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Design and Synthesis of Stereochemically Defined Novel Spirocyclic P2-Ligands for HIV-1 Protease Inhibitors

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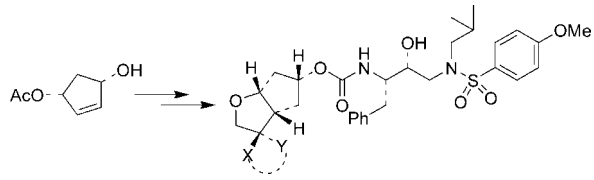
ABSTRACT



The synthesis of a series of stereochemically defined spirocyclic compounds and their use as novel P2-ligands for HIV-1 protease inhibitors are described. The bicyclic core of the ligands was synthesized by an efficient *n*Bu$_3$SnH-promoted radical cyclization of a 1,6-enyne followed by oxidative cleavage. Structure-based design, synthesis of ligands, and biological evaluations of the resulting inhibitors are reported.

The introduction of highly active antiretroviral therapy (HAART) in 1996, in combination with HIV-1 protease inhibitors and reverse transcriptase inhibitors, has dramatically changed the management of HIV/AIDS.[1] The advent of HAART has significantly reduced morbidity and mortality and has improved the quality of life for HIV-infected patients, particularly in developed nations.[2] Despite this important breakthrough, current and future management of HIV/AIDS is being challenged by the rapid emergence of multi-drug-resistant HIV-1 strains and drug-related side effects.[3] Consequently, development of novel and effective treatment regimens are critically important.

In our continuing effort to design a new generation of HIV-1 protease inhibitors (PIs) that combat drug resistance, we developed a series of exceedingly potent PIs. A number of these nonpeptidyl PIs have shown superb antiviral activity and drug-resistance profiles. In our structure-based design strategies, we introduced the "backbone binding concept" with the presumption that an inhibitor that makes maximum interactions in the protease active site, particularly hydrogen bonding with the backbone atoms, may retain its potency against mutant strains.[4] Darunavir (**1**, Figure 1), which has been approved by the FDA for the treatment of patients harboring multi-drug-resistant HIV-1 strains, has emerged from this approach.[5,6] Our detailed X-ray structural analysis of protein−ligand complexes revealed an extensive hydrogen

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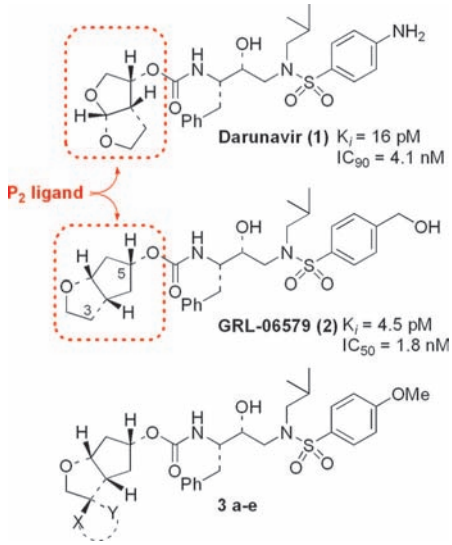

Figure 1. Structure of HIV protease inhibitors.

bonding network with HIV-1 protease backbone atoms and most notably with the designed bis-THF P2-ligand.[7]

More recently, we reported another novel PI, GRL-06579 (**2**), which features a stereochemically defined bicyclic hexahydrocyclopentylfuran (Cp-THF) P2-ligand in the hydroxyethylsulfonamide isostere core.[8] The X-ray crystallographic analysis of **2**-bound HIV-1 protease documented extensive hydrogen bonding interactions including the Cp-THF oxygen with the backbone atoms in the S2-subsite.[8]

The favorable drug-resistance profile of this PI containing the Cp-THF ligand logically prompted us to design several structural analogs. We set out to introduce new functionalities on this bicyclic core that could create additional interactions within the enzyme catalytic site. The 3-position of the Cp-THF ligand appeared particularly suitable for this purpose, because of its proximity to the flap region and the S2-subsite of the protease. Based upon our analysis of the X-ray crystal structure of **2**-bound protease, we planned to investigate the effect of a structurally constrained spirocyclic motif at the 3-position of the Cp-THF ring. We speculated that a cyclic ether oxygen or an oxazolidinone carbonyl oxygen may be positioned in this cyclic motif to accept a hydrogen bond from the enzyme active site residues or a backbone NH. Such a functionality would fill in the hydrophobic pocket in the S2-subsite as well. Furthermore, this structural feature may improve the pharmacological profile of these inhibitors.[9,10]

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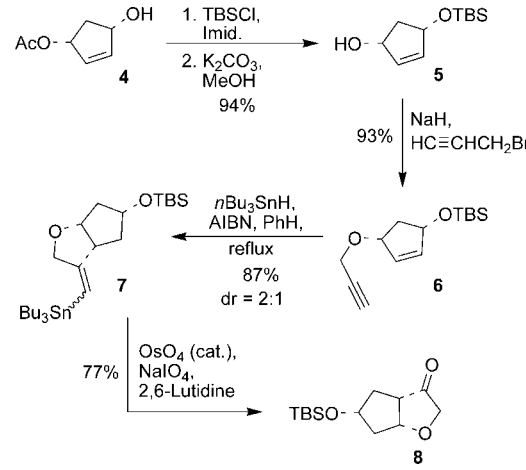
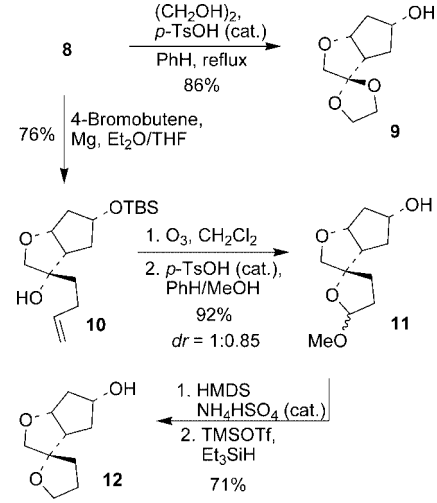
Scheme 1. Synthesis of Bicyclic Ketone **8**



We initially set out to synthesize a series of spirocyclic Cp-THF-derived P2-ligands and their corresponding HIV-protease inhibitors (**3a**−**e**). A synthetic strategy was devised so that all analogs could be synthesized from a common precursor that gives rapid access to new polycyclic molecular probes. The general synthesis of the bicyclic core of our new P2-ligands was accomplished in enantiomerically pure form as shown in Scheme 1. Optically active monoacetate **4** was obtained in 95% ee by desymmetrization of the corresponding *meso*-diacetate with acetyl cholinesterase.[11] Protection of alcohol **4** as a TBS ether followed by methanolysis of the acetyl group furnished compound **5**. Propargylation of **5** using propargyl bromide in the presence of NaH provided alkyne **6** in excellent yield.

The construction of the bicyclic core was accomplished by an intramolecular radical cyclization of alkyne **6** using nBu$_3$SnH and AIBN in benzene at reflux. This provided vinyl stannane **7** as a mixture of *cis*/*trans* diastereoisomers (2:1),

Scheme 2. Synthesis of Spirocyclic Ketal and Ether Ligands



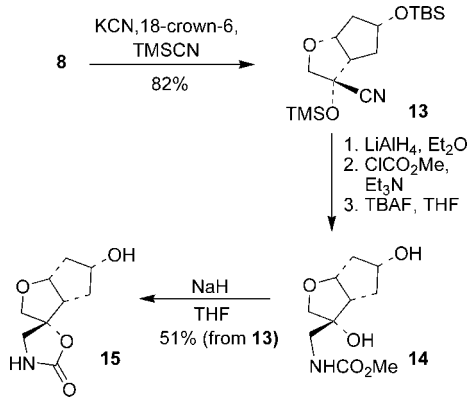
along with trace amounts of the olefin, which presumably formed during purification on silica gel. The mixture of isomers was directly oxidized with a catalytic amount of OsO_4 in the presence of $NaIO_4$ and 2,6-lutidine to afford the key intermediate, ketone **8** in 77% yield.

We first turned our attention to the synthesis of spirocyclic ketal **9** and ether **12**. Molecular modeling of the corresponding inhibitors suggested that the ligand oxygens could be within hydrogen bonding distance to the NH amide bonds of both Asp30 and Asp29 residues.

Spirocyclic dioxolane ligand **9** was obtained in 86% yield by treatment of ketone **8** with ethylene glycol in benzene with a catalytic amount of *p*-TsOH. Synthesis of ether **12** was achieved in four consecutive steps starting from ketone **8**. Reaction of **8** with homoallyl magnesium bromide furnished compound **10** in 76% yield. Ozonolysis of the terminal alkene and refluxing the resulting crude aldehyde in benzene/methanol followed by azeotropic distillation of the excess methanol afforded methyl acetal **11** as a mixture (1:0.85) of diastereoisomers. Reduction of this acetal intermediate **11** furnished the desired alcohol **12** by applying a one-pot procedure involving (1) TMS-protection of the alcohol with hexamethyldisilazane and (2) subsequent reduction of the acetal with triethylsilane.[12]

We have designed spirocyclic oxazolidinone ligands that could potentially exploit polar interactions with the backbone atoms and residues in the HIV-1 protease active site. Their respective syntheses are highlighted in Scheme 3 and 4.

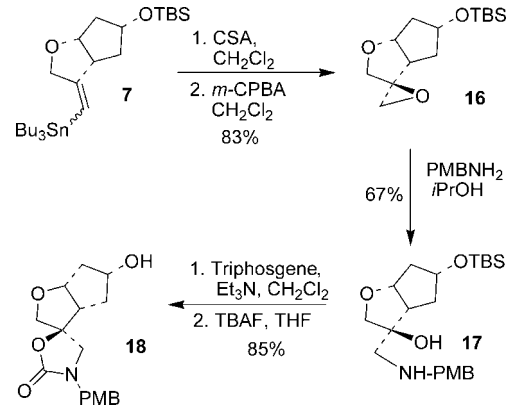
Scheme 3. Synthesis of Spirooxazolidinone Ligand **15**



Cyanohydrin **13** was synthesized in 82% yield from ketone **8**. $LiAlH_4$-reduction of the cyanide provided the corresponding amine, which exhibited partial TMS-deprotection. Therefore, the crude mixture was directly submitted to the next steps with (1) formation of the methyl carbamate derivative and (2) removal of the silyl ethers with TBAF in THF. The resulting diol **14** was then treated with NaH in THF to give oxazolidinone ligand **15** in 51% yield over four steps (from **13**).

Synthesis of oxazolidinone **18** started with vinylstannane **7** (Scheme 4). Proto-destannylation of **7** was carried out with CSA in CH_2Cl_2. Epoxidation of the resulting olefin with *m*-CPBA gave epoxide **16** as a major diastereomer (93:7 ratio). Opening of the epoxide with *p*-methoxybenzylamine

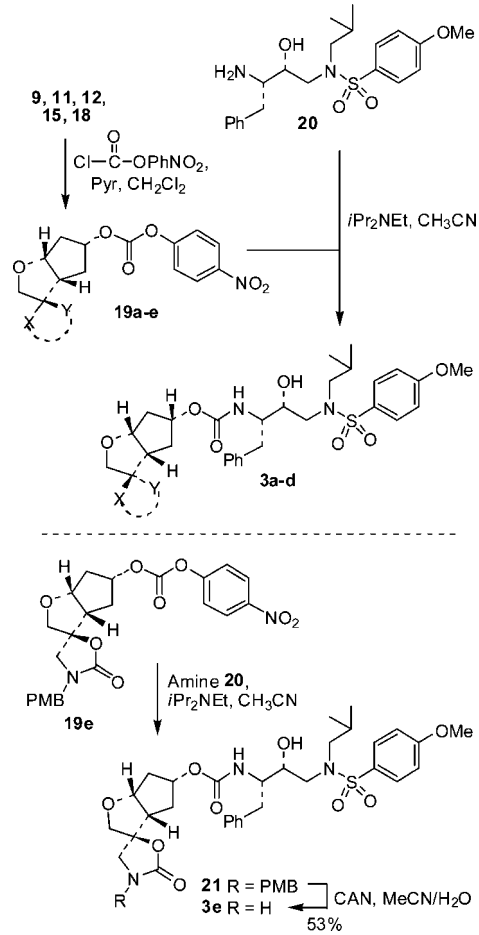
Scheme 4. Synthesis of Spirooxazolidinone Ligand **18**



gave amino alcohol **17** in 67% yield. The carbonyl was installed using triphosgene and Et_3N in CH_2Cl_2. Deprotection of the TBS-group provided the desired oxazolidinone **18**.

The synthesis of polycyclic PIs is shown in Scheme 5. Various synthetic ligands were reacted with 4-nitrophenylchloroformate and pyridine to form the corresponding activated carbonates, **19a**−**e**. Reaction of the respective

Scheme 5. Synthesis of Inhibitors **3a**−**e**

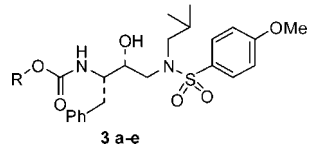


active carbonate with known[8] amine **20** in the presence of diisopropylethylamine afforded PIs **3a**−**d**. For the synthesis of inhibitor **3e**, amine **20** was reacted with active carbonate **19e** to provide urethane **21**. Removal of the PMB group from **21** by exposure to ceric ammonium nitrate (CAN) afforded inhibitor **3e**.

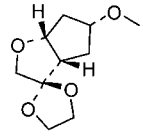
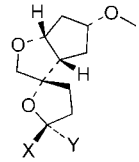
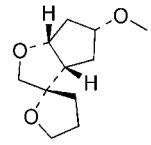
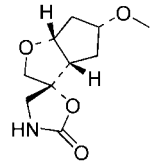
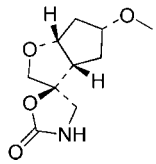
We examined all inhibitors for their enzymatic potency as well as their cellular activity, and the results are displayed in Table 1. As shown, most inhibitors exhibited excellent enzymatic potency. Dioxolane-based analogue **3a** displayed a K_i value of 0.16 nM. Inhibitor **3b** contains the spirocyclic methyl acetal as a mixture (1:0.85 ratio) of diastereomers. These diastereomers were separated by HPLC, and the stereochemical identity of each diastereomer was determined by extensive NOESY experiments. Diastereomer **3b**-(*S*) showed an enzymatic K_i of 0.81 nM. The **3b**-(*R*) isomer is slightly more potent (K_i = 0.38 nM). The removal of the methoxy group from **3b** resulted in inhibitor **3c**, which showed a loss of enzyme inhibitory activity. Both inhibitors **3b** and **3c** have shown comparable antiviral activity. We have examined stereochemically defined oxazolidinone derivatives as P2-ligands. Inhibitor **3d** displayed a K_i of 0.29 nM. Diastereomeric inhibitor **3e** is slightly more potent than **3d** in both enzyme inhibitory as well as in antiviral assays (IC_{50} = 21 nM in MT-2 cells). The inhibitors in Table 1 in general are significantly less potent than UIC-PI (TMC-126),[14] the corresponding methoxysulfonamide derivative of darunavir or Cp-THF-containing inhibitor **2**.[8]

In conclusion we have designed and synthesized a series of inhibitors containing stereochemically defined novel spirocyclic P2-ligands. The syntheses of these ligands were carried out from the key intermediate **8**, which was efficiently prepared in optically active form by using a radical cyclization as the key step. The spirooxazolidinone-derived inhibitor **3e** is the most potent inhibitor in this series. While these inhibitors contain novel P2-ligands, it appears that the spirocyclic motif at the 3-position of the Cp-THF ring resulted in a significant reduction in potency. Further design and optimization of the ligand binding site interactions are in progress.

Table 1. Enzymatic and Antiviral Activity of PIs



3 a-e

Inhibitor	R	K_i (nM)[a]	IC_{50} (μM)[b]
3a		0.16	0.28
3b		**3b**-(*S*) isomer (X=OMe,Y=H): 0.81 **3b**-(*R*) isomer (X=H,Y=OMe): 0.38	0.23[c]
3c		2.22	0.17
3d		0.29	0.093
3e		0.17	0.021

[a] K_i determined following protocol as described by Toth and Marshall, mean values of at least four determinations.[13] [b] MT-2 cells (2×10^4/mL) were exposed to 100 $TCID_{50}$ of HIV-1_{LAI} and cultured in the presence of various concentrations of PIs, and the IC_{50}'s were determined by using the MTT assay on day 7 of culture.[6] [c] Tested as a 1:0.85 mixture.

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Supporting Information Available: Experimental procedures, spectral data, and ^{1}H NMR and ^{13}C NMR spectra for compounds **5**−**21** and **3a**−**e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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