a number of nonpeptidal high-affinity ligands for the HIV protease substrate-binding site.[6−10] These ligands are designed based upon various available three-dimensional structures of the protein−ligand complexes. The key feature in our ligand design is the incorporation of a conformationally constrained functionality that replaces a peptide bond and mimics the biological mode of action. As exemplified, a stereochemically defined tetrahydrofuran ring can serve as a surrogate (inhibitor **2**) for the asparagine side chain of Ro 31-8959-based HIV protease inhibitors.[7] An examination of the X-ray crystal structure of inhibitor Ro 31-8959 bound to HIV protease led us to further speculate that a fused bicyclic ligand with oxygens positioned properly could effectively hydrogen bond to the NH of the Asp 29 and 30 residues corresponding to the quinaldic amide−asparagine amide fragment of the Ro 31-8959 inhibitor.[5a] Furthermore, we presumed that due to considerable rotational freedom about the four bonds connecting the two carbonyls involved, such constrained ligands may provide additional gains in binding energy and thereby offset the loss of the P_3 hydrophobic binding of the quinoline ring. Indeed, as described[8] in a recent communication, the structure-based design of a stereochemically defined fused bicyclic tetrahydrofuran effectively replaced two amide bonds and a 10π-aromatic system of **1** (Ro 31-8959).[5a] Subsequently, structure−activity studies established that the position of ring oxygens, ring size, and stereochemistry are all important to effective binding. In this article, we report the structure-based design, synthesis, and structure−activity studies of a new class of protease inhibitors incorporating novel nonpeptidal ligands which interact specifically at the HIV protease substrate-binding site.

Chemistry

The enantioselective synthesis of bis-tetrahydrofuran (bis-Thf) **7** is illustrated in Scheme 1. Allylation of 3(*R*)-diethyl malate (**3**) according to Seebach's procedure[11] afforded the desired diasteoreomer **4** as the major (selectivity 12:1) product after distillation (85% yield). The above mixture was reduced by LAH in diethyl ether, and the resulting triol was treated with a catalytic amount of *p*-TsOH in acetone to provide the isopropylidene derivative **5** (74% yield). Swern oxidation of **5** provided the aldehyde which upon treatment with camphorsulfonic acid (CSA) in methanol furnished the methyl acetal **6** as a mixture (ratio 4:1) in 50% yield. The acetal mixture **6** was converted to bis-Thf ligand **7** by the following reaction sequence: (1) ozonolytic cleavage of the terminal olefin, (2) $NaBH_4$ reduction of the resulting aldehyde in ethanol at 0 °C, and (3) exposure of the corresponding alcohol with CSA in methylene chloride at 23 °C for 12 h (74% from **6**). The mixed acetal **6** was also converted to the bicyclic ligand **8** by

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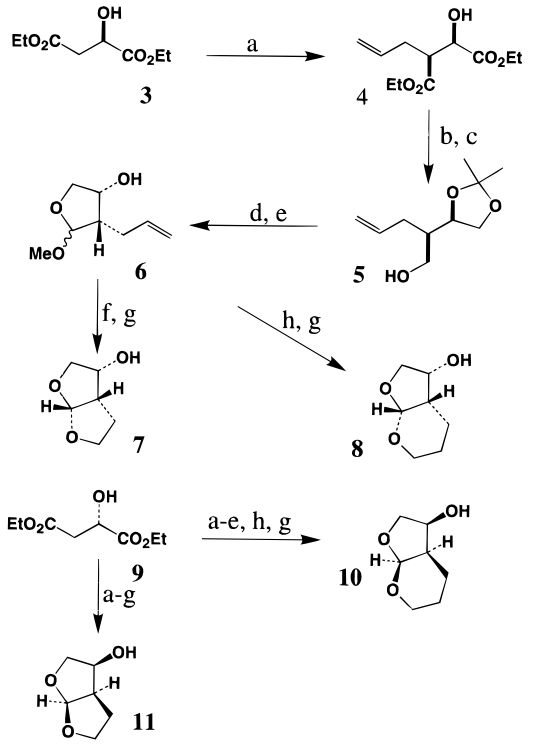
Scheme 1. Enantioselective Synthesis of the Bicyclic Ligands[a]

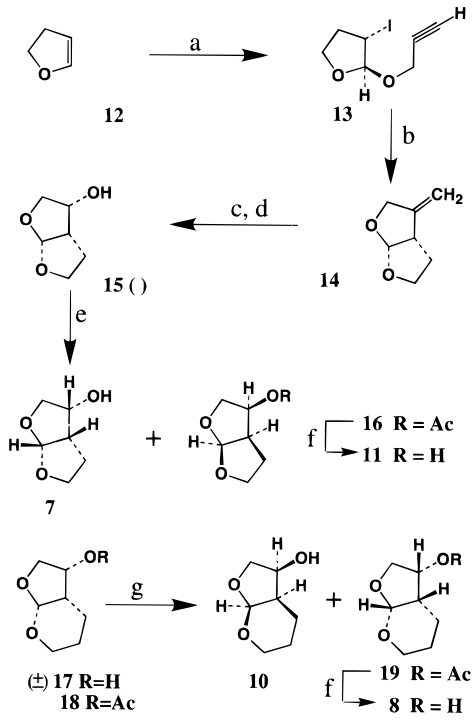


[a] Key: (a) LDA, CH=CHCH$_2$Br; (b) LAH, Et$_2$O; (c) acetone, pTsOH; (d) Swern oxidation; (e) CSA, MeOH; (f) ozonolysis then NaBH$_4$; (g) CSA, CH$_2$Cl$_2$; (h) 9-BBN, THF, aqueous NaOH, H$_2$O$_2$.

hydroboration with 9-BBN followed by reaction of the resulting alcohol with CSA in methylene chloride. Similarly, enantiomeric bicyclic ligands **10** and **11** were synthesized, starting from optically pure 3(*S*)-diethyl malate (**9**) following the sequence of reactions described above.

Alternatively, racemic synthesis of these ligands followed by their enzymatic resolution provided an easy access to these ligands in optically active form.[12] As shown in Scheme 2, reaction of commercial 2,3-dihydrofuran (**12**) with *N*-iodosuccinimide and propargyl alcohol in methylene chloride at 0−23 °C for 3 h resulted in the iodo ether **13** in excellent yields (91−95%). Radical cyclization of the iodo ether **13** with tributyltin hydride[13] in refluxing toluene in the presence of a catalytic amount of AIBN afforded the bicyclic acetal **14** in good yield (70−80%) after silica gel chromatography. This radical cyclization was more conveniently effected with sodium borohydride reduction in the presence of a catalytic amount (10 mol %) of cobaloxime (Scheme 2)[14] in 95% ethanol at 65 °C for 3 h affording the bicyclic acetal **14** in comparable yield (70−75%). Ozonolytic cleavage followed by the reduction of the resulting ketone with sodium borohydride in ethanol at −15 °C furnished the racemic endo alcohol **15** (74−78%) after chromatography.[16] The optical resolution of the racemic alcohol **15** was carried out efficiently by exposure to Amano lipase[17]-mediated acylation as well as the hydrolysis of the corresponding acetate. Thus, acylation of **15** with immobilized[18] lipase PS-30 (25% by weight with respect to lipase PS30) in the presence of acetic anhydride in dimethoxyethane at 23 °C for 3 h afforded the unacylated alcohol **7** (42% yield) and the acylated alcohol **16** (45% yield) which were separated by silica gel chromatography. The optical purity of the

Scheme 2. Synthesis and Optical Resolution of the Bicyclic Ligands[a]



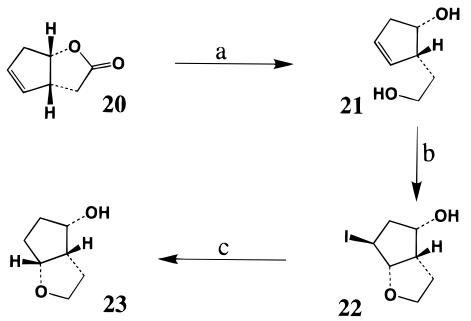
[a] Key: (a) *N*-iodosuccinimide, propargyl alcohol, CH$_2$Cl$_2$, 0−23 °C; (b) cobaloxime (cat), NaBH$_4$, EtOH; (c) O$_3$, CH$_2$Cl$_2$−MeOH, Me$_2$S, −78−23 °C; (d) NaBH$_4$, EtOH, −15 °C; (e) immobilized lipase 30, Ac$_2$O, DME, 23 °C; (f) aqueous LiOH, THF−H$_2$O; (g) immobilized lipase 30, pH 7 buffer, 23 °C.

alcohol **7** (95% ee, $[\alpha]^{23}_D$ −11.9°, MeOH) was determined by formation of Mosher ester and ^{19}F-NMR analysis.[19] The acylated alcohol **16** was hydrolyzed by treatment with aqueous lithium hydroxide to provide optically active **11** (87% ee, $[\alpha]^{23}_D$ +11.7°, MeOH). Similarly, racemic **17** was synthesized utilizing dihydropyran as the starting material. The resolution of **17** was effected by formation of the corresponding acetate **18** followed by the enzymatic hydrolysis with immobilized lipase PS-30 in phosphate buffer (pH = 7.0) at 23 °C for 24 h. The hydrolyzed alcohol **10** (yield 34%, 90% ee) and the acetate **19** (40%) were separated by silica gel chromatography. Ester hydrolysis of **19** furnished the alcohol **8** in optically active form (94% ee). The represented absolute configurations of the resolved alcohols were assigned based on comparison of their optical rotation with the ligands synthesized utilizing 3(*S*)- and 3(*R*)-diethyl malates as described above.

Enantiomerically pure fused tetrahydrofuran **23** was synthesized from commercial *cis*-(−)-3,3a,6,6a-tetrahydro-2*H*-cyclopenta[*b*]furan-2-one (**20**) according to Scheme 3. As shown, reduction of **20** with LAH in tetrahydrofuran at 23 °C afforded the diol **21** (isolated yield 96%). Treatment of the diol **21** with iodine and potassium iodide in methylene chloride at 23 °C furnished the iodo ether **22**.[20] Radical dehalogenation of the iodine with tributyltin hydride in refluxing dioxane in the presence of a catalytic amount of AIBN provided the bicyclic ligand **23** with defined absolute configuration.

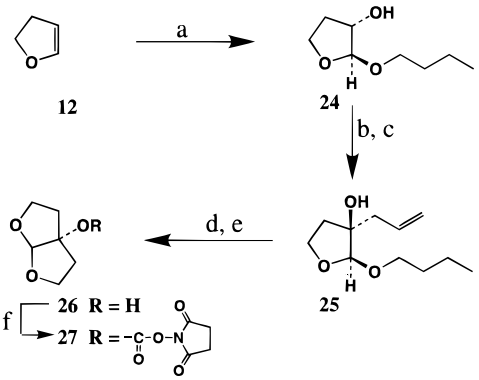
Synthesis of symmetric bicyclic ligand **26** is outlined in Scheme 4. Reaction of 2,3-dihydrofuran (**12**) with mCPBA in 1-butanol at −15−0 °C for 2 h afforded the alcohol **24** after distillation (65%). Oxidation of **24** with

Scheme 3. Enantioselective Synthesis of the Bicyclic Ligands[a]



[a] Key: (a) $LiAlH_4$, THF, 23 °C; (b) KI, I_2, $NaHCO_3$, CH_2Cl_2, 23 °C; (c) nBu_3SnH, AIBN, dioxane, reflux.

Scheme 4. Synthesis of the Symmetric Bicyclic Ligand[a]



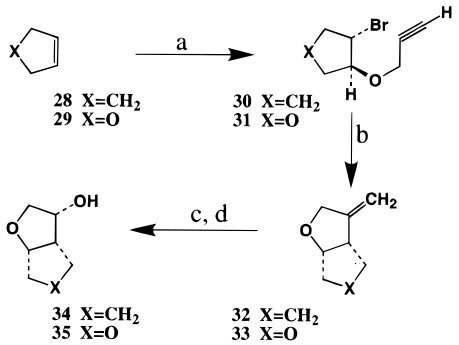
[a] Key: (a) mCPBA, *n*-butanol, 0 °C; (b) Pyr·SO_3, DMSO, Et_3N; (c) allylmagnesium bromide, Et_2O, 0−23 °C; (d) O_3, CH_2Cl_2−MeOH, −78−0 °C, then $NaBH_4$, EtOH, 0 °C; (e) pTsOH, CH_2Cl_2, 23 °C; (f) $COCl_2$, pyridine, $PhCH_3$, then *N*-hydroxysuccinimide, CH_3CN, Et_3N, 23 °C.

pyridine·SO_3 complex in methylene chloride gave the corresponding ketone which was reacted with allylmagnesium bromide in diethyl ether at 0−23 °C for 4 h to furnish the alcohol **25**.[21] Ozonolysis of the terminal olefin followed by reduction of the ozonide with sodium borohydride in ethanol at 0 °C provided the corresponding alcohol which was treated with *p*-TsOH in methylene chloride to afford the symmetric ligand **26**. Reaction of **26** with phosgene and pyridine in toluene followed by reaction with *N*-hydroxysuccinimide in acetonitrile furnished the mixed active carbonate **27** after silica gel chromatography.[22]

Racemic bicyclic ligands **34** and **35** were prepared (Scheme 5) utilizing a similar synthetic route as described for racemic **15**. Reaction of cyclopentene with *N*-bromosuccinimide and propargyl alcohol provided good yield of the corresponding bromo ether **30** (yield 72%). However, the reaction with 2,5-dihydrofuran proceeded with modest yield (35%) of the corresponding bromo ether **31**. Tributyltin hydride-mediated radical cyclization of **30** and **31** provided the bicyclic olefins **32** and **33** which were converted to racemic bicyclic ligands **34** and **35** for structure−activity studies.

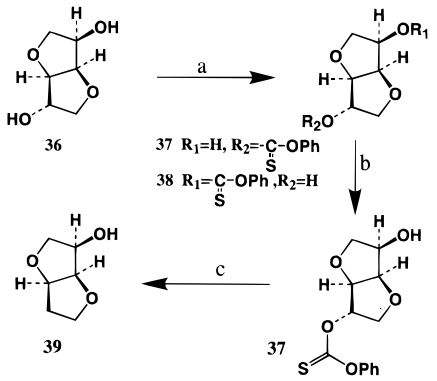
Bicyclic ligand **39** with oxygens in a vicinal relationship was synthesized in enantiomerically pure form starting from commercially available 1,4:3,6-dianhydro-D-sorbitol (**36**) (Scheme 6). Treatment of **36** with commercial chlorothionoformate and triethylamine in methylene chloride at 23 °C for 4 h afforded a mixture (2:1) of thionocarbonates **37** and **38**. The isomers were separated on silica gel by column chromatography, and

Scheme 5. Synthesis of Racemic Bicyclic Ligands[a]



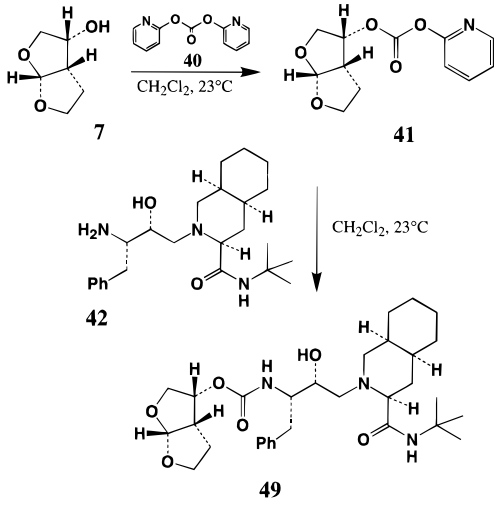
[a] Key: (a) *N*-bromosuccinimide, propargyl alcohol, CH_2Cl_2, 0−23 °C; (b) nBu_3SnH, AIBN, PhH, reflux; (c) O_3, CH_2Cl_2−MeOH, Me_2S, −78−23 °C; (d) $NaBH_4$, EtOH, −15 °C.

Scheme 6. Synthesis of the Bicyclic Ligand[a]



[a] Key: (a) PhOC(S)Cl, Et_3N, CH_2Cl_2; (b) separated by silica gel chromatography; (c) nBu_3SnH, AIBN, PhMe, reflux.

Scheme 7



the major isomer **37** was exposed to the radical deoxygenation[23] conditions to provide the ligand **39**.

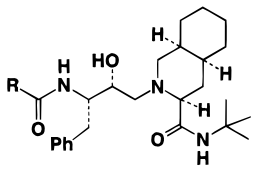
Synthesis of various inhibitors with bicyclic ethers as the P_2 ligands and decahydroisoquinolinecarboxamide as the P_1' ligand was carried out according to Scheme 7. The previously described[6,7] hydroxyethylamine isostere **42** was transformed into the various target inhibitors listed in Tables 1 and 2 by an alkoxycarbonylation of the respective alcohol.[24] For example, reaction of bis-Thf ligand **7** with dipyridyl carbonate (**40**) and triethylamine in methylene chloride afforded the active carbonate **41** after chromatography. Reaction of the mixed carbonate **41** with amine **42** in methylene chloride

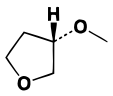
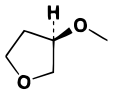
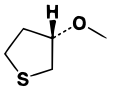
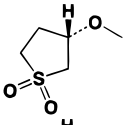
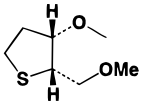
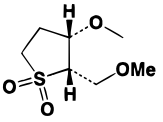
afforded only the inhibitor **49** (white solid, mp 98−101 °C) by ^{1}H-NMR and HPLC analysis (isolated yield 76%). For the preparation of inhibitor **55**, the mixed active carbonate **27** was reacted with amine **42** in methylene chloride in the presence of 3 equiv of triethylamine at 23 °C for 12 h to provide **55** after silica gel chromatography. Similarly, various nonpeptidal ligands were converted to other inhibitors in Tables 1 and 2.

Results and Discussion

As described previously,[9] the preliminary X-ray crystal structure of the enzyme−inhibitor complex of **43** and HIV-1 protease[25] has suggested a number of structural changes on the tetrahydrofuran ring that could lead to improved binding. The urethane of 3(*S*)-hydroxysulfolane (**46**) has exhibited nearly a 2-fold potency enhancement compared to **43**, presumably due to the close proximity of the sulfolane oxygen cis to the 3-hydroxyl group to Asp 29 and Asp 30 NH. It is evident in the crystal structure that the oxygen atom of the tetrahydrofuran ring in **43** is oriented toward the Asp 29 and Asp 30 NH. Recently, researchers from Vertex Laboratories have reported a similar observation in their X-ray crystal structure of protein−ligand complex containing an inhibitor with 3(*S*)-tetrahydrofuranyloxy group as the P_2 ligand.[26] The protein−ligand structure of inhibitor **43** further provided rationale for incorporation of the *cis*-2-alkyl substitutent of the sulfolane ring of inhibitor **46**. Optimization of these findings resulted in inhibitors with reduced molecular weight and comparable in vitro potency to **1** (Ro 31-8959). A detailed account of these investigations has been reported recently.[8] Subsequently, during the introduction of a *cis*-2-methoxymethyl substitutent of the sulfolane ring of inhibitor **46**, we have observed a very intriguing structure−activity relationship. As evident in Table 1, replacement of the ring oxygen in **43** with sulfur (inhibitor **45**) resulted in no change in inhibitory potency. Oxidation of the ring sulfur to sulfone has provided nearly a 2-fold improvement over **45**. Thus far, our observation is that the ring sulfones, in general, are 2−5-fold more potent than their corresponding ring sulfides.[27] Consistent with our earlier observation, incorporation of a *cis*-2-methoxymethyl substitutent in cyclic sulfide **45** afforded inhibitor **47** with a 6-fold potency enhancement. However, in contrast, oxidation of ring sulfur to the corresponding sulfone resulted in inhibitor **48** with reduction in potency (IC_{50} 23.8 nM). To gain insight into the molecular binding properties, an energy-minimized active model of inhibitors **46** and **47** was created[28] utilizing the X-ray crystal structure of the enzyme−inhibitor complex of L-689,502 bound to HIV-1 protease (2.25 Å resolution).[30] On the basis of superimposition of these modeled structures (Figure 1), it appeared that in addition to filling the hydrophobic pocket in the S_2 region, the methoxyl oxygen of inhibitor **47** is in close proximity to hydrogen bonding with the Asp 29 and Asp 30 NH. Thus, the methoxyl oxygen is effectively competing for the same binding site as the sulfolane oxygen cis to the 3-hydroxyl group of inhibitor **46**. Therefore, oxidation of the ring sulfide in inhibitor **47** did not provide any additional potency enhancement as was observed earlier. These findings subsequently provided the basis for a conformationally constrained and structurally new class of ligand design.

Table 1. Structure and Inhibitory Potencies of Various Heterocyclic Derivatives



Comp.	R	IC_{50} (nM)	CIC_{95} (nM)
43.		132±30 (n=72)	800 (n=3)
44.		694	>800
45.		132	---
46.		76±12 (n=14)	364±44 (n=17)
47.		19.7	---
48.		23.8	---

As can be seen from the superimposed stereoviews of **46** and **47** (Figure 1), the conformation of methoxymethyl side chain of **47** can be further restricted to form another ring cycle to the existing five-membered ring. On the basis of this possible molecular insight, we speculated that a bicyclic ligand with oxygens positioned appropriately in the ring would interact effectively with the Asp 29 and Asp 30 residues of the enzyme active site. Also, superimposition of the X-ray crystal structure of the protein−ligand complex[31] of Ro 31-8959 and the modeled structure of **47** (Figure 2) revealed the potential benefit of such ligand design. It appears that a fused bicyclic tetrahydrofuran not only could replace the quinaldic amide and asparagine amide of Ro 31-8959 inhibitor, but it may also provide additional binding energy to offset the loss of P_3-hydrophobic binding corresponding to the quinoline ring. Indeed, incorporation of bis-tetrahydrofuran as the P_2 ligand with 3(*R*),3a(*S*),6a(*R*)-configurations (inhibitor **49**), as speculated from the ligand-binding site interactions of inhibitors **47** and **1**, has shown impressive in vitro potencies.[9] As presented in Table 2, inhibitor **49** has shown an enzyme inhibitory potency (IC_{50}) of 1.8 ± 0.2 nM (n = 6). In comparison, the inhibitor with 3(*S*),3a(*R*),6a(*S*)-bis-Thf as the P_2 ligand (inhibitor **50**, IC_{50} 6.4 nM) is less potent than **49**. The difference in enzyme inhibitory potencies is also reflected in their antiviral activities. Inhibitor **49** has prevented the spread of HIV-1 in MT4 human T-lymphoid cells infected with IIIb isolate[32] at an average concentration (n = 4) of 46 ± 4 nM (CIC_{95}). Inhibitor **50**, in contrast, has shown an antiviral potency of 200 nM. In head to head

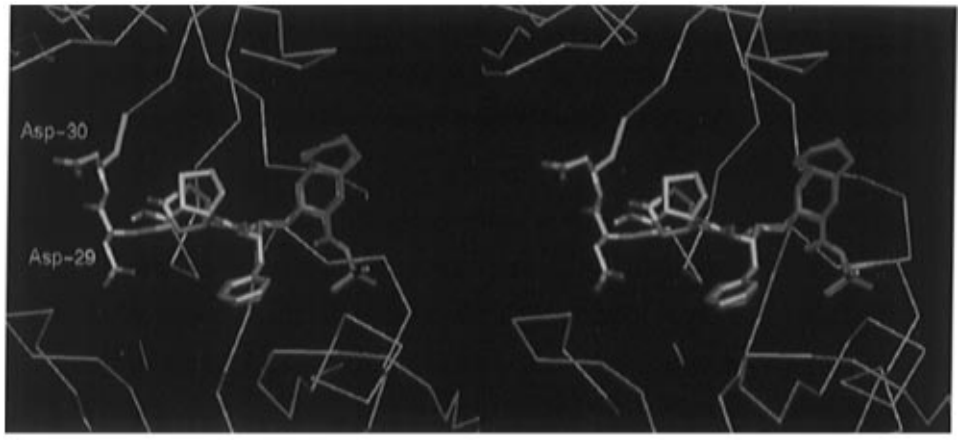


Figure 1. Stereoview of the optimized bound conformations of inhibitors **46** (magenta) and **47** (green) superimposed in the HIV-1 protease active site.[30]

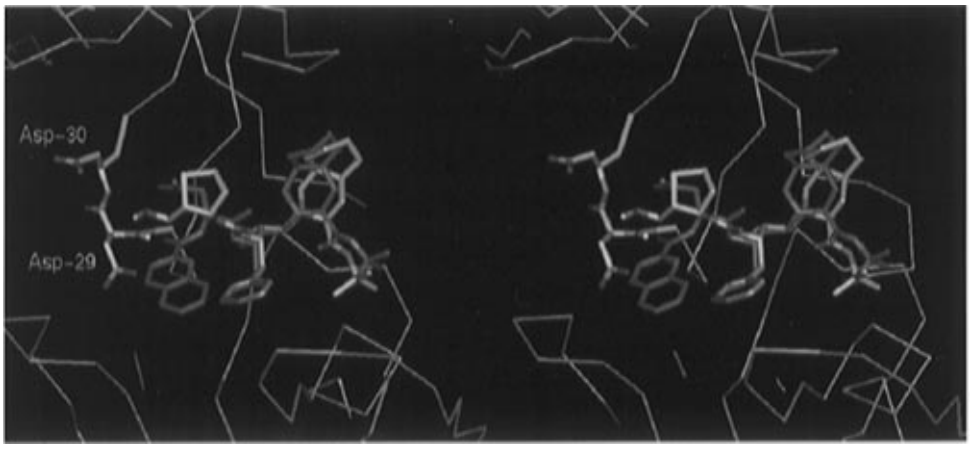
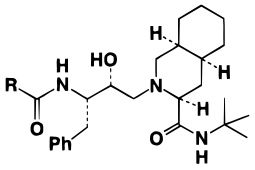


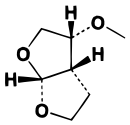
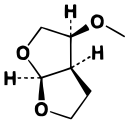
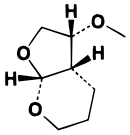
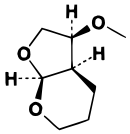
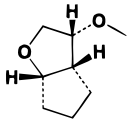
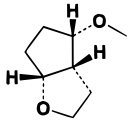
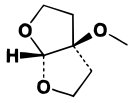
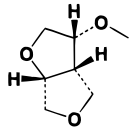
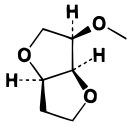
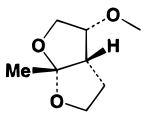
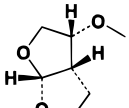
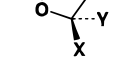
Figure 2. Stereoview of the X-ray structure of inhibitor **1** (magenta) bound to HIV-1 protease and optimized bound conformation of inhibitor **47** (green).

comparison, inhibitor **49** was shown to be equipotent to **1** (Ro 31-8959; CIC_{95} 23 ± 7 nM).[33] The enhanced inhibitory potency as well as the stereochemical preference for 3(*R*),3a(*S*),6a(*R*)-configurations in bis-Thf ligand (inhibitor **49)** indicated a specific hydrogen-bonding interaction with the residues of the S_2 region of the enzyme active site. Incorporation of 3-hexahydrofuropyran as the P_2 ligand has shown reversal of stereochemical trends. Inhibitor **52** with 3(*S*),3a(*R*),7a(*S*)-configurations has shown an IC_{50} value of 1.2 nM, a greater than 3-fold potency increase over inhibitor **51** (IC_{50} 4.2 nM). Antiviral potency of this inhibitor (compound **50**; CIC_{95} 50 nM) is also comparable to that of Ro 31-8959 or inhibitor **49**. Evaluation of compounds **53** and **54** established that both oxygens are involved in binding. Incorporation of symmetric bis-Thf ligand (inhibitor **55**) resulted in at least a 225-fold loss (IC_{50} 410 nM) in inhibitory potency. Similarly, change of the ring oxygen positions (inhibitors **56** and **57**) also resulted in significant loss of potency. Various substitution at the bicyclic ring (inhibitors **58**−**60**) did not improve enzyme inhibitory or antiviral potencies. Thus, the above structure−activity studies provided ample evidence that in the bis-Thf ligand of inhibitor **49**, the ring stereochemistry, ring size, and position of ring oxygens all are critical to effective binding in the S_2 region of the substrate-binding site.

To gain further insight into the molecular binding properties, the three-dimensional structure of both inhibitors **1** (Ro 31-8959) and **49** was determined by X-ray diffraction at a resolution of 2.2 and 2.10 Å, respectively.[34] A superimposed stereoview of these inhibitors (Figure 3) was then created by superimposition of the X-ray crystal structure of **1** (green) on the X-ray crystal structure of **49** (magenta) bound to HIV-1 protease in the same frame of reference. The (*R*)-hydroxyl group of inhibitor **49** is positioned symmetrically between the catalytic aspartates of the HIV-1 protease. The P_1 benzyl side chain and the P_1' decahydroisoquinoline moiety of **49** are located at the S_1 and S_1' regions, respectively, in the enzyme active site. The *N*-*tert*-butyl group is positioned in the S_2' subsite. A comparison of binding properties of the bis-Thf ligand of **49** and the asparagine of **1** is very intriguing. As shown, both the bis-Thf oxygen-1 and asparagine carbonyl of **1** are within hydrogen-bonding distance (3.2 and 3.5 Å, respectively, between the heavy atoms) to the Asp 30 NH of the HIV-1 protease. The bis-Thf oxygen-6 and the P_3 quinoline amide carbonyl of **1** are also appropriately positioned for hydrogen-bonding interaction with the Asp 29 NH (bonding distance 3.0 and 3.3 Å, respectively, between the heavy atoms) present in the S_2-binding domain of the enzyme. Like most reported protein−ligand complex structures, the P_2 bis-Thf urethane carbonyl and the *tert*-butyl amide carbonyl of **49** hydrogen bond to the structural water molecule that interacts with the flap Ile 50 NH residues.[35] Thus, incorporation of a stereochemically defined bis-Thf or fused Thf−Thp ligand into the Ro 31-8959-based hydroxyethylamine isostere provided

Table 2. Structure and Inhibitory Potencies of Inhibitors Containing Novel Bicyclic P_2 Ligands



Comp.	R	IC_{50} (nM)	CIC_{95} (nM)
49.		1.8±0.2 (n=7)	46±4 (n=4)
50.		6.4	200
51.		4.2	100
52.		1.2	50
53.		190	---
54.		17	---
55.		410	>200
56.		1700	---
57.		>3000	>200
58.		18.1	>200
59.		4.1 (X=Me, Y=H)	200
60.		27.1 (X=H, Y=Me)	200

inhibitors (compounds **49** and **52**) with comparable in vitro antiviral potencies to inhibitors with both P_2 and P_3 ligands. As shown in Figure 3, the bis-Thf ligand essentially replaces two amide bonds and a 10π-aromatic system of the Ro 31-8959 inhibitor which is currently in advanced clinical trials.[36]

In addition to reduction of molecular weight and replacement of amide bonds, inhibitors incorporating the fused cyclic ethers as P_2 ligands have shown significant improvement in aqueous solubility compared to the Ro 31-8959 class of inhibitors. As can be seen in Table 3, inhibitor **49** has shown an aqueous solubility of 0.235 mg/mL in phosphate buffer (pH = 7.4), a greater than 23-fold improvement over **1** (Ro 31-8959; aqueous solubility less than 0.01 mg/mL). The 3(*S*)-tetrahydrofuranyl carbamate in inhibitor **43** also exhibited solubility enhancement (aqueous solublity 0.15 mg/mL). The inhibitor containing bis-Thf ligand (inhibitor **49**) also shows a decreased log *P* value compared to inhibitor **1**. The log *P* values of inhibitors **1** and **49** were measured to be 5.7 and 3.5, respectively. Pharmacokinetic studies in dogs with inhibitor **52** are encouraging. Inhibitor **52** was administered in dogs at an oral dose of 10 mg/kg dissolved in a 10% citric acid solution. The average (two dogs) C_{max} was 4380 nM after 20 min. After 3 h, the plasma level was above 200 nM, more than 4-fold over its CIC_{95} value. Oral bioavailability was estimated by comparing the area under the curve following oral administration with that of iv administration at a dose of 2 mg/kg in DMSO. The oral bioavailability was determined to be 17% in dogs under the formulation protocol described above.[37]

Conclusion

In summary, on the basis of X-ray crystal structures of various protein−ligand complexes, we have designed and synthesized a novel class of nonpeptidal high-affinity P_2 ligands for the HIV-1 protease substrate-binding site. The designed ligands are conformationally constrained with a fused bicyclic ether-like feature. The inhibitor incorporating a 3(*R*),3a(*S*),6a(*R*)-bis-Thf as the P_2 ligand (inhibitor **49**) is the most potent compound in the series. This inhibitor has exhibited in vitro antiviral activities comparable to inhibitors in the Ro 31-8959 (inhibitor **1**)-based hydroxyethylamine series with both P_2 and P_3 ligands. Based on the inhibitor-bound X-ray crystal structures of **1** and **49** with the HIV-1 protease, it appears that the bis-Thf ring oxygens essentially compete for the same binding site as the P_2 asparagine carboxamide and the P_3 quinaldic amide carbonyls of inhibitor **1** (Ro 31-8959). Through various structure−activity studies, we have also shown that the stereochemistry, position of oxygens, substitution in the ring, and ring size all are critical to optimum binding. Incorporation of bis-Thf ligand (inhibitor **49**) not only provided a protease inhibitor with reduced molecular weight but also led to improved aqueous solubility and decreased log *P* value (Figure 4). Basically, fusion of a tetrahydrofuran ring to inhibitor **44** resulted in inhibitor **49** (Figure 4) with an impressive 300-fold enhancement in its inhibitory potency and more than 30-fold enhancement of its antiviral potency. The molecular weight of the bis-Thf is essentially one-half the combined molecular weight of the P_2 asparagine and P_3 quinoline ligands of inhibitor **1**. Further molecular design is currently underway in our laboratories.

Experimental Section

All melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton

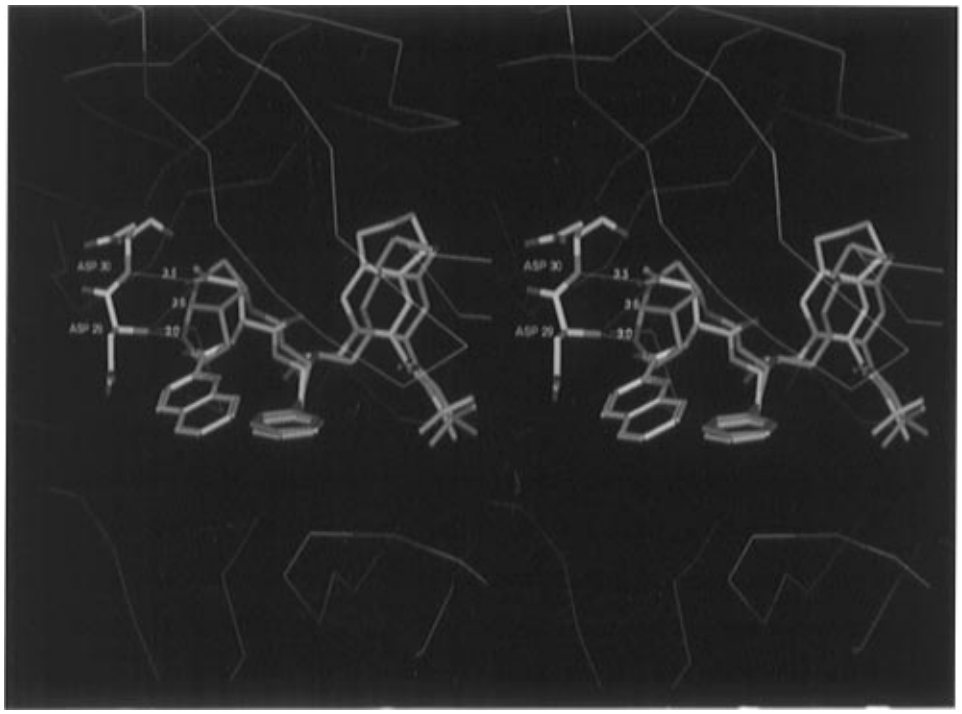


Figure 3. Stereoview of the X-ray structure of the inhibitors **1** (green) and **49** (magenta) bound to HIV-1 protease.

Table 3. Aqueous Solubilities of Selected Inhibitors

inhibitors	solubility (mg/mL)
1	0.01
43	0.15
49	0.235
50	0.130
52	0.107

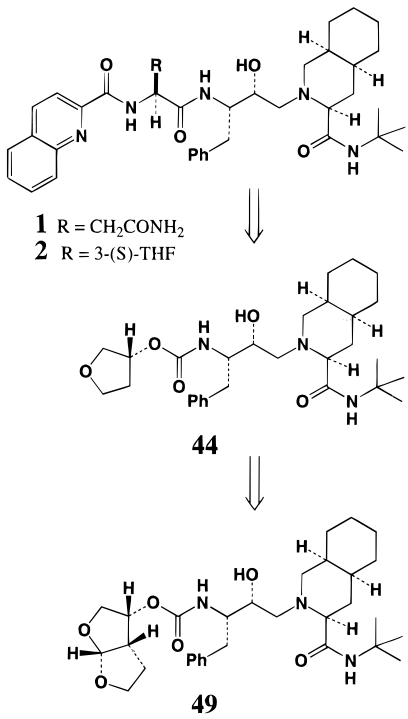


Figure 4.

magnetic resonance spectra were recorded on a Varian XL-300 spectrometer using tetramethylsilane as the internal standard. Significant ^{1}H-NMR data for representative compounds are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in hertz. FAB mass spectra were recorded on a VG Model 7070 mass spectrometer, and relevant data are tabulated as *m/z*. Elemental analyses were performed by the Analytical Department, Merck Research Laboratories, West Point, PA, and were within ±0.4% of the theoretical values. Anhydrous solvents were obtained as follows: methylene chloride, distillation from P_4O_{10}; tetrahydrofuran, distillation from sodium/benzophenone; dimethylformamide and pyridine, distillation from CaH_2. All other solvents were HPLC grade. Column chromatography was performed with E. Merck 240−400 mesh silica gel under a low pressure of 5−10 psi. Thin-layer chromatography (TLC) was carried out with E. Merck silica gel 60 F-254 plates.

(2*R*,3*S*)-Ethyl 2-Hydroxy-3-allylsuccinate (4). (−)-Diethyl (2*R*,3*S*)-3-allyl-2-hydroxysuccinate (7.2 g) was prepared according to the procedure of D. Seebach, J. Aebi, and D. Wasmuth, *Organic Syntheses* **1985**, *63*, 109−120. Compound **4**: ^{1}H-NMR ($CDCl_3$) δ 5.8 (m, 1 H), 5.15 (m, 2 H), 4.3 (m, 1 H), 4.1−4.2 (m, 4 H), 3.0 (m, 1 H), 2.4−2.7 (m, 2 H), 1.25 (t, 3 H, J = 7.0 Hz), 1.2 (t, 3 H, J = 6.9 Hz).

(2*R*,3*R*)-1,2-*O*-Isopropylidene-3-allylbutane-1,4-diol (5). A solution of 9.4 g (40.8 mmol) of (2*R*,3*S*)-ethyl 2-hydroxy-3-allylsuccinate (**2**) in 15 mL of diethyl ether was added dropwise to a suspension of 3.1 g (81.6 mmol) of $LiAlH_4$ in ether (70 mL) at 0 °C. The resulting mixture was stirred at room temperature for 12 h. After this period, the mixture was heated to reflux for 1 h and then cooled to 0 °C. The reaction was quenched by sequential dropwise addition of water (3.1 mL), 20% aqueous NaOH (3.1 mL), and then water (8 mL). The mixture was stirred for 1 h, and THF (50 mL) and anhydrous Na_2SO_4 were added. The mixture was filtered through Celite, and the filter cake was throughly washed with THF. Evaporation of the solvents gave 6.3 g of the corresponding triol, as a colorless oil which was utilized directly without further purification.

The resulting triol was dissolved in acetone (500 mL), and 150 mg of *p*-toluenesulfonic acid monohydrate was added. The resulting mixture was stirred at 24 °C for 3 h. After this period, the mixture was concentrated under reduced pressure, and the resulting residue was taken up in ethyl acetate and washed with saturated aqueous $NaHCO_3$. The layers were separated, and the organic layer was dried over Na_2SO_4. Filtration and evaporation of the solvent provided a residue which was chromatographed over silica gel (50% ethyl acetate/hexane) to furnish 4.1 g (59% yield) of the title compound as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 5.75 (m, 1 H), 5.1 (m, 2 H),

4.1 (m, 2 H), 3.7 (m, 4 H), 2.0 (m, 1 H), 2.0 (m, 1 H), 1.45 (s, 3 H), 1.4 (s, 3 H).

(2*S*,3*S*,4*R*)-2-Methoxy-3-allyl-4-hydroxytetrahydrofuran (6). To a stirred solution of oxalyl chloride (1.1 mL) in CH_2Cl_2 (40 mL) at −50 °C was added a mixture (1:2) of dimethyl sulfoxide and CH_2Cl_2 (6 mL). The resulting mixture was stirred for 3 min, and a solution of 2.0 g of (2*R*,3*R*)-1,2-*O*-isopropylidene-3-allylbutane-1,4-diol in CH_2Cl_2 (6 mL) was added dropwise over a period of 5 min. The mixture was allowed to warm to −20 °C over a period of 1.5 h, and triethylamine (10 mL) was added. The reaction mixture was stirred at −20 °C to room temperature for 1 h, and water (30 mL) was added. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed over silica gel (25% ethyl acetate/hexane) to provide 1.56 g of the corresponding aldehyde as a colorless oil.

To a stirred solution of 1.3 g of the aldehyde in methanol (25 mL) was added camphorsulfonic acid (300 mg), and the resulting mixture was stirred at 24 °C for 12 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (5 mL), and the mixture was concentrated under reduced pressure. The residue was partitioned between ethyl acetate and brine solution. The layers were separated, and the aqueous layer was extracted with ethyl acetate (50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a residue which was chromatographed over silica gel (25% ethyl acetate/hexane) to provide (815 mg) the methyl acetal **6** (4:1 mixture) as an oil.

(3*R*,3a*S*,6a*R*)-3-Hydroxyhexahydrofuro[2,3-*b*]furan (7). To a stirred solution of 500 mg (3.2 mmol) of (2*R*,3*S*,4*R*)-2-methoxy-3-allyl-4-hydroxytetrahydrofuran (**4**) in a mixture (1:1) of methanol and methylene chloride (25 mL) at −78 °C was bubbled through a stream of ozonized oxygen until the blue color persisted (1 h). After the solution was flushed with nitrogen for 5 min, dimethyl sulfide (3 mL) was added. The cooling bath was removed, and the mixture was allowed to warm to room temperature. Evaporation of the solvent gave the crude aldehyde as a colorless oil. The resulting aldehyde was dissolved in absolute ethanol (20 mL) and cooled to 0 °C, and solid $NaBH_4$ (180 mg, 4.7 mmol) was added in three portions. The mixture was stirred for 15 min, and the reaction was quenched with 10% aqueous citric (3 mL) acid. The mixture was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate and brine solution. The layers were separated, and the aqueous layer was extracted with ethyl acetate (25 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a residue which was chromatographed over silica gel (75% ethyl acetate/hexane) to provide 430 mg of (2*R*,3*S*,4*R*)-2-methoxy-3-(2′-hydroxyethyl)-4-hydroxytetrahydrofuran as a colorless oil.

To stirred a solution of 400 mg of (2*R*,3*S*,4*R*)-2-methoxy-3-(2′-hydroxyethyl)-4-hydroxytetrahydrofuran in dry CH_2Cl_2 (100 mL) was added camphorsulfonic acid (200 mg). The resulting mixture was stirred at 23 °C for 12 h. After this period, the reaction was quenched by additon of saturated aqueous $NaHCO_3$ (5 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed over silica gel (75% ethyl acetate/hexanes) to give 350 mg of **7** as a colorless oil; $[\alpha]^{23}_D$ −12.4°, MeOH; ^{1}H-NMR ($CDCl_3$) δ 5.7 (d, 1 H, J = 5.13 Hz), 4.45 (dd, 1 H, J = 6.8, 14.6 Hz), 3.9−4.0 (m, 3 H), 3.65 (dd, 1 H, J = 7, 9.1 Hz), 2.9 (m, 1 H), 2.3 (m, 1 H), 1.85 (m, 2 H); MS (70 eV) m/z 131 (M^+ + H).

(3*R*,3a*S*,7a*R*)-3-Hydroxyhexahydrofuro[2,3-*b*]pyran (8). To a stirred solution of 225 mg of (2*R*,3*S*,4*R*)- and (2*S*,3*S*,4*R*)-2-methoxy-3-allyl-4-hydroxytetrahydrofuran (**6**) in 5 mL of THF cooled to −10 °C was added dropwise 4.3 mL of 0.5 M 9-BBN in THF. After stirring at 24 °C for 12 h, another 2.5 mL of 0.5 M 9-BBN was added, and stirring was continued for 24 h. The reaction was quenched with a mixture of 1 mL of 30% H_2O_2 and 1 mL of 20% NaOH. The resulting mixture was heated to 50 °C for 1.5 h and then cooled to 24 °C and extracted with three portions of ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (75% ethyl acetate/hexanes) to afford a mixture of (2*R*,3*S*,4*R*)- and (2*S*,3*S*,4*R*)-2-methoxy-3-(3′-hydroxypropyl)-4-hydroxytetrahydrofuran (140 mg) as a clear colorless oil.

A solution of 140 mg of (2*R*,3*S*,4*R*)- and (2*S*,3*S*,4*R*)-2-methoxy-3-(3′-hydroxypropyl)-4-hydroxytetrahydrofuran and camphorsulfonic acid (100 mg) in 100 mL of CH_2Cl_2 was stirred at 24 °C for 12 h. The reaction was quenched with 3 mL of saturated aqueous $NaHCO_3$ and the mixture stirred for 10 min. The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by chromatography over silica gel (75% ethyl acetate/hexanes) afforded the title compound **8** (100 mg) as a clear colorless oil; $[\alpha]^{23}_D$ +10.1°, MeOH; ^{1}H-NMR ($CDCl_3$) δ 5.0 (s, 1 H), 4.3 (m, 1 H), 3.8−4.1 (m, 3 H), 3.5 (m, 1 H), 3.0 (br s, 1 H), 2.5 (br d, 1 H), 1.8−2.2 (m, 3 H), 1.4 (m, 1 H); MS (70 eV) m/z 145 (M^+ + H).

(3*S*,3a*R*,7a*S*)-3-Hydroxyhexahydrofuro[2,3-*b*]pyran (10). From (+)-diethyl (2*S*,3*R*)-3-allyl-2-hydroxysuccinate using the procedure substantially as described above for preparation of (3*R*,3a*S*,7a*R*)-3-hydroxyhexahydrofuro[2,3-*b*]pyran, there was obtained a clear colorless oil: $[\alpha]^{23}_D$ −10.8°, MeOH; ^{1}H-NMR ($CDCl_3$) δ 5.0 (s, 1 H), 4.3 (m, 1 H), 3.8−4.1 (m, 3 H), 3.5 (m, 1 H), 3.0 (br s, 1 H), 2.5 (br d, 1 H), 1.8−2.2 (m, 3 H), 1.4 (m, 1 H); MS (70 eV) m/z 145 (M^+ + H).

(3*S*,3a*R*,6a*S*)-3-Hydroxyhexahydrofuro[2,3-*b*]furan (11). From (+)-diethyl (2*S*,3*R*)-3-allyl-2-hydroxysuccinate using the procedure substantially as described above for preparation of (3*R*,3a*S*,6a*R*)-3-hydroxyhexahydrofuro[2,3-*b*]furan, there was obtained a clear colorless oil: $[\alpha]^{23}_D$ +12.1°, MeOH; ^{1}H-NMR ($CDCl_3$) δ 5.7 (d, 1H, J = 5.1 Hz), 1.85 (m, 2 H), 4.45 (dd, 1H, J = 6.8, 14.6 Hz), 3.9−4.0 (m, 3 H), 3.65 (dd, 1 H, J = 7, 9 Hz), 2.9 (m, 1 H), 2.3 (m, 1 H); MS (70 eV) m/z 131 (M^+ + H).

***trans*-2-(Propargyloxy)-3-iodotetrahydrofuran (13).** To a stirred, ice cold suspension of 15 g (66.6 mmol) of *N*-iodosuccinimide in 150 mL of CH_2Cl_2 was added a mixture of dihydrofuran (66.6 mmol, 4.67 g, 5.1 mL) and propargyl alcohol (100 mmol, 5.0 g, 5.2 mL) in 50 mL of CH_2Cl_2 over 20 min. After warming to 24 °C with stirring over 2 h, 200 mL of water was added, and the stirring was continued for 1 h. The layers were separated and the aqueous layer extracted with 2 × 100 mL of CH_2Cl_2. The combined organic extracts were washed with brine solution containing a small amount of $Na_2S_2O_3$ (70 mg), dried over anhydrous Na_2SO_4, filtered, and concentrated. Chromatography over silica gel using 30% ethyl acetate in hexane afforded (15.4 g, 92%) the title iodo ether as an oil: ^{1}H-NMR ($CDCl_3$) δ 5.4 (br s, 1 H), 4.0−4.3 (m, 5 H), 2.7 (m, 1 H), 2.48 (br s, 1 H), 2.25 (m, 1 H); IR (neat) 2956, 2180, 1621, 1440 cm^{-1}.

(3a*R*,6a*S*)- and (3a*S*,6a*R*)-3-Methylene-4*H*-hexahydrofuro[2,3-*b*]furan (14) (Tributyltin hydride procedure). To a refluxing solution of tributyltinhydride (20.7 mL, 77 mmol) containing AIBN (100 mg) in toluene (200 mL) was added dropwise a solution of 15.4 g (61 mmol) of iodotetrahydrofuran **13** in toluene (50 mL) over a period of 1 h. The resulting mixture was stirred at reflux for an additional 4 h (monitored by TLC). The mixture was then cooled to 23 °C and concentrated under reduced pressure. The residue was partitioned between petroleum ether and acetonitrile (200 mL of each), and the acetonitrile (lower) layer was concentrated. The residue was purified by chromatography on silica gel, using 10% ethyl acetate in hexane as the eluent to provide the title product **14** (5.84 g, 76%) as an oil: ^{1}H-NMR ($CDCl_3$) δ 5.7 (d, 1 H, J = 4.9 Hz), 4.9−5.1 (m, 2 H), 4.3−4.6 (m, 2 H), 3.7−4.0 (m, 2 H), 3.3 (m, 1 H), 1.8−2.2 (m, 2 H); IR (neat) 2970, 1645, 1430 cm^{-1}.

(3a*R*,6a*S*)- and (3a*S*,6a*R*)-3-Methylene-4*H*-hexahydrofuro[2,3-*b*]furan (14) (Catalytic cobaloxime procedure).

To a solution of iodo ether **13** (6.4 g, 25.4 mmol) in 95% ethanol (80 mL) were added solid $NaBH_4$ (1.06 g, 28 mmol) and 10 N NaOH (2.6 mL, 26 mmol). The solution was flushed with N_2, and several portions of finely powered cobaloxime (611 mg, 1.5 mmol) were added during a 1 h period at 50 °C (bath temperature 65 °C). The resulting mixture was stirred for an additional 1 h, and the reaction mixture was concentrated under reduced pressure. The resulting residue was diluted with brine, and the mixture was thoroughly extracted with ether (3 × 150 mL). The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue which was chromatographed over silica gel to provide the title product (2.3 g, 73%) as an oil: ^{1}H-NMR ($CDCl_3$) δ 5.7 (d, 1 H, J = 4.9 Hz), 4.9−5.1 (m, 2 H), 4.3−4.6 (m, 2 H), 3.7−4.0 (m, 2 H), 3.3 (m, 1 H), 1.8−2.2 (m, 2 H); IR (neat) 2970, 1645, 1430 cm^{-1}; MS (70 eV) m/z 126 (m^+).

(3*S*,3a*R*,6a*S*)- and (3*R*,3a*S*,6a*R*)-3-Hydroxy-4*H*-hexahydrofuro[2,3-*b*]furan (15). A stream of ozone was dispersed into a solution of **14** (5.84 g, 46.4 momol) in methanol (150 mL) and CH_2Cl_2 (150 mL) at −78 °C for 30 min. The resulting blue solution was purged with nitrogen until colorless, then the reaction was quenched with 20 mL of dimethyl sulfide, and the resulting mixture was allowed to warm to 23 °C. The mixture was concentrated under reduced pressure to afford the crude ketone. This ketone was dissolved in ethanol (50 mL) and cooled to 0 °C, and sodium borohydride (2.1 g, 55.6 mmol) was added. The reaction mixture was stirred for an additional 2 h at 0 °C, and then the reaction was quenched with 10% aqueous citric acid (10 mL). The resulting mixture was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate and brine. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated carefully under reduced pressure. The resulting residue was chromatographed over silica gel using 30% ethyl acetate in hexane as the eluent to furnish (4.52 g, 75%) the title racemic alcohol **15** as an oil: ^{1}H-NMR ($CDCl_3$) δ 5.7 (d, J = 5.13 Hz, 1 H), 4.45 (dd, J = 6.8, 14.6 Hz, 1 H), 3.9−4.0 (m, 3 H), 3.65 (dd, 1 H, J = 7 Hz, 9.1), 2.9 (m, 1 H), 2.3 (m, 1 H), 1.85 (m, 2 H); IR (neat) 2951, 1640, 1346, 1210 cm^{-1}; MS (70 eV) m/z 131 (M^+ + H).

Preparation of Immobilized Amano Lipase 30. Commercially available Celite 521 (4 g; Aldrich) was loaded on a Buchner funnel and washed successively with 50 mL of deionized water and 50 mL of 0.05 N phosphate buffer (pH = 7.0; Fisher Scientific). The washed Celite was then added to a suspension of 1 g of Amano lipase 30 in 20 mL of 0.05 N phosphate buffer. The resulting slurry was spread on a glass dish and allowed to dry in the air at 23 °C for 48 h (weight 5.4 g, water content about 2% by Fisher method).

(3*R*,3a*S*,6a*R*)-3-Hydroxyhexahydrofuro[2,3-*b*]furan (7) by Immobilized Lipase-Catalyzed Acylation. To a stirred solution of racemic alcohol **15** (2 g, 15.4 mmol) and acetic anhydride (4 g, 42.4 mmol) in 100 mL of DME was added 2.7 g (about 25% by weight of lipase PS30) of immobilized Amano lipase, and the resulting suspension was stirred at 23 °C. The reaction was monitored by TLC and ^{1}H-NMR analysis until 50% conversion was reached. The reaction mixture was filtered, and the filter cake was washed repeatedly with ethyl acetate. The combined filtrate was carefully concentrated in a rotary evaporator, keeping the bath temperature below 15 °C. The residue was chromatographed over silica gel to provide 843 mg (42%) of **7** (95% ee; $[\alpha]^{23}_D$ −11.9°, c 1.24, MeOH); ^{1}H-NMR ($CDCl_3$) δ 5.7 (d, 1 H, J = 5.1 Hz), 4.45 (dd, 1 H, J = 6.8, 14.6 Hz), 3.85−4.0 (m, 3 H), 3.65 (dd, 1 H, J = 7.0, 9.1 Hz), 2.9 (m, 1 H), 2.3 (m, 1 H), 1.85 (m, 2 H). Also, 1.21 g of **16** was obtained after washing with 5% aqueous sodium carbonate (45%; $[\alpha]^{23}_D$ +31.8°, c 1.86, MeOH); ^{1}H-NMR ($CDCl_3$) δ 5.7 (d, 1 H, J = 5.2 Hz), 5.2 (dd, 1 H, J = 6.4, 14.5 Hz), 3.8−4.1 (m, 3 H), 3.75 (dd, 1 H, J = 6.6, 9.2 Hz), 3.1 (m, 1 H), 2.1 (s, 3 H), 1.85−2.1 (m, 2 H); IR (neat) 2947, 1750, 1630, 1338, 1220 cm^{-1}.

(1*S*,2*R*)-2-(2′-Hydroxyethyl)-3-cyclopenten-1-ol (21). To a stirring mixture of $LiAlH_4$ (0.667 g, 17.58 mmol) in dry THF (12 mL) at 0 °C under nitrogen was added dropwise a solution of commercial *cis*-(−)-3,3a,6,6a-tetrahydro-2*H*-cyclopenta[*b*]-furan-2-one (1.09 g, 8.78 mmol) in THF (15 mL) over 15 min. The mixture was brought to room temperature, and after 4 h acetone (2 mL) was added followed by aqueous NaOH (8%, 1.2 mL) and water (1 mL). The mixture was diluted with ethyl acetate (50 mL), and anhydrous sodium sulfate was added. The resulting mixture was stirred for 30 min and filtered. The filter cake was washed with ethyl acetate, and the solvent was evaporated under reduced pressure. The resulting residue was purified by column chromatography (EtOAc/hexane) over silica gel to yield the title compound (1.08 g, 96.4%) as a white oil: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.74 (dt, J = 2.4, 8.2 Hz, 1 H), 5.55 (d, J = 4.1 Hz, 1 H), 4.48 (dt, J = 3.0, 10.0 Hz, 1 H), 3.83 (m, 1 H), 3.70 (dt, J = 4.8, 15.8 Hz, 1 H), 2.60−2.79 (m, 3 H), 2.36 (br d, J = 17.1 Hz, 1 H), 1.71−1.95 (m, 3 H).

(3a*S*,4*S*,6*S*,6a*S*)-4-Hydroxy-6-iodohexahydro-2*H*-cyclopenta[*b*]furan (22). To a vigorously stirring heterogeneous mixture of **21** (0.517 g, 4.04 mmol) dissolved in CH_2Cl_2 (20 mL) and water (2 mL) was added solid $NaHCO_3$ (1.35 g, 16.07 mmol) followed by solid KI (1.34 g, 8.07 mmol) and I_2 (2.04 g, 8.04 mmol). The mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 (25 mL), and washed with sodium thiosulfate solution (2 × 20 mL), water (15 mL), and brine (15 mL). Evaporation of CH_2Cl_2 followed by column chromatogrophy (EtOAc/hexane) yielded the title compound (0.882 g, 86%) as a yellow oil: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.74 (d, 1 H, J = 6.6 Hz), 4.67 (m, 1 H), 4.23 (br s, 1 H), 3.76−3.90 (m, 2 H), 2.95 (br s, 1 H), 2.26−2.35 (dd, 1 H, J = 2.8, 5.8 Hz), 2.02−2.21 (m, 2 H), 1.6−2.0 (m, 2 H).

(3a*S*,4*S*,6a*R*)-4-Hydroxyhexahydro-2*H*-cyclopenta[*b*]-furan (23). To a vigorously refluxing mixture of *n*-tributyltin hydride (0.572 g, 1.97 mmol) and AIBN (10 mg) in dioxane (10 mL) under nitrogen was added dropwise a solution of **22** (0.25 g, 0.984 mmol) in dioxane (1.8 mL). The mixture was refluxed overnight, diluted with acetonitrile (50 mL), and washed with hexane (2 × 25 mL). Evaporation of the solvents followed by column chromatography (1:1 EtOAc/hexane) over silica gel yielded the title compound (0.102 g, 81%) as a pale yellow oil: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.35 (t, 1 H, J = 6.2 Hz), 4.19 (m, 1 H), 3.95 (m, 1 H), 3.62 (q, 1 H, J = 1.7, 8.35 Hz), 2.69 (m, 1 H), 1.98−2.09 (m, 1 H), 1.76−1.90 (m, 3 H), 1.57−1.73 (m, 3 H); IR (neat) 3410, 2982, 1500 cm^{-1}.

Hexahydrofuro[2,3-*b*]furan-3a-ol (26). Into a stirred solution of (2-*n*-butyloxy)-3-allyltetrahydrofuran-3-ol (**25**) (10 g; prepared as described by Jalali-Naini and co-workers[21]) in methanol (10 mL) and methylene chloride (220 mL) at −78 °C was passed a stream of ozone until a blue color persisted. The mixture was purged with nitrogen, warmed to 0 °C, and diluted with ethanol (100 mL). To this mixture was added $NaBH_4$ (5 g), and the resulting mixture was stirred at 23 °C for 6 h. After this period, the solvents were removed under reduced pressure and the residue was partitioned between 10% citric acid (50 mL) and CH_2Cl_2 (100 mL). The layers were separated, the aqueous layer was extracted with additional CH_2Cl_2 (2 × 100 mL), and the combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated. The residue was dissolved in CH_2Cl_2 (100 mL), and *p*-toluenesulfonic acid monohydrate (0.10 g) was added. The resulting mixture was heated at reflux for 24 h and then concentrated to a small volume under reduced pressure. Distillation of the residue (110−130 °C, at 0.1 mmHg) furnished (2 g) the title compound as an oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.4 (s, 1 H), 4.0−4.2 (m, 4 H), 3.0 (br s, 1 H), 2.2 (m, 4 H); IR (neat) 2948, 1656, 1460, 1210 cm^{-1}; MS (70 eV) m/z 131 (M^+ + H).

Hexahydrofuro[2,3-*b*]furan-3a-yl Succinimidyl Carbonate (27). To a stirred solution of 1 g of hexahydrofuro-[2,3-*b*]furan-3a-ol in 25 mL of 12.5% phosgene in toluene cooled to −10 °C was added 1 mL of pyridine. The mixture was allowed to warm to 25 °C and stir for 4 h and then was concentrated to dryness under reduced pressure. The oily residue after drying under vacuum (1.3 g) was dissolved in 30 mL of anhydrous acetonitrile and then cooled in an ice bath. To this cold solution were added 1.14 g of *N*-hydroxysuccinimide and 1.3 mL of triethylamine. The mixture was aged for 48 h at 25 °C and then concentrated to dryness. The residue was dissolved in 200 mL of ethyl acetate, washed with

2 × 50 mL of water, dried over $MgSO_4$, and concentrated to dryness under reduced pressure. Chromatography of the residue with 20% ethyl acetate in methylene chloride gave 0.49 g of product as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.7 (s, 1 H), 4.0−4.25 (m, 4 H), 2.85 (s, 4 H), 2.5 (m, 4 H); MS (70 eV) m/z 272 ($M^+ + H$).

***trans*-3-(Propargyloxy)-4-bromocyclopentane (30).** To a stirred suspension of 26.1 g (0.14 mol) of *N*-bromosuccinimide and propargyl alcohol (61 mL) in CH_2Cl_2 (25 mL) at −30 °C was added a solution of cyclopentene (64 mmol, 5.0 g, 6.5 mL) in 25 mL of CH_2Cl_2 over a period of 2 h. The resulting mixture was warmed to 23 °C and stirred at that temperature for 24 h. After this period, aqueous NaOH solution (10%, 100 mL) was added and the mixture was stirred for 15 min. The layers were separated and the aqueous layer extracted with CH_2CL_2 (2 × 100 mL). The combined organic extracts were washed with brine containing a small amount of $Na_2S_2O_3$ (70 mg) and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a residue which was chromatographed over silica gel (10% ethyl acetate in hexane) to provide (9.6 g, 92%) the title bromo ether **30** as an oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.2−4.35 (m, 2 H), 4.18 (m, 2 H), 2.45 (m, 1 H), 2.1−2.4 (m, 2 H), 1.7−2.1 (m, 4 H).

Bicyclic Olefin 32. To a refluxing solution of 17.3 mL (64 mmol) of tributyltin hydride containing AIBN (400 mg) in toluene (200 mL) was added dropwise a solution of 9.6 g (54 mmol) of bromocyclopentane **30** in toluene (200 mL) over a period of 1 h. The resulting mixture was stirred at reflux for an additional 24 h. The mixture was then cooled to 23 °C and concentrated under reduced pressure. The residue was partitioned between petroleum ether and acetonitrile (200 mL of each), and the acetonitrile (lower) layer was concentrated. The residue was purified by chromatography on silica gel, using 10% ethyl acetate in hexane as the eluent to provide the *exo*-olefin **32** (1.48 g, 22%) as an oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.8 (br s, 2 H), 4.5 (m, 1 H), 2.2−2.4 (m, 2 H), 3.0 (m, 1 H), 1.4−1.8 (m, 6 H).

Bicyclic Alcohol 34. A stream of ozone was dispersed into a solution of **32** (1.48 g, 11.9 mmol) in methanol (40 mL) and CH_2Cl_2 (40 mL) at −78 °C for 30 min. The resulting blue solution was purged with nitrogen until colorless, then the reaction was quenched with 5 mL of dimethyl sulfide at −78 °C, and the resulting mixture was allowed to warm to 23 °C. The mixture was concentrated under reduced pressure to afford the crude ketone. The resulting crude ketone was dissolved in ethanol (30 mL), the solution was cooled to 0 °C, and sodium borohydride (1.05 g, 27.6 mmol) was added. The reaction mixture was stirred for an additional 2 h at 0 °C and then the reaction quenched with 10% aqueous citric acid (10 mL). The resulting mixture was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate and brine. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated carefully under reduced pressure. The resulting residue was chromatographed over silica gel (30% ethyl acetate in hexane) to furnish (193 mg, 14%) the title racemic alcohol **34** as an oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.4 (m, 1 H), 4.35 (br s, 1 H), 3.75 (dd, 1 H, J = 4.5, 8 Hz), 3.65 (dd, 1 H, J = 8, 11 Hz), 2.65 (m, 1 H), 1.8−2.0 (m, 2 H), 1.4−1.8 (m, 4 H).

***trans*-3-Bromo-4-(propargyloxy)tetrahydrofuran (31).** To a stirred suspension of *N*-bromosuccinimide (50.8 g, 0.28 mol) and propargyl alcohol (120 mL) at −30 °C was added a solution of dihydrofuran (140 mmol, 10 g) in CH_2Cl_2 (50 mL) over a period of 2 h. The resulting mixture was warmed to 23 °C and stirred at that temperature for 24 h. Following the workup and chromatography according to the procedure described for compound **30**, the bromo ether **31** (10.2 g, 35%) was obtained as an oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.6−4.75 (m, 3 H), 4.45 (m, 2 H), 4.2−4.35 (m, 3 H), 2.5 (m, 1 H).

Bicyclic Olefin 33. To a refluxing solution of tributyltin hydride (16.9 mL, 63 mmol) containing AIBN (400 mg) in toluene (200 mL) was added dropwise a solution of 10.2 g (40 mmol) of bromotetrahydrofuran **31** in toluene (50 mL) over a period of 1 h. The resulting mixture was stirred at reflux for an additional 24 h. Following the workup and chromatography according to the procedure described for compound **32**, the bicyclic olefin **33** (1.21 g, 16%) was obtained as an oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.0 (br s, 2 H), 4.75 (m, 1 H), 4.5 (m, 1 H), 4.3 (m, 1 H), 4.05 (br d, 1 H, J = 10 Hz), 3.8−3.95 (m, 2 H), 3.4 (dd, 1 H, J = 4.1, 10 Hz), 3.2 (m, 1 H).

Bicyclic Alcohol 35. A stream of ozone was dispersed into a solution of **33** (1.21 g, 10.5 mmol) in methanol (40 mL) and CH_2Cl_2 (40 mL) at −78 °C for 30 min. The resulting blue solution was purged with nitrogen until colorless, then the reaction was quenched with 5 mL of dimethyl sulfide at −78 °C, and the resulting mixture was allowed to warm to 23 °C. The mixture was concentrated under reduced pressure to afford the crude ketone. The resulting crude ketone was dissolved in ethanol (30 mL), the solution was cooled to 0 °C, and sodium borohydride (1.2 g, 31 mmol) was added. The reaction mixture was stirred for an additional 2 h at 0 °C and then the reaction quenched with 10% aqueous citric acid (10 mL). Following the workup and chromatography according to the procedure described for compound **34**, racemic bicyclic alcohol **35** (150 mg, 10%) was obtained as an oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.6 (m, 1 H), 4.3−4.4 (m, 2 H), 4.05 (br d, 1 H, J = 11 Hz), 3.9 (d, 1 H, J = 8.8 Hz), 3.6 (dd, 1 H, J = 2.1, 8.8 Hz), 3.45 (m, 2 H), 2.9 (m, 1 H), 2.2 (br, 1 H).

Thionocarbonates 37 and 38. To a mixture of isosorbide (**36**) (7.31 g, 50.0 mmol) and triethylamine (607.2 mg, 6.0 mmol) in dry methylene chloride (25 mL) was added phenyl chlorothionoformate (863.2 mg, 692 mL, 5.0 mmol) over a period of 10 min at 23 °C, and the resulting mixture was stirred under nitrogen atmosphere for 12 h. The reaction was quenched with aqueous $NaHCO_3$ solution, and the layers were separated. The aqueous layer was extracted with methylene chloride (2 × 20 mL). The combined extracts were washed successively with water and brine and dried with anhydrous Na_2SO_4. Removal of the solvent under reduced pressure furnished a thick oil which was purified by flash chromatography over silica gel (1:1 ethyl acetate/hexanes) to afford pure thionocarbonates **37** (577 mg, 41%) and **38** (273 mg, 19%) as white solids: ^{1}H-NMR ($CDCl_3$) (**37**) δ 7.55−7.41 (m, 2 H), 7.31 (t, 1 H, J = 7.5 Hz), 7.11 (d, 2 H, J = 7.65 Hz), 5.69 (d, 1 H, J = 3.38 Hz), 4.76−4.71 (m, 2 H), 4.39−4.32 (m, 2 H), 4.13 (dd, 1 H, J = 3.55, 11.16 Hz), 3.94 (dd, 1 H, J = 5.96, 9.51 Hz), 3.63 (dd, 1 H, J = 5.86, 9.52 Hz), 2.58 (d, 1 H, J = 7.15 Hz); (thionocarbonate **38**) δ 7.44−7.4 (m, 2 H), 7.3 (t, 1 H, J = 7.43 Hz), 7.13 (d, 2 H, J = 7.84 Hz), 5.66 (dd, 1 H, J = 5.37, 10.59 Hz), 5.03 (t, 1 H, J = 5.03 Hz), 4.47 (d, 1 H, J = 4.6 Hz), 4.4 (d, 1 H, J = 5.38 Hz), 4.06−3.98 (m, 4 H), 1.83 (d, 1 H, J = 5.57 Hz).

Bicyclic Furanofuran 39. To a refluxing solution of tri-*n*-butyltin hydride (277 mg, 0.95 mmol) and 2,2′-azobis-(isobutyronitrile) (8 mg, 0.048 mmol) in dry toluene (3 mL) under nitrogen atmosphere was added dropwise a solution of thionocarbonate **37** (130 mg, 0.46 mmol) in toluene (2 mL) over a period of 1 h. The resulting mixture was heated under reflux for 4 h. The mixture was cooled to 23 °C, and toluene was removed under reduced pressure. The residue was partitioned between acetonitrile (15 mL) and hexanes (15 mL). After separating the acetonitrile layer, the hexane layer was extracted with additional acetonitrile (15 mL). The combined acetonitrile layers were evaporated under reduced pressure to obtain a residue which was purified by flash chromatography over silica gel (1:3 ethyl acetate/hexanes) to obtain **39** (40 mg, 67%): ^{1}H-NMR ($CDCl_3$) δ 4.59 (dt, 1 H, J = 1.1, 4.7 Hz), 4.46 (t, 1 H, J = 5.15 Hz), 4.3−4.18 (m, 1 H), 4.05 (dt, 1 H, J = 2.72, 8.2 Hz), 3.89−3.76 (m, 2 H), 3.69−3.62 (m, 1 H), 2.78 (d, 1 H, J = 6.35 Hz), 2.2−1.9 (m, 2 H).

(3*R*,3a*S*,6a*R*)-3-Hydroxyhexahydrofuro[2,3-*b*]furanyl 2-Pyridyl Carbonate (41). To a stirred solution of 380 mg (2.9 mmol) of (3*R*,3a*S*,6a*R*)-3-hydroxyhexahydrofuro[2,3-*b*]-furan in dry CH_2Cl_2 (25 mL) at 23 °C was added di-2-pyridyl carbonate (950 mg, 4.4 mmol) and triethylamine (0.62 mL). The resulting mixture was stirred for 12 h and the reaction quenched with saturated aqueous $NaHCO_3$ (15 mL). The layers were separated, and the organic layer was washed with brine (15 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a residue which

was chromatographed over silica gel (75% ethyl acetate/hexane) to furnish 460 mg of (3*R*,3a*S*,6a*R*)-3-hydroxyhexahydrofuro[2,3-*b*]furanyl 2-pyridyl carbonate (**41**) as a brown oil. Unreacted starting alcohol (120 mg) was also recovered from this reaction. Carbonate **41**: ^{1}H-NMR ($CDCl_3$) δ 8.5 (m, 1 H), 7.8 (m, 1 H), 7.3 (m, 1 H), 7.1 (m, 1 H), 5.75 (m, 1 H), 5.25 (m, 1 H), 4.2 (m, 1 H), 4.0 (m, 3 H), 3.1 (m, 1 H), 2.2 (m, 1 H), 2.0 (m, 1 H).

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*,3″*R*,3″a*S*,6″a*R*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(3″-hexahydrofuro[2,3-*b*]furanyl)oxy]carbonyl]amino]butyl]decahydroisoquinoline-3-carboxamide (49). To a stirred solution of 400 mg (1.6 mmol) of (3*R*,3a*S*,6a*R*)-3-hydroxyhexahydrofuro[2,3-*b*]furanyl 2-pyridyl carbonate in dry CH_2Cl_2 (30 mL) was added decahydroisoquinoline-3-carboxamide derivative **42** (430 mg, 1.1 mmol). The resulting solution was stirred at 23 °C for 12 h. After this period, the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL) and the mixture diluted with CH_2Cl_2 (25 mL). The layers were separated, and the organic layer was washed with brine (15 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure afforded a residue which was purified by silica gel chromatography (75% ethyl acetate/hexane) to furnish 620 mg of inhibitor **49** as a white crystalline solid: mp 98−101 °C; ^{1}H-NMR ($CDCl_3$) δ 7.18−7.3 (br m, 5 H), 5.77 (s, 1 H), 5.61 (d, 1 H), 5.59 (d, 1 H), 5.0 (br q, 1 H), 4.05 (br m, 1 H), 3.75−3.95 (br m, 3 H), 3.7 (m, 2 H), 3.1−3.2 (m, 1 H), 2.8−3.0 (br m, 4 H), 2.5−2.7 (br m, 2 H), 2.25 (br d, 2 H), 1.4−1.9 (br m, 14 H), 1.35 (s, 9 H); MS (70 eV) *m*/*z* 558 (m^+ + H). Anal. ($C_{31}H_{47}N_3O_6$) C, H, N.

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*,3″*S*,3″a*R*,6″a*S*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(3″-hexahydrofuro[2,3-*b*]furanyl)oxy]carbonyl]amino]butyl]decahydroisoquinoline-3-carboxamide (50). From (3*S*,3a*R*,6a*S*)-3-hydroxyhexahydrofuro[2,3-*b*]furan using the procedure substantially as described above for preparation of compound **49**, there was obtained a white solid: mp 85−9 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.2−7.3 (br m, 5 H), 5.8 (s, 1 H), 5.7 (m, 1 H), 5.4 (m, 1 H), 5.0 (br q, 1 H), 3.95 (m, 3 H), 3.8−3.9 (m, 2 H), 3.5 (m, 1 H), 2.8−3.1 (m, 4 H), 2.6 (m, 2 H), 2.3 (m, 2 H), 2.0 (m, 1 H), 1.4−1.9 (br m, 14 H), 1.3 (s, 9 H); MS (70 eV) *m*/*z* 558 (m^+ + H). Anal. ($C_{31}H_{47}N_3O_6$) C, H, N.

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*,3″*R*,3″a*S*,7″a*R*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(3″-hexahydro-4″*H*-furo[2,3-*b*]pyranyl)oxy]carbonyl]amino]butyl]decahydroisoquinoline-3-carboxamide (51). From (3*R*,3a*S*,7a*R*)-3-hydroxy-4*H*-hexahydrofuro[2,3-*b*]pyran using the procedure substantially as described above for preparation of compound **49**, there was obtained a white solid: mp 147−53 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.3−7.4 (m, 5 H), 6.9 (s, 1 H), 5.5 (br d, 1 H), 5.25 (m, 2 H), 5.0 (br d, 1 H), 4.1 (m, 1 H), 3.9 (m, 4 H), 3.8 (m, 2 H), 3.4 (m, 1 H), 2.9−3.1 (m, 4 H), 2.5−2.7 (m, 2 H), 2.3 (m, 2 H), 2.1 (m, 2 H), 1.6−2.0 (m, 6 H), 1.3−1.5 (m, 6 H), 1.3 (s, 9 H); MS (70 eV) *m*/*z* 572 (m^+ + H). Anal. ($C_{32}H_{49}N_3O_6$) C, H, N.

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*,3″*S*,3″a*R*,7″a*S*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(3″-hexahydro-4″*H*-furo[2,3-*b*]pyranyl)oxy]carbonyl]amino]butyl]decahydroisoquinoline-3-carboxamide (52). From (3*S*,3a*R*,7a*S*)-3-hydroxy-4*H*-hexahydrofuro[2,3-*b*]pyran (**10**) using the procedure substantially as described above for preparation of compound **49**, there was obtained a white solid: mp 82−6 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.3 (m, 5 H), 6.85 (s, 1 H), 5.2 (m, 1 H), 5.05 (m, 3 H), 4.1 (m, 1 H), 3.8 (m, 4 H), 3.85−4.0 (m, 4 H), 2.95 (m, 4 H), 2.6 (m, 2 H), 2.3 (m, 2 H), 2.15 (m, 1 H), 1.6−2.0 (m, 6 H), 1.3−1.5 (m, 6 H), 1.3 (s, 9 H); MS (70 eV) *m*/*z* 572 (m^+ + H). Anal. ($C_{32}H_{49}N_3O_6$) C, H, N.

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*,4″*R*,3″a*R*,6″a*S*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(3″-hexahydro-2*H*-cyclopenta[*b*]furanyl)oxy]carbonyl]amino]butyl]decahydroisoquinoline-3-carboxamide (53). From racemic bicyclic alcohol **34** (100 mg, 0.78 mmol) using the procedure substantially as described above for preparation of compound **49**, there was obtained a 1:1 mixture of diastereomers which were separated by silica gel chromatography (25% ethyl acetate in hexane) to afford **53** (40 mg, R_f = 0.45) and diastereomer (R_f = 0.6). Inhibitor **53**: MS (70 eV) *m*/*z* 556 (m^+ + H). Anal. ($C_{32}H_{49}N_3O_5$·0.25EtOAc) C, H, N.

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*,3″a*S*,4″*S*,6″a*R*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(4″-hexahydro-2*H*-cyclopenta[*b*]furanyl)oxy]carbonyl]amino]butyl]decahydroisoquinoline-3-carboxamide (54). From (3a*S*,4*S*,6a*R*)-4-hydroxyhexahydro-2*H*-cyclopenta[*b*]furan using the procedure substantially as described above for preparation of compound **49**, there was obtained a white solid: mp 87−8 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.2−7.4 (m, 5 H), 5.8 (s, 1 H), 5.35 (m, 1 H), 4.8 (m, 1 H), 4.35 (m, 1 H), 3.6−4.0 (m, 3 H), 3.5 (m, 1 H), 2.8−3.2 (m, 3 H), 2.5−2.8 (m, 3 H), 2.25 (m, 1 H), 2.0 (m, 1 H), 1.2−1.9 (m, 18 H), 1.34 (s, 9 H); MS (70 eV) *m*/*z* 556 (m^+ + H). Anal. ($C_{32}H_{49}N_3O_5$·0.5EtOAc) C, H, N.

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(3a″-hexahydrofuro[2,3-*b*]furanyl)oxy]carbonyl]-amino]butyl]decahydroisoquinoline-3-carboxamide (55). From hexahydrofuro[2,3-*b*]furan-3a-yl succinimidyl carbonate (**27**) using the procedure substantially as described above for preparation of compound **49**, there was obtained inhibitor **55** as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.2−7.4 (m, 5 H), 5.8 (br s, 1 H), 5.4 (m, 1 H), 3.7−4.0 (m, 6 H), 2.8−3.2 (m, 3 H), 2.2−2.6 (m, 4 H), 1.95−2.1 (m, 2 H), 1.2−1.9 (m, 16 H), 1.34 (s, 9 H); MS (70 eV) *m*/*z* 558 (m^+ + H). Anal. ($C_{31}H_{47}N_3O_6$) C, H, N.

Inhibitor 56. To a stirred solution of bicyclic alcohol **35** (150 mg, 1.1 mmol) in dry CH_2Cl_2 (5 mL) at 23 °C were added di-2-pyridyl carbonate (368 mg, 1.7 mmol) and triethylamine (0.24 mL). The resulting mixture was stirred for 12 h and then the reaction quenched with saturated aqueous $NaHCO_3$ (5 mL). The layers were separated, and the organic layer was washed with brine (5 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a residue which was chromatographed over silica gel (75% ethyl acetate/hexane) to furnish the corresponding mixed carbonate of **35** (79 mg) as a brown oil. From the above mixed carbonate (79 mg) and decahydroisoquinoline-3-carboxamide derivative **42** (124 mg, 0.31 mmol) using the procedure substantially as described above for preparation of compound **49**, there was obtained inhibitor **56** (140 mg) as a mixture (1:1) of diastereomers after silica gel chromatography (25% ethyl acetate in hexane). The mixture was assayed for enzyme inhibitory potency. Inhibitor **56**: MS (70 eV) *m*/*z* 558 (m^+ + H). Anal. ($C_{31}H_{47}N_3O_6$) C, H, N.

(3*R*,3a*S*,5*S*,6a*R*)- and (3*R*,3a*S*,5*R*,6a*R*)-3-Hydroxy-5-methylhexahydrofuro[2,3-*b*]furan for Inhibitors 59 and 60: (1*S*,3*S*,4*R*,1′*R*,2″*SR*)-, (1*S*,3*S*,4*R*,1′*S*,2″*SR*)-, (1*S*,3*S*,4*R*,-1′*R*,2″*SR*)-, and (1*R*,3*S*,4*R*,1′*S*,2″*SR*)-2-Methoxy-3-(1′-oxiranylmethyl)-4-[(2″-tetrahydropyranyl)oxy]tetrahydrofuran. To a stirred solution of (2*R*,3*S*,4*R*)- and (2*S*,3*S*,4*R*)-2-methoxy-3-allyl-4-hydroxytetrahydrofuran (**6**) (0.5 g, 3.2 mmol) and dihydropyran (0.87 mL, 9.6 mmol) in dry THF (25 mL) was added *p*-toluensulfonic acid (10 mg). The resulting solution was stirred at 23 °C for 12 h. After this period, the reaction was quenched with saturated $NaHCO_3$ solution. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed over slilica gel (50% ethyl acetate/hexane as eluent) to furnish the tetrahydropyranyl ether (625 mg) as an oil. To a solution of the above tetrahydropyranyl ether (300 mg) in CH_2Cl_2 (25 mL) was added mCPBA (60%, 461 mg, 1.6 mmol), and the resulting mixture was stirred at 23 °C for 12 h. The reaction was quenched with aqueous $NaHCO_3$ solution, the layers were separated, and the aqueous layer was extracted with an additional amount of CH_2Cl_2 (25 mL). The combined extracts were dried over Na_2SO_4 and concentrated. Chromatography of the residue over silica gel (50% ethyl acetate/hexane) afforded the corresponding epoxide (238 mg) as an oil.

(2*R*,3*S*,4*R*,2′*SR*,2″*SR*)-2-Methoxy-3-(2′-hydroxypropyl)-4-[(2-tetrahydropyranyl)oxy]tetrahydrofuran. To a stirred, ice cold suspension of $LiAlH_4$ (50 mg, 1.4 mmol) in THF (10 mL) was added a solution of the above epoxide (230 mg) in THF (2 mL). The resulting mixture was warmed to 23 °C and stirred for 12 h. After this period, the reaction was quenched

with water (0.1 mL) and then 20% NaOH (0.1 mL) and the resulting mixture was stirred for 30 min. The mixture was then filtered through diatomaceous earth, and the filtrate was concentrated under reduced pressure. The residue was chromatographed over silica gel (75% ethyl acetate/hexane) to provide a mixture of the corresponding mixture of secondary alcohols (180 mg) as an oil.

(3*R*,3a*S*,5*S*,6a*R*)- and (3*R*,3a*S*,5*R*,6a*R*)-3-Hydroxy-5-methylhexahydrofuro[2,3-*b*]furan. To a stirred solution of the above alcohols (180 mg) in a mixture (10:1) of CH_2Cl_2 and methanol (50 mL) was added camphorsulfonic acid (50 mg), and the resulting mixture was stirred at 23 °C for 14 h. After this period, the mixture was concentrated to dryness, the residue was redissolved in CH_2Cl_2 (70 mL), and the resulting solution was stirred for an additional 12 h. It was then washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatography of the residue over silica gel (75% ethyl acetate/hexane) provided (3*R*,3a*S*,5*R*,6a*R*)-3-hydroxy-5-methylhexahydrofuro[2,3-*b*]furan (60 mg, for inhibitor **59**) and (3*R*,3a*S*,5*S*,6a*R*)-3-hydroxy-5-methylhexahydrofuro[2,3-*b*]furan (60 mg for inhibitor **60**) as oils.

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*,3″*R*,3″a*S*,5″*R*,6″a*R*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(5″-methyl-3″-hexahydrofuro-[2,3-*b*]furanyl)oxy]carbonyl]amino]butyl]decahydroisoquinoline-3-carboxamide (59). From (3*R*,3a*S*,5*R*,6a*R*)-3-hydroxy-5-methylhexahydrofuro[2,3-*b*]furan using the procedure substantially as described above for preparation of compound **49**, there was obtained a white solid: mp 100−2 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.3−7.4 (m, 5 H), 5.75 (s, 1 H), 5.5−5.6 (m, 3 H), 4.9 (m, 1 H), 3.8−4.0 (m, 4 H), 3.7 (m, 1 H), 3.2 (m, 1 H), 2.9 (m, 4 H), 2.6 (m, 3 H), 2.3 (m, 2 H), 1.5−2.0 (m, 6 H), 1.4−1.6 (m, 6 H), 1.3 (s, 9 H), 1.28 (d, 3 H, J = 6.9 Hz); MS (70 eV) m/z 572 (m^+ + H). Anal. ($C_{32}H_{49}N_3O_6$·0.1$CHCl_3$) C, H, N.

(3*S*,4a*S*,8a*S*,2′*R*,3′*S*,3″*R*,3″a*S*,5″*S*,6″a*R*)-*N*-*tert*-Butyl-2-[2′-hydroxy-4′-phenyl-3′-[[[(5″-methyl-3″-hexahydrofuro-[2,3-*b*]furanyl)oxy]carbonyl]amino]butyl]decahydroisoquinoline-3-carboxamide (60). From (3*R*,3a*S*,5*S*,6a*R*)-3-hydroxy-5-methylhexahydrofuro[2,3-*b*]furan using the procedure substantially as described above for preparation of compound **49**, there was obtained a white solid: mp 105−7 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.3−7.4 (m, 5 H), 5.8 (s, 1 H), 5.6 (m, 3 H), 5.0 (m, 1 H), 4.05 (m, 4 H), 3.9 (m, 2 H), 3.65 (m, 1 H), 2.2 (m, 1 H), 2.95 (m, 4 H), 2.6 (m, 2 H), 2.25 (m, 2 H), 1.5−2.9 (m, 4 H), 1.4−1.6 (m, 6 H), 1.3 (s, 9 H), 1.2 (m, 3 H); MS (70 eV) m/z 572 (m^+ + H). Anal. ($C_{32}H_{49}N_3O_6$·0.35$CHCl_3$) C, H, N.

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References

(1) Erickson, J. W.; Fesik, S. W. Macromolecular X-Ray Crystallography and NMR as Tools for Structure-based Drug Design. *Annu. Rep. Med. Chem*. **1992**, *26*, 271 and references cited therein.

(2) (a) Thaisrivongs, S. HIV Protease Inhibitors. *Annu. Rep. Med. Chem*. **1994**, *29*, 133. (b) Darke, P. L.; Huff, J. R. HIV Protease as an Inhibitor Target for the Treatment of AIDS. In *Advances in Pharmacology*; August, J. T., Anders, M. W., Murad, F., Eds.; Academic Press: San Diego, CA, 1994; Vol. 25, pp 399−454. (c) Clare, M. HIV Protease: Structure-based Design. *Perspect. Drug Discovery Des.* **1993**, *1*, 49−68. (d) Debouck, C. The HIV-1 Protease as a Therapeutic Target for AIDS. *AIDS Res. Hum. Retroviruses* **1992**, *8* (2), 153−64. (e) Martin, J. A. Recent Advances in the Design of HIV Protease Inhibitors. *Antiviral Res*. **1992**, *17*, 265−78. (f) Huff, J. R. HIV Protease: A Novel Chemotherapeutic Target for AIDS. *J. Med. Chem*. **1991**, *34*, 2305−14. (g) Norbeck, D. W.; Kempf, D. J. HIV Protease Inhibitors. *Annu. Rep. Med. Chem*. **1991**, *26*, 141−50. (h) Tomasselli, A. G.; Howe, W. J.; Sawyer, T. K.; Wlodawer, A.; Heinrikson, R. L. The complexities of AIDS: An Assessment of the HIV Protease as a Therapeutic Target. *Chimicaoggi* **1991**, 6−27.

(3) Wlodawer, A.; Erickson, J. W.; Structure-based Inhibitors of HIV-1 Protease. *Annu. Rev. Biochem*. **1993**, *62*, 543−85.

(4) (a) Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.-W.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. L-735, 524: An Orally Bioavailable Human Immunodeficiency Virus Type I Protease Inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096−100. (b) Dorsey, B. D.; Levin, R. B.; McDaniel, S. L.; Vacca, J. P.; Guare, J. P.; Darke, P. L.; Zugay, J.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; Lin, J. H.; Chen, I.-W.; Holloway, M. K.; Fitzgerald, P. M. D.; Axel, M. G.; Ostovic, D.; Anderson, P. S.; Huff, J. R. L-735, 524: The Design of a Potent and Orally Bioavailable HIV Protease Inhibitor. *J. Med. Chem*. **1994**, *37*, 3443−51. (c) Kempf, D. J.; Marsh, K. C.; Denissen, J.; McDonald, E.; Vasavononda, S.; Flentge, C.; Green, B. G.; Fino, L.; Park, C.; Kong, X.-P.; Wideburg, N. E.; Saldivar, A.; Ruiz, L.; Kati, W. M.; Sham, H. L.; Robins, T.; Stewart, K. D.; Hsu, A.; Plattner, J. J.; Leonard, J.; Norbeck, D. ABT-538 is a Potent Inhibitor of Human Immunodeficiency Virus Protease with High Oral Bioavailability in Humans. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2484−8. (d) Lam, P. Y. S.; Jadhav, P. K.; Eyermann, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, Y. N.; Chang, C.-H.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Erickson-Viitanen, S. Rational Design of Potent, Bioavailable, Nonpeptide Cyclic Ureas as HIV Protease Inhibitors. *Science* **1994**, *263*, 380−4.

(5) (a) Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. Rational Design of Peptide-Based HIV Proteinase Inhibitors. *Science* **1990**, *248*, 358−61. (b) Williams, P. E. O.; Muirhead, G. J.; Madigan, M. J.; Mitchell, A. M.; Shaw, T. Disposition and Bioavailability of the HIV Proteinase Inhibitor, R0 31-8959, After Single doses in Healthy Volunteers. Proceedings of British Pharmacology Society Meeting, Univ. of Newcastle, Upon Tyne, Apr. 8−10, 1992. (c) Thaisrivongs, S.; Tomich, P. K.; Watenpaugh, K. D.; Chong, K. T.; Howe, W. J.; Yang, C.-P.; Strohbach, J. W.; Turner, S. R.; McGrath, J. P.; Bohanon, M. J.; Lynn, J. C.; Mulichack, A. M.; Spinelli, P. A.; Hinshaw, R. R.; Pagano, P. J.; Moon, J. B.; Ruwart, M. J.; Wilkinson, K. F.; Rush, B. D.; Zipp, G. L; Dalga, R. J.; Schwende, F. J.; Howard, G. M.; Padbury, G. E.; Toth, L. N.; Zhao, Z.; Koeplinger, K. A.; Kakuk, T. J.; Cole, S. L.; Zaya, R. M.; Piper, R. C.; Jeffery, P. Structure-based Design of HIV Protease Inhibitors: 4-Hydroxycoumarins and 4-Hydroxy-2-pyrones as Non-peptidic Inhibitors. *J. Med. Chem*. **1994**, *37*, 3200−4.

(6) Ghosh, A. K.; Thompson, W. J.; McKee, S. P.; Duong, T. T.; Lyle, T. A.; Chen, J. C.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. 3-Tetrahydrofuran and Pyran Urethanes as High Affinity P_2-Ligands for HIV-1 Protease Inhibitors. *J. Med. Chem*. **1993**, *36*, 292−4.

(7) (a) Ghosh, A. K.; Thompson, W. J.; Holloway, M. K.; Mckee, S. P.; Duong, T. T.; Lee, H. Y.; Munson, P. M.; Smith, A. M.; Wai, J. M.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. Potent HIV Protease Inhibitors: The Development of Tetrahydrofuranylglycines as Novel P_2-Ligands and Pyrazine Amides as P_3-Ligands. *J. Med. Chem*. **1993**, *36*, 2300−10. (b) Thompson, W. J.; Ghosh, A. K.; Holloway, M. K.; Lee, H. Y.; Munson, P. M.; Schwering, J. E.; Wai, J. M.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. 3′-Tetrahydrofuranylglycine as a Novel, Unnatural Amino Acid Surrogate for Asparagine in the Design of Inhibitors of the HIV Protease. *J. Am. Chem. Soc.* **1993**, *115*, 801−3.

(8) (a) Ghosh, A. K.; Lee, H. Y.; Thompson, W. J.; Culberson, C.; Holloway, M. K.; McKee, S. P.; Munson, P. M.; Duong, T. T.; Smith, A. M.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. The Development of Cyclic Sulfolanes as Novel and High Affinity P_2-Ligands for HIV-1 Protease Inhibitors. *J. Med. Chem*. **1994**, *37*, 1177−88. (b) Ghosh, A. K.; Thompson, W. J.; Lee, H. Y.; McKee, S. P.; Munson, P. M.; Duong, T. T.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. Cyclic Sulfolanes as Novel and High Affinity Ligands for HIV-1 Protease Inhibitors. *J. Med. Chem*. **1993**, *36*, 924−7.

(9) Ghosh, A. K.; Thompson, W. J.; Fitzgerald, P. M. D.; Culberson, C. J.; Axel, M. G.; McKee, S. P.; Huff, J. R.; Anderson, P. S. Structure-Based Design of HIV-1 Protease Inhibitors: Replacement of Two Amides and a 10π-Aromatic System by a Fused Bis-tetrahydrofuran. *J. Med. Chem*. **1994**, *37*, 2506−8.

(10) Ghosh, A. K.; Thompson, W. J.; Munson, P. M.; Liu, W.; Huff, J. R. Cyclic Sulfone-3-carboxamides as Novel P_2-Ligands for Ro 31-8959 Based HIV-1 Protease Inhibitors. *Biorg. Med. Chem. Lett.* **1995**, *5*, 83−8.
(11) Seebach, D.; Aebi, J.; Wasmuth, D. Diastereoselective α-Alkylation of *β*-Hydroxycarboxylic Esters Through Alkoxide Enolates: (+)-Diethyl (2s, 3R)-3-Allyl-2-Hydroxysuccinate from (−)-Diethyl-S-Malate. *Org. Synth.* **1985**, *63*, 109−20.
(12) Ghosh, A. K.; Chen, Y. Synthesis and Optical Resolution of High Affinity P_2-Ligands for HIV-1 Protease Inhibitors. *Tetrahedron Lett.* **1995**, *36*, 505−8.
(13) Dulcere, J. P.; Rodriguez, J.; Santelli, M.; Zahra, J. P. Preparation D'acyl-4-Methylene-3-Tetrahydrofurans: Un Acces Aux α-Methylene *γ*-Lactones et Butenolides Fonctionnalies. (Preparation of 4-acyl-3-methylenetetrahydrofurans: an access to functionalized α-methylene-*γ*-lactones and butenolides.) *Tetrahedron Lett.* **1987**, *28*, 2009−12 and references cited therein.
(14) Okabe, M.; Abe, M.; Tada, M. Reductive Cyclization of 2-[(2-Propynyl)oxy]ethyl Bromides by a Cobalt Complex, Cobaloxime (I). A New Method for the Synthesis of α-Methylene-*γ*-butyrolactones. *J. Org. Chem.* **1982**, *47*, 1775−7. For preparation of cobaloxime, see ref 15.
(15) Schrauzer, G. N. Bis(dimethylglyoximato)cobalt Complexes. *Inorg. Synth.* **1968**, *11*, 62−4.
(16) The stereochemical assignment of alcohol **15** was made based on comparison with alcohol **7**.
(17) Purchased from Amano International Enzyme Co., Troy, VA.
(18) A slightly modified procedure was used; see: Bianchi, D.; Cesti, P.; Battistel, E. Anhydrides as Acylating Agents in Lipase-Catalyzed Stereoselective Esterification of Racemic Alcohols. *J. Org. Chem.* **1988**, *53*, 5531−34.
(19) Enantiomeric excess (90% ee) was determined by ^{19}F-NMR spectroscopy using the Mosher ester; see: Dale, J. A.; Dull, D. L.; Mosher, H. S. α-Methyl-α-trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. *J. Org. Chem.* **1969**, *34*, 2543−9.
(20) Tokoroyama, T.; Matsuo, K.; Kanazawa, R. Synthetic Studies on Terpenic compounds. XII. Selective Degradation of the Side Chain in Portulal. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 3383−4.
(21) Jalali-Naini, M.; Lallemand, J. Y. Iodocyclization of Unsaturated Lactols and Acetals, a New Route to Furo-2,3-b-Furans and Pyrans. *Tetrahedron Lett.* **1986**, 497−500.
(22) Shute, R.; Rich, D. H. Synthesis and Evaluation of Novel Activated Mixed carbonate Reagents for the Introduction of the 2-(Trimethylsilyl)ethoxycarbonyl(Teoc)-Protecting Group. *Synthesis* **1987**, 346.
(23) Barton, D. H. R.; Motherwell, W. B. New and Selective Reactions and Reagents in carbohydrate Chemistry. *Pure Appl. Chem.* **1981**, *53*, 15−31.
(24) (a) Ghosh, A. K.; Duong, T. T.; McKee, S. P. Di(2-Pyridyl) Carbonate Promoted Alkoxycarbonylation of Amines: A Convenient Synthesis of Functionalized Carbamates. *Tetrahedron Lett.* **1991**, *32*, 4251−4. (b) Ghosh, A. K.; Duong, T. T.; McKee, S. P.; Thompson, W. J. N,N′-Disuccinimidyl Carbonate: A Useful Reagent for Alkoxycarbonylation of Amines. *Tetrahedron Lett.* **1992**, *33*, 2781−4.
(25) A preliminary model of the X-ray crystal structure of the inhibitor **43** complexed with the HIV-1 protease at 2.5 Å resolution has been provided by Dr. Paula Fitzgerald, Department of Biophysical Chemistry. The final coordinates of the fully refined structure will be published elsewhere.
(26) Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. Crystal Structure of HIV-1 Protease in Complex with VX-478, a Potent and Orally Bioavailable Inhibitor of the Enzyme. *J. Am. Chem. Soc.* **1995**, *117*, 1181−2.
(27) The ligands corresponding to the inhibitors **47** and **48** were prepared according to the procedure described in Scheme 4 in ref 8a.
(28) The structure was energy minimized in the active site using the Merck molecular force field, OPTIMOL, which is a variant of the MM2 program; see ref 29.
(29) Halgren, T. A. Merck Sharp and Dohme Research Laboratories, Rahway, NJ. Unpublished work on the development of the force field program OPTIMOL. OPTIMOL differs from MM2 mainly in the use of partial charges on atoms, instead of bond dipoles, and in the absence of unshared pairs of electrons on certain nitrogen and oxygen atoms. Also, see: Allinger, N. L.; Conformational Analysis. 130. MM2. A Hydrocarbon Force Field Utilizing V1 and V2 Torsional Terms. *J. Am. Chem. Soc.* **1977**, *99*, 8127−34.
(30) Thompson, W. J.; Fitzgerald, P. M. D.; Holloway, M. K.; Emini, E. A.; Darke, P. L.; McKeever, B. M.; Schleif, W. A.; Quintero, J. C.; Zugay, J. A.; Tucker, T. J.; Schwering, J. E.; Homnick, C. F.; Nunberg, J.; Springer, J. P.; Huff, J. R. Synthesis and Antiviral Activity of a Series of HIV-1 Protease Inhibitors with Functionality Tethered to the P_1 or P_1' Phenyl Substituents : X-ray Crystal Structure Assisted Design. *J. Med. Chem.* **1992**, *35*, 1685−1701.
(31) A complex between **1** (Ro 31-8959) and HIV-1 protease was crystallized in the space group $P6_1$, $a = b = 63.33$ Å, $c = 83.31$ Å. A model for the complex was partially refined against data extending from 8.0 to 2.2 Å in resolution; the *R*-value for the current model is 0.196, and deviations from ideal bond distances are 0.018 Å. Full details of the crystallographic analysis will be published elswhere.
(32) For details of the assay protocol, see ref 30.
(33) Craig, J. C.; Duncan, I. B.; Hockley, D.; Grief, C.; Roberts, N. A.; Mills, J. S.; *Antiviral Res.* **1991**, *16*, 295−305 and references cited therein. These authors report IC_{90} values of 6−30 nM in cell culture. However, the assay protocol differs widely in that syneytia formation rather than p^{24} production was monitored as end point, and cell types other than MT4 were employed.
(34) A complex between **49** and HIV-1 protease was crystallized in the space group $P6_1$, $a = b = 63.40$ Å, $c = 83.48$ Å. A model for the complex was refined to completion against data extending from 8.0 to 2.1 Å in resolution; the *R*-value for the final model is 0.166, and deviations from ideal bond distances are 0.018 Å. Full details of the crystallographic analysis will be published in due course.
(35) Vazquez, M. L.; Bryant, M. L.; Clare, M.; DeCrescenzo, G. A.; Doherty, E. M.; Freskos, J. N.; Getman, D. P.; Houseman, K. A.; Julien, J. A.; Kocan, G. P.; Mueller, R. A.; Shieh, H.-S..; Stallings, W. C.; Stegeman, R. A.; Talley, J. J. Inhibitors of HIV-1 Protease Containing the Novel and Potent (R)-(Hydroxyethyl)-sulfonamide Isostere. *J. Med. Chem.* **1995**, *38*, 581−4. Also, see ref 3.
(36) (a) Delfraissy, J. F.; Sereni, D.; Brun-Vezinet, F.; Dussaix, E.; Krivine, A.; Dormont, J.; Bragman, K. A Phase I-II Dose Ranging Study of the Safety and Activity of Ro 31-8959 (HIV Proteinase Inhibitor) in Previously Zidovudine (ZDV) Treated HIV-Infected Individuals. Skinner, C.; Sedwick, A.; Bragman, K.; Pinching, A. J.; Weber, J. A Phase I-II Dose Ranging Study of the Safety and Activity of Ro 31-8959 (HIV Proteinase Inhibitor) in Asymptomatic or Mildly Symptomatic HIV-Infection. Abstracts at the 9th Int. Conf. on AIDS, Berlin, 1993. (b) Teppler, H.; Pomerantz, R.; Bjornsson, T.; Pientka, J.; Osborne, B.; Woolfe, E.; Yeh, K.; Duetsch, P.; Emini, E.; Squires, K.; Saag, M.; Waldman, S. 1st Natl. Conf. on Human Retroviruses and Related Infections, Washington, DC, Dec. 12−16, 1993; Session 77, L8.
(37) Personal communication: Dr. Juinn Lin, Department of Animal Pharmacology, Merck Research Laboratories, West Point, PA. Details of these experiments will be published elsewhere.

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