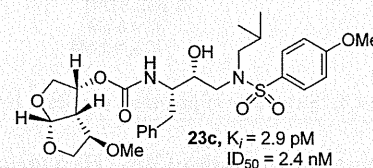


## Design, Synthesis, and X-ray Structure of Substituted Bis-tetrahydrofuran (Bis-THF)-Derived Potent HIV-1 Protease Inhibitors

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## Supporting Information

**ABSTRACT:** We investigated substituted bis-THF-derived HIV-1 protease inhibitors in order to enhance ligand-binding site interactions in the HIV-1 protease active site. In this context, we have carried out convenient syntheses of optically active bis-THF and C4-substituted bis-THF ligands using a [2,3]-sigmatropic rearrangement as the key step. The synthesis provided convenient access to a number of substituted bis-THF derivatives. Incorporation of these ligands led to a series of potent HIV-1 protease inhibitors. Inhibitor **23c** turned out to be the most potent ( $K_i = 2.9$  pM;  $IC_{50} = 2.4$  nM) among the inhibitors. An X-ray structure of **23c**-bound HIV-1 protease showed extensive interactions of the inhibitor with the protease active site, including a unique water-mediated hydrogen bond to the Gly-48 amide NH in the S2 site.



**KEYWORDS:** HIV-1 protease inhibitors, Darunavir, bis-THF, drug-resistant, design, synthesis, X-ray structure

In our continuing studies aimed at the design and synthesis of novel HIV-1 protease inhibitors to combat drug resistance, we have developed a variety of exceedingly potent inhibitors.<sup>1</sup> One of these inhibitors, darunavir (**1**,  $K_i = 16$  pM, antiviral  $IC_{50} = 4.1$  nM, Figure 1) was first approved in June 2006 for the treatment of HIV/AIDS patients harboring drug-resistant HIV-1 variants.<sup>2</sup> Later, darunavir received an expanded approval for the treatment of all therapy-naïve HIV/AIDS patients, including pediatric patients.<sup>3</sup> Darunavir incorporates a stereochemically defined (3*R*,3*aS*,6*aR*)-bis-tetrahydrofuran (bis-THF) urethane as the P2 ligand in a (*R*)-hydroxyethyl sulfonamide isostere.<sup>4</sup> Structural studies of darunavir documented that the bis-THF ligand is involved in a network of hydrogen bonding interactions with the protein-backbone of the HIV-1 protease, as formulated in our “backbone binding” concept.<sup>5</sup> A number of other clinical protease inhibitors (**2**, Breacanavir; **3**, GS-8374) have also incorporated this bis-THF ligand and demonstrated marked antiviral potency against a panel of multidrug-resistant HIV-1 variants.<sup>6,7</sup>

In our continuing studies toward maximizing ligand-binding site interactions, based upon the X-ray structure of **1**-bound HIV-1 protease, we further speculated that the incorporation of an alkoxy substituent at the C4-position of bis-THF could lead to new interactions with the backbone NH of Gly-48. We therefore required a stereoselective synthesis that would provide access to C4-substituted bis-THF derivatives for further optimization. The bis-THF ligand contains three contiguous stereogenic centers. A

number of syntheses of the bis-THF ligand have been reported, including several optically active routes from our research group.<sup>8–12</sup> Quaedflieg and co-workers reported a chiral synthesis that involved a conjugate addition of nitromethane.<sup>13</sup> Other syntheses have been attempted with the use of various catalysts to obtain the desired product.<sup>14</sup> Interestingly, many of these syntheses require late-stage resolution to obtain the enantiomerically pure ligand. Furthermore, the reported syntheses are not convenient for the preparation of C4-substituted derivatives. In view of this shortcoming, we investigated a new optically active synthesis utilizing an efficient [2,3]-sigmatropic rearrangement as the key step and an inexpensive chiral precursor as the starting material. The synthetic route provided convenient access to optically active bis-THF and functionalized bis-THF ligands. We incorporated these ligands in the hydroxyethylsulfonamide isostere, and the resulting HIV-1 protease inhibitors were evaluated in enzyme inhibitory and antiviral assays.

The synthesis of the bis-THF ligand started with known compound **4**.<sup>11</sup> It was prepared in multigram quantities by Wittig olefination of (*S*)-glyceraldehyde acetonide with (ethoxycarbonylmethylene) triphenylphosphorane, as described previously.<sup>11</sup> The requisite (*S*)-glyceraldehyde can be obtained from ascorbic

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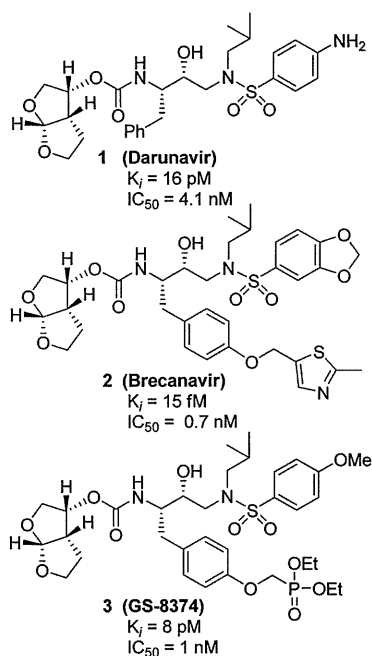
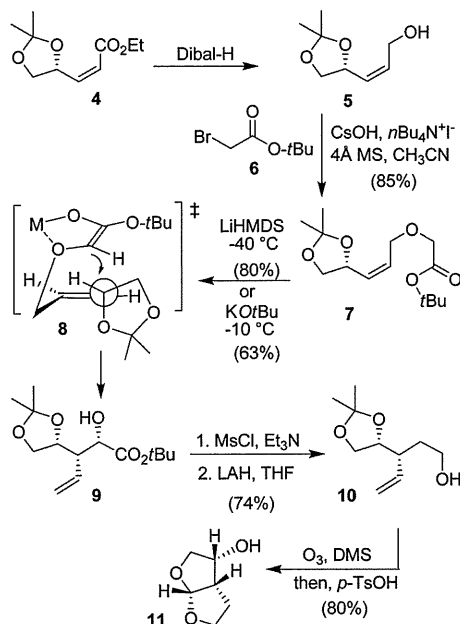


Figure 1. Structures of protease inhibitors 1–3.

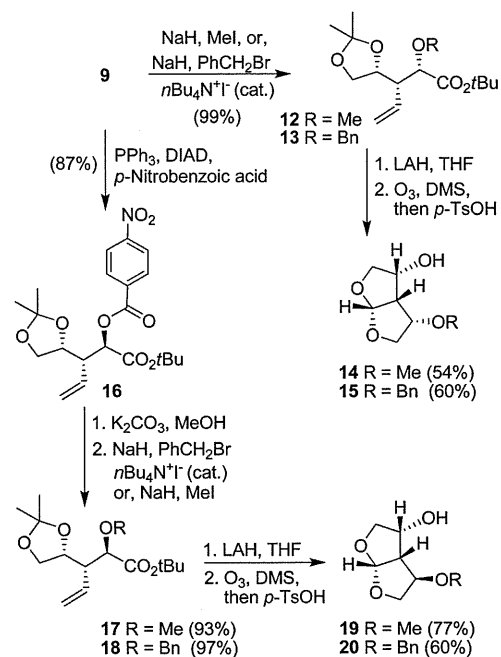
## Scheme 1. Synthesis of Bis-THF Ligand 11



acid,<sup>15,16</sup> L-arabinose,<sup>17</sup> L-serine,<sup>18</sup> and L-tartaric acid,<sup>19</sup> as reported. We used the L-tartaric acid-derived procedure for our synthesis. Dibal-H reduction of 4 resulted in alcohol 5 (Scheme 1).<sup>20,21</sup>

We investigated the O-alkylation of 5 using *tert*-butyl bromoacetate 6 under a variety of conditions. The use of KOtBu in THF at 23 °C for 2 h resulted in a single product, 7 in 56% yield. O-alkylation, using Cs<sub>2</sub>CO<sub>3</sub> in DMF also proceeded with a moderate yield (59%). However, the use of CsOH–H<sub>2</sub>O and activated molecular sieves, in the presence of tetrabutylammonium iodide in acetonitrile, provided the best results.<sup>22</sup> These conditions afforded the desired O-alkylated product 7 in 85%

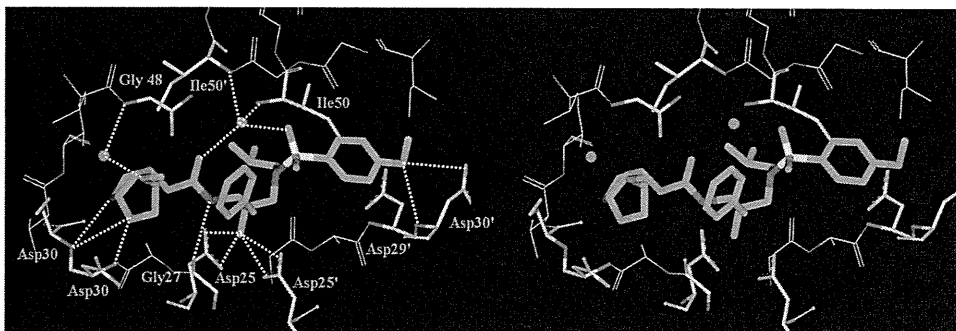
## Scheme 2. Syntheses of Substituted Bis-THF Ligands



yield. Only a small amount of the transesterification product (<5%) and a small amount (<5%) of starting material were recovered in this reaction.

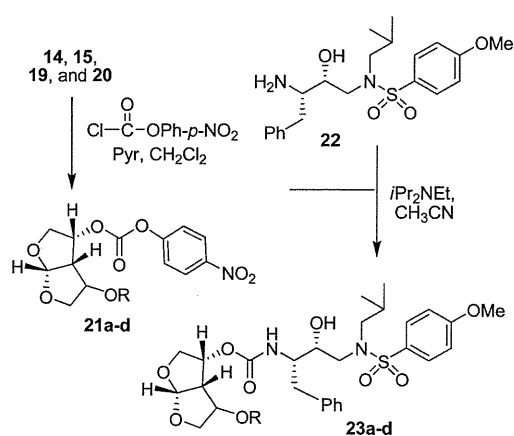
We then investigated a [2,3]-sigmatropic rearrangement of 7 under a variety of conditions. Reaction of 7 with KOtBu at 23 °C proceeded smoothly but provided 9 a 4:1 diastereoselective ratio at the C2-chiral center. Lowering the temperature to –10 °C resulted in a marked increase in selectivity (17:1) with a moderate yield (63%). It should be noted that the C2-chiral center would be eliminated en route to the bis-THF ligand; however, stereoselectivity is important for the synthesis of C4-derivatives. Reactions with LiHMDS in THF at –20 °C provided 9 in 55% yield and 6:1 diastereoselectivity. However, the same reaction at –40 °C to –30 °C for 1 h provided the best yield. Diastereomer 9 was obtained as a single product in 80% yield. The stereochemical outcome of the [2,3]-sigmatropic rearrangement can be rationalized by the proposed transition-state model 8, in which the allylic C–O bond is orthogonal to the plane of the allylic C=C and is antiperiplanar with respect to the approaching carbanion, as described by Bruckner and co-workers.<sup>23</sup> For the synthesis of the bis-THF ligand, alcohol 9 was converted to the corresponding mesylate. LAH reduction of the mesylate provided alcohol 10 in 74% yield, over 2 steps. Oxidative cleavage of the terminal alkene followed by acid-catalyzed cyclization, in the presence of a catalytic amount of *p*-TsOH in CHCl<sub>3</sub> at reflux, afforded the bis-THF alcohol 11 in 80% yield.

As shown in Scheme 2, rearrangement product 9 was utilized for the synthesis of C4-substituted bis-THF ligands. Alcohol 9 can be conveniently functionalized into derivatives which can further interact with the HIV-1 protease backbone atoms. Thus, protection of the free hydroxyl group provided methyl and benzyl ethers 12 and 13, respectively. LAH reduction, followed by ozonolysis, and acid-catalyzed cyclization resulted in compounds 14 and 15, respectively. Inversion of the C2-hydroxyl group of 9 using Mitsunobu's protocol<sup>24</sup> gave 16 in 87% yield.



**Figure 2.** Stereoview of the X-ray structure of inhibitor **23c** (green)-bound HIV-1 protease (pdb code: 3QAA). All strong hydrogen bonding interactions are shown as dotted lines.

### Scheme 3. Syntheses of Protease Inhibitors



Selective deprotection of the nitro benzoate with potassium carbonate in methanol at 0 °C, and subsequent protection with methyl iodide or benzyl bromide provided methyl and benzyl ethers **17** and **18**, respectively. LAH reduction, ozonolysis, and cyclization resulted in the desired substituted bis-THF ligands **19** and **20**.

As shown in Scheme 3, alcohols **14**, **15**, **19**, and **20** were activated with *p*-nitrophenyl chloroformate to provide mixed activated carbonates **21a–d**. These carbonates were reacted with amine **22** to afford HIV-1 protease inhibitors **23a–d**.<sup>8</sup> These inhibitors were all evaluated for their activity in both enzyme inhibitory and antiviral assays. The results are shown in Table 1. The enzyme inhibitory activity ( $K_i$ ) was determined according to an assay protocol reported by Toth and Marshall.<sup>25</sup> The inhibitors displayed extremely potent enzyme inhibitory activity. Compound **23c** ( $K_i = 0.0029$  nM) is the most potent compound in this series. Its diastereomer **23a** ( $K_i = 0.035$  nM) displayed a loss in enzyme inhibitory activity. The benzyl derivatives **23b** and **23d** have also shown reduced enzyme activity. Antiviral  $IC_{50}$  values were determined using the MTT assay.<sup>26,27</sup> Consistent with its enzyme inhibitory potency, inhibitor **23c** exhibited an impressive antiviral activity ( $IC_{50} = 2.4$  nM).

To gain molecular insights into ligand-binding site interactions responsible for the potent antiviral activity of inhibitor **23c**, we have determined an X-ray crystal structure of GRL-04410 (**23c**) complexed with the wild-type protease. The structure was refined to an *R*-factor of 0.175 at a high resolution of 1.40 Å. The structure comprises the protease dimer and inhibitor in two orientations related by a 180° rotation, with 55/45 relative

**Table 1. Structure and Activity of Inhibitors**

Entry	Inhibitor	$K_i$ (nM)	$IC_{50}$ (nM) <sup>a,b</sup>
1.	<b>24</b> (TMC-126) <sup>27</sup>	0.014	1.4
2.	<b>23a</b>	0.035	55
3.	<b>23b</b>	0.073	335
4.	<b>23c</b> (GRL-04410)	0.0029	2.4
5.	<b>23d</b>	0.3	—

<sup>a</sup> Values are means of at least three experiments. <sup>b</sup> Human T-lymphoid (MT-2) cells were exposed to 100 TCID<sub>50</sub> values of HIV-1<sub>LAI</sub> and cultured in the presence of each PI, and  $IC_{50}$  values were determined using the MTT assay.

occupancies (pdb code: 3QAA). The protease dimer structure is essentially identical to that in the protease–darunavir complex with a rmsd of 0.14 Å on the C $\alpha$  atoms.<sup>28</sup> The inhibitor interactions in the active site cavity consist of a series of hydrogen bonds and weaker CH $\cdots$ O interactions, as described previously for darunavir and GRL-09865.<sup>28,29</sup> The critical differences are provided by the methoxy group on the bis-THF ligand in **23c**. As shown in Figure 2, the methoxy oxygen forms a water-mediated hydrogen bond with the amide NH of Gly-48. Furthermore, the methyl group forms a CH $\cdots$ O interaction with the carbonyl oxygen of Gly-48 that could stabilize the conformation of the flexible flaps. The flexibility of the flaps is important for the binding of substrates or inhibitors and for the catalytic activity of HIV protease. The flap conformation and flexibility

can be altered by sequence polymorphisms and drug resistant mutations.<sup>30–32</sup> Consequently, inhibitors such as **23c** with strong flap interactions are expected to retain high affinity for drug resistant variants of the protease.

In conclusion, we investigated C4-alkoxy substituted bis-THF-derived HIV-1 protease inhibitors in order to enhance ligand-binding site interactions in the HIV-1 protease active site. In this context, we have developed an optically active synthesis of the bis-THF and C4-substituted bis-THF ligands using a [2,3]-sigmatropic rearrangement as the key step. Incorporation of C4-substituted bis-THF ligands resulted in a series of highly potent HIV protease inhibitors. Compound **23c** is remarkably potent ( $K_i = 2.9$  pM;  $IC_{50} = 2.4$  nM). The stereochemical importance of the methoxy substituent is evident, as the corresponding epimer is significantly less potent. A protein–ligand X-ray structure of **23c**-bound HIV-1 protease revealed extensive interactions of the inhibitor in the active site of HIV-1 protease. It maintained all key backbone hydrogen bonding interactions similar to those of darunavir. Of particular importance, the methoxy oxygen on the bis-THF ligand is involved in a unique water-mediated hydrogen bond to the Gly-48 amide NH. Further design and ligand optimization involving this interaction is in progress.

## ■ ASSOCIATED CONTENT

**S** Supporting Information. Experimental procedures and <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## Design and Synthesis of Potent HIV-1 Protease Inhibitors Incorporating Hexahydrofuropyranol-Derived High Affinity P<sub>2</sub> Ligands: Structure–Activity Studies and Biological Evaluation

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The design, synthesis, and evaluation of a new series of hexahydrofuropyranol-derived HIV-1 protease inhibitors are described. We have designed a stereochemically defined hexahydrofuropyranol-derived urethane as the P<sub>2</sub>-ligand. The current ligand is designed based upon the X-ray structure of **1a**-bound HIV-1 protease. The synthesis of (3*aS*,4*S*,7*aR*)-hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol, (–)-**7**, was carried out in optically active form. Incorporation of this ligand provided inhibitor **35a**, which has shown excellent enzyme inhibitory activity and antiviral potency. Our structure–activity studies have indicated that the stereochemistry and the position of oxygens in the ligand are important to the observed potency of the inhibitor. Inhibitor **35a** has maintained excellent potency against multidrug-resistant HIV-1 variants. An active site model of **35a** was created based upon the X-ray structure of **1b**-bound HIV-1 protease. The model offers molecular insights regarding ligand-binding site interactions of the hexahydrofuropyranol-derived novel P<sub>2</sub>-ligand.

### Introduction

HIV-1 protease inhibitors are critical components of highly active antiretroviral therapy (HAART<sup>a</sup>).<sup>1–3</sup> The HAART treatment regimens significantly reduced HIV/AIDS-related mortality.<sup>4,5</sup> However, the rapid emergence of drug-resistant HIV-1 strains and the appearance of cross-resistance are severely limiting long-term treatment options.<sup>6–8</sup> An estimated 10–25% of newly infected patients harbor at least one viral strain that is resistant to current medications.<sup>9–11</sup> In addition, PI regimens suffer from a number of other drawbacks including high pill burden, treatment cost, poor AD-MET properties, debilitating side effects, and toxicity issues.<sup>12</sup> Therefore, the development of novel PIs with broad-spectrum activity against multidrug-resistant HIV-1 variants remains a major therapeutic objective.<sup>13</sup>

In our continuing interest to develop novel protease inhibitors (PI) with broad-spectrum activity against multidrug-resistant HIV-1 variants, we have reported a series of PIs including PIs **1a**, **1b**, **2**, and **3**.<sup>14–16</sup> These inhibitors exhibited excellent antiviral activity against multidrug-resistant HIV-1 variants. Darunavir (TMC-114, Figure 1) has been recently approved by the FDA.<sup>17,18</sup> It has displayed a high genetic barrier to resistance and retained high potency against multidrug resistant HIV-1 strains. It has been demonstrated that

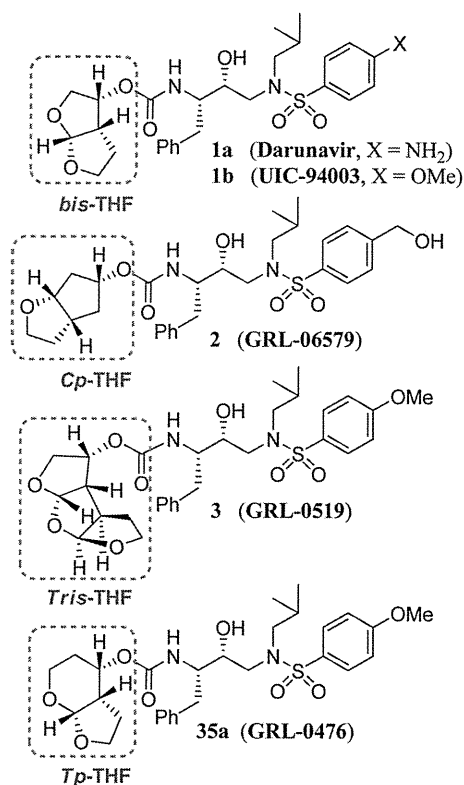
resistance to **1a** is significantly delayed compared to other approved PIs.<sup>19–21</sup>

Our structure-based design of **1a** and other PIs is inspired by the premise that an inhibitor engaged in multiple interactions, especially hydrogen bonding with the HIV protease backbone atoms, should retain these affinities with mutant strains.<sup>22</sup> As the enzyme backbone conformation is only minimally distorted when mutations occur, backbone atoms–PI interactions are likely maintained, therefore sustaining the inhibitor affinity and potency. Inhibitor **1a**'s superb resistance profile likely originates from the extensive interactions the inhibitor makes within the HIV-1 protease's binding site and particularly with the backbone atoms of the enzyme.<sup>22–24</sup> Extensive studies of **1a**-bound HIV-1 protease crystal structures have consistently revealed tight hydrogen bonding between the inhibitor and the protease backbone.<sup>23–25</sup> The stereochemically defined bis-tetrahydrofuran (bis-THF) P<sub>2</sub> ligand in **1a** forms a strong hydrogen bonding network between its two cyclic ether oxygens and the backbone amide NH bonds of the protease residues, Asp29 and Asp30.<sup>22</sup> These observations likely provide explanations for **1a**'s outstanding antiviral activity. Not surprisingly, several other protease inhibitors featuring the bis-THF as the P<sub>2</sub> ligand have exhibited equally impressive antiviral activities and resistance profiles.<sup>22,26</sup>

The bis-THF ligand represents an intriguing pharmacophoric scaffold for the development of PIs to combat drug resistance. To further optimize the bis-THF structural template, we have now investigated ligands that could enhance the backbone-binding as well as improve hydrophobic interactions with the protease active site. The X-ray structure of **1a**-bound HIV-1 protease has shown a distance of about 3.0–3.2 Å

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<sup>a</sup>Abbreviations: bis-THF, bis-tetrahydrofuran; Cp-THF, cyclopentanyltetrahydrofuran; Tp-THF, tetrahydropyranyltetrahydrofuran; PI, protease inhibitor; HAART, highly active antiretroviral therapy; APV, amprenavir; DRV, darunavir; SQV, saquinavir; IDV, indinavir; LPV, lopinavir; RTV, ritonavir; ATV, atazanavir.



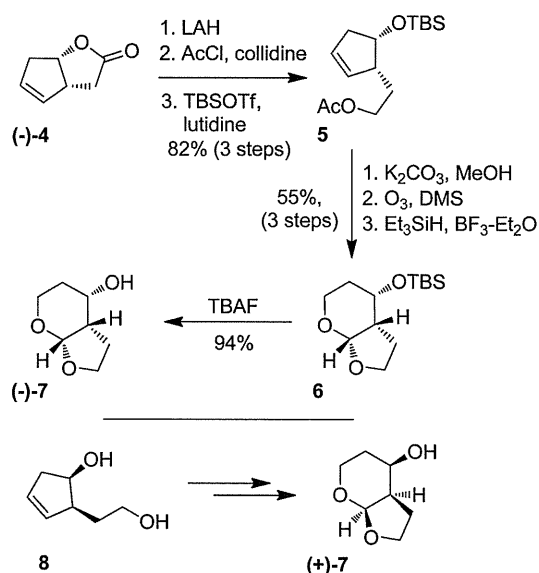
**Figure 1.** Structures of inhibitors **1–3** and **35a**.

between the bis-THF cyclic oxygens and the Asp30 NH amide bond, while a shorter 2.9 Å distance was observed with the Asp29 NH bond.<sup>23,25</sup> In order to maximize and promote closer hydrogen bonding with the Asp30 backbone NH bond, we thought a larger ring on the P<sub>2</sub> ligand should increase the dihedral angle of the bicyclic acetal, bring the oxygen closer, give more flexibility to the structure, and offer a more optimal alignment of the cyclic oxygen with the Asp30 NH bond. Such factors could realistically promote tighter hydrogen bonding with the Asp30 backbone NH bond. Besides, this extra methylene group in the “inner” ring would also provide more favorable van der Waals interactions within the hydrophobic pocket created by Ile47, Val32, Ile84, Leu76, and Ile50' residues in the protease S<sub>2</sub> subsite. In addition, a larger ring could potentially lead to better flexibility and adaptability to protease mutations. Herein, we report the design, synthesis, and biological evaluation of a series of highly potent PIs that combined a (*R*)-hydroxyethylsulfonamide isostere with the furopyranol ligand (–)-**7**. Among all inhibitors of the series, **35a** showed the most impressive inhibitory and antiviral activity (*K*<sub>i</sub> = 2.7 pM, IC<sub>50</sub> = 0.5 nM). Moreover, inhibitor **35a** was evaluated against a panel of multidrug-resistant HIV-1 viruses. It retained potent activity against a variety of multidrug-resistant clinical HIV-1 strains with EC<sub>50</sub> values in low nanomolar range, which is superior to other PIs and comparable to **1a**. Modeling of **35a** based upon the X-ray structure of **1b**-bound HIV-1 protease active site has provided critical molecular insight into the ligand-binding site interactions.

## Chemistry

The synthesis of enantiomerically pure (3*aS*,4*S*,7*aR*)-hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol is shown in Scheme 1. It was achieved starting from known enantiomerically pure lactone **4**.<sup>27</sup> Lactone **4** was reduced into the corresponding

**Scheme 1.** Synthesis of Ligand (–)-**7** and Its Respective Enantiomer (+)-**7**

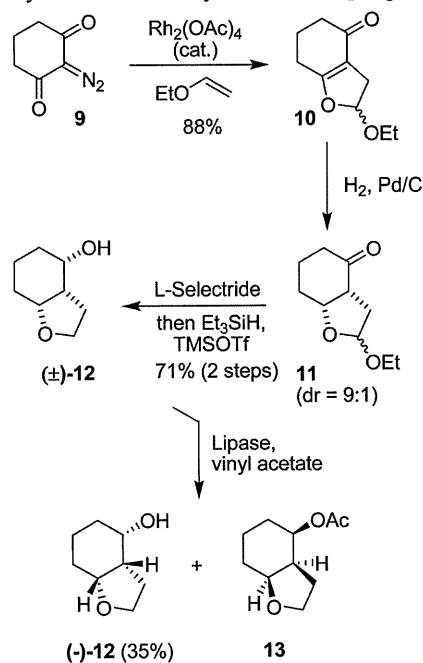


diol using lithium aluminum hydride in 95% yield. Selective monoacetylation at the primary alcohol using AcCl and 2,4,6-collidine at –78 °C<sup>28</sup> and subsequent silylation of the remaining free hydroxyl furnished intermediate **5** in 86% yield (two steps). Removal of the acetate group, followed by ozonolysis of the olefin, furnished a bicyclic bis-acetal intermediate. Reduction of the hemiacetal moiety using Et<sub>3</sub>SiH and BF<sub>3</sub>·Et<sub>2</sub>O afforded bicyclic intermediate **6** in 55% yield in three steps. Removal of the silyl group with TBAF in THF furnished the desired hexahydrofuropyran-4-ol ligand (–)-**7**.

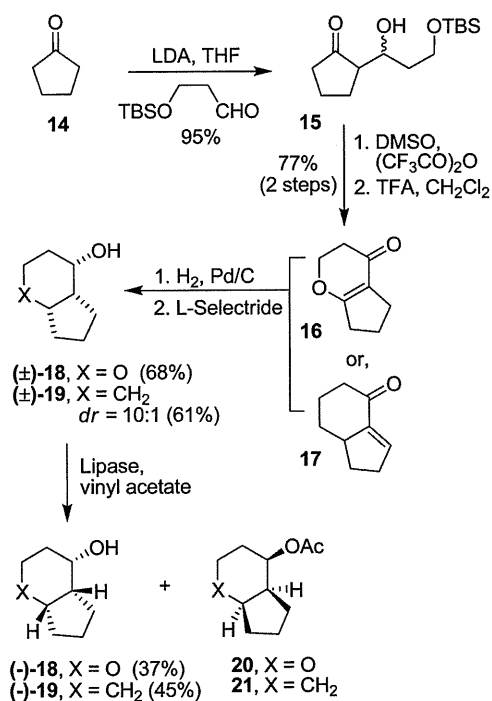
To demonstrate the importance of the absolute stereochemistry of the bicyclic structure of ligand (–)-**7**, its corresponding enantiomer (+)-**7** was synthesized starting from intermediate **8** (Scheme 1). Intermediate **8** was synthesized by an enzyme-catalyzed desymmetrization of cyclopentene *meso*-diacetate followed by a Claisen rearrangement step.<sup>27b,29</sup> The resulting diester was reduced by LAH to provide **8**. It was used for the synthesis of (+)-**7** and subjected to the same synthetic sequence applied from lactone (–)-**4** in the synthesis of (–)-**7** (Scheme 1). To examine the importance of each of the two cyclic ether oxygens in the furopyranol ligand (–)-**7**, we prepared the corresponding cyclohexane and cyclopentane derivatives (Schemes 2 and 3).

The synthesis of 4-hydroxyoctahydrobenzofuran ligand (–)-**12** is shown in Scheme 2. Reaction of diazocyclohexanone **9**<sup>30</sup> with ethyl vinyl ether in presence of a catalytic amount of Rh<sub>2</sub>(OAc)<sub>4</sub> at 23 °C gave derivative **10**.<sup>31</sup> Hydrogenation of the ketofuran in the presence of Pd/C under H<sub>2</sub> (1 atm) furnished the corresponding crude ketone **11** as a 9:1 mixture of diastereoisomers. A one-pot procedure involving L-selectride reduction of the ketone followed by Et<sub>3</sub>SiH/TMSOTf-promoted reduction of the acetal furnished the racemic alcohol (±)-**12** (71% from **10**). Enzymatic resolution of (±)-**12** using lipase Amano PS-30 provided the desired enantiopure alcohol (–)-**12** (98.8% ee by chiral HPLC analysis of the 2,4-dinitrobenzoate derivative), after ~55% conversion to the acetate.

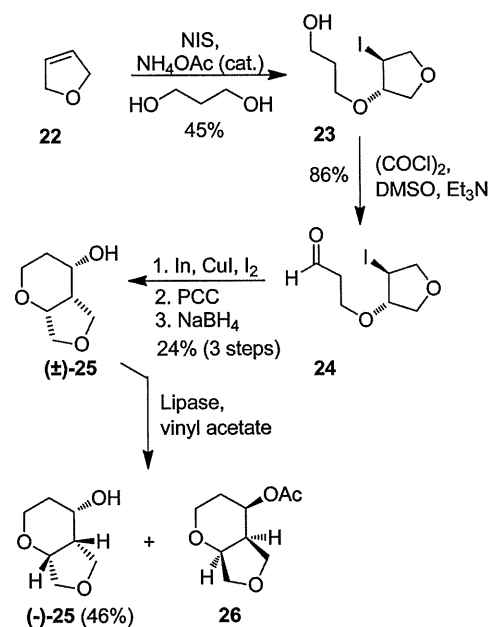
The synthesis of cyclopentapyranol ligand (–)-**18** is shown in Scheme 3. Pentanone **14** was treated with LDA and then reacted with *tert*-butyldimethylsilyloxypropionaldehyde<sup>32</sup>

Scheme 2. Synthesis of Furocyclohexanol P<sub>2</sub> Ligand (–)-12

Scheme 3. Syntheses of Ligands (–)-18 and (–)-19



to furnish intermediate **15** (dr 3:1) in 95% yield. A DMSO-TFAA promoted oxidation of the free hydroxy group followed by TFA-promoted cyclocondensation furnished the bicyclic  $\alpha,\beta$ -unsaturated ketone **16**. Hydrogenation in presence of 10% Pd/C followed by L-selectride reduction of the ketone gave racemic alcohol ( $\pm$ )-**18** as a single diastereomer in 68% yield over two steps. Lipase-catalyzed resolution of the alcohol provided enantiomerically pure alcohol (–)-**18**. For the synthesis of a P<sub>2</sub> ligand devoid of any cyclic oxygen, known tetrahydroindanone **17**<sup>33</sup> was similarly hydrogenated in presence of 10% Pd/C to give the corresponding bicyclic ketone. Accordingly, L-selectride-promoted reduction of the

Scheme 4. Synthesis of Hexahydrofuro[3,4-*b*]pyran-4-ol Ligand **25**

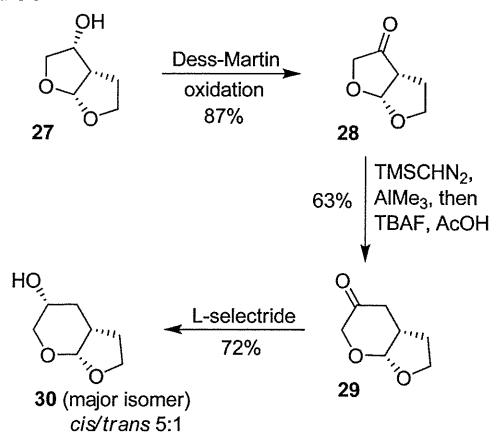
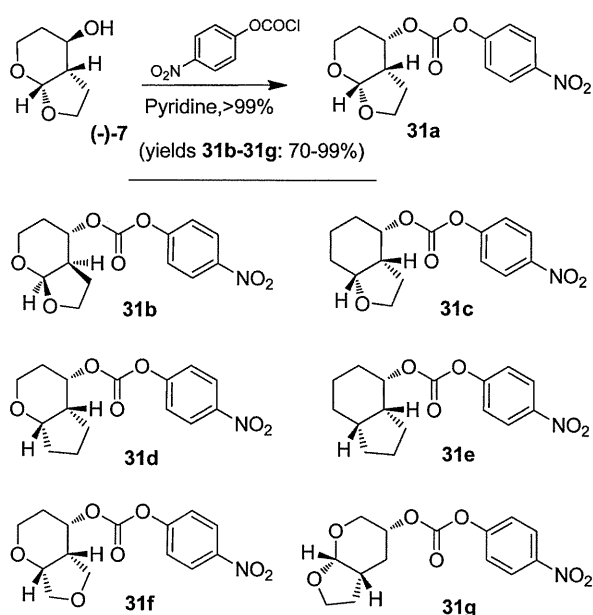
ketone provided the corresponding alcohol (dr = 10:1, as observed by <sup>1</sup>H and <sup>13</sup>C NMR). Lipase-mediated resolution of the major *cis*-alcohol gave the respective chiral ligand (–)-**19** (90% ee determined by chiral HPLC).

Since the introduction of a six-membered ring in the P<sub>2</sub> ligand structure may introduce more structural flexibility, we set out to explore ligands in which the cyclic oxygens were moved to adjacent positions. Such ligands would also demonstrate the importance of the oxygen positions in the bicyclic structure of ligand (–)-**7**. Thus, isomeric ligand **25** was synthesized with the furan oxygen moved to its vicinal position. The synthesis of 4-hydroxyhexahydro-2*H*-furo[3,4-*b*]pyran **25** is shown in Scheme 4. Iodoalkoxylation of the 2,5-dihydrofuran **22** using propanediol in the presence of *N*-iodosuccinimide and catalytic NH<sub>4</sub>OAc provided iodo alcohol **23**. Swern oxidation gave aldehyde **24** in 86% yield. An intramolecular Barbier-type reaction was then conducted using indium in the presence of copper(I) iodide and iodine to furnish a mixture of diastereoisomeric alcohols.<sup>34</sup> Oxidation followed by stereoselective reduction using NaBH<sub>4</sub> furnished the racemic *cis,cis*-bicyclic alcohol ( $\pm$ )-**25** as the sole product. Lipase-mediated resolution finally gave the enantiomerically pure alcohol **25**.

To ascertain the importance of the position of the urethane in (–)-**7**, we have synthesized hexahydrofuro[3,4-*b*]pyran-5-ol ligand **30** shown in Scheme 5. The free hydroxyl on the pyran ring was moved to the C3 position. The synthesis was accomplished starting from enantiomerically pure bis-THF ligand **27** synthesized by us previously.<sup>35</sup> Dess–Martin oxidation of **27** provided the corresponding ketone. Homologation of the resulting ketone using trimethylsilyldiazomethane in the presence of AlMe<sub>3</sub> followed by treatment of the crude mixture with TBAF and acetic acid provided furanopyranone **29**. Stereoselective reduction of ketone **29** using L-selectride furnished alcohol **30** as a mixture of inseparable diastereoisomers (dr = 5:1). Both isomers were separated after formation of the corresponding activated mixed carbonate **31g**.

The synthesis of the protease inhibitors was accomplished in a two-step sequence shown in Schemes 6 and 7. Each ligand alcohol synthesized above was reacted with 4-nitrophenyl

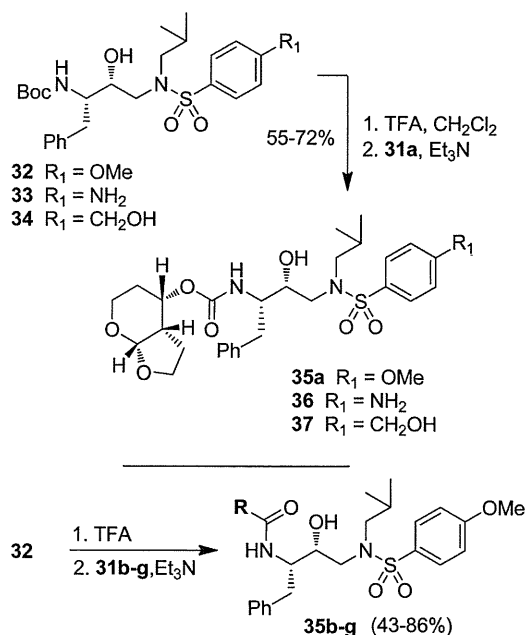


**Scheme 5.** Synthesis of Hexahydrofuro[2,3-*b*]pyran-5-ol Ligand **30****Scheme 6.** Synthesis of Activated Mixed Carbonates **31a–g**

chloroformate in the presence of pyridine to form mixed activated carbonates **31a–g** in 70–99% yield. The syntheses of the corresponding protease inhibitors were achieved by coupling the mixed activated carbonates with previously reported hydroxyethylsulfonamide isosteres **32–34** (Scheme 7).<sup>15,35</sup> The syntheses of various HIV-PIs containing the Tp-THF (–)-**7** were achieved by respectively treating the Boc-protected isosteres **32–34** with TFA in  $\text{CH}_2\text{Cl}_2$  and subsequently by coupling the resulting free amine isosteres with activated mixed carbonate **31a** in THF/ $\text{CH}_3\text{CN}$  in the presence of  $\text{Et}_3\text{N}$ . The corresponding inhibitors **35a**, **36**, and **37** were obtained in good yields (Scheme 7). Inhibitors **35b–g** were made in a similar manner.

## Results and Discussion

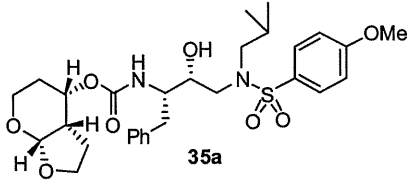
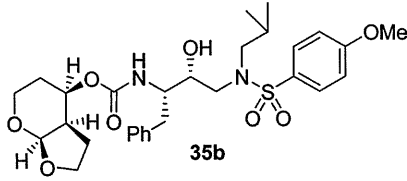
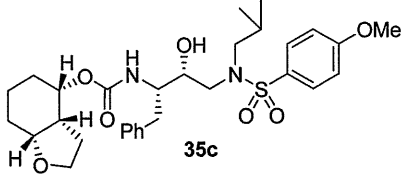
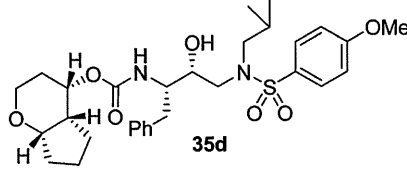
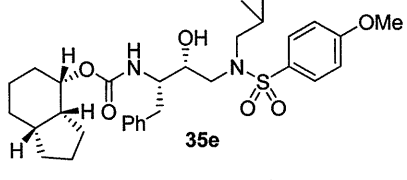
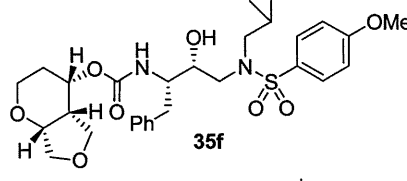
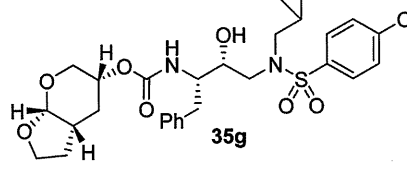
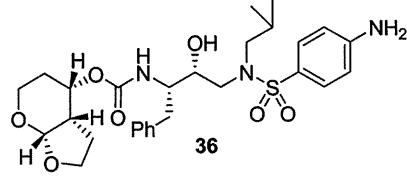
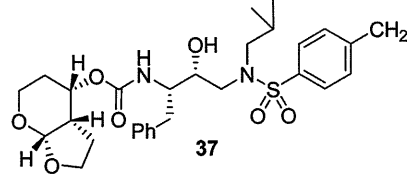
As mentioned above, our preliminary modeling suggested that a hexahydrofuro[2,3-*b*]pyranol (–)-**7** ligand may interact with backbone atoms and residues in the protease S2-site. All inhibitors in Table 1 were evaluated in enzyme inhibitory assays following a protocol described by Toth and Marshall.<sup>36</sup> Inhibitors that showed potent  $K_i$  values were further evaluated through in vitro antiviral assays. As can be seen, inhibitor **35a**,

**Scheme 7.** Syntheses of Inhibitors **35a–g**, **36**, and **37**

with Tp-THF (–)-**7**, exhibited an enzyme  $K_i$  value of 2.7 pM. Antiviral activity of **35a** and other inhibitors were determined in MT-2 human-T-lymphoid cells exposed to HIV-1<sub>LAI</sub>.<sup>19</sup> As shown, **35a** has displayed remarkable antiviral potency ( $\text{IC}_{50} = 0.5 \text{ nM}$ ), comparable to those of PIs **1a** and **1b**. The bicyclic ring stereochemistry of the P<sub>2</sub> ligand proved to be important as inhibitor **35b**, with enantiomeric ligand (+)-**7**, displayed a significant reduction in enzyme inhibitory potency (> 20-fold increase in  $K_i$ ) as well as antiviral activity ( $\text{IC}_{50} = 19 \text{ nM}$ ).

To probe the importance of the cyclic ether oxygens in the bicyclic structure of (–)-**7**, inhibitors **35c–e** were synthesized and evaluated. As shown, inhibitor **35c**, with a cyclohexane ring in place of the tetrahydropyran ring, only displayed a 2-fold reduction in  $K_i$  values but a 16-fold decrease in antiviral activity compared to inhibitor **35a**. A more dramatic loss of enzymatic potency was observed with compound **35d** with a cyclopentane ring in place of a THF ring in the P<sub>2</sub> ligand. The  $K_i$  value dropped to 1.43 nM. Inhibitor **35e**, which lacks both cyclic ether oxygens, displayed even lower  $K_i$  and no appreciable antiviral activity. Those results clearly demonstrated the critical role of both cyclic ether oxygens in ligand (–)-**7**. Furthermore, the difference of activity observed between **35a** and **35c** suggests that the O1 oxygen on the THF-ring of (–)-**7** exerts a stronger interaction with the enzyme compared to the pyran oxygen. Inhibitor **35f**, in which the THF-oxygen of the P<sub>2</sub> ligand is located at a vicinal position, also exhibited a substantial loss of potency (i.e.,  $K_i = 5.3 \text{ nM}$ ) and no antiviral activity. These results corroborated our previous observations with the bis-THF ligand in PIs **1a** and **1b**. The THF-oxygen in (–)-**7** likely has a stronger hydrogen bonding interaction with the Asp29 backbone NH and may form a weak hydrogen bond with Asp30, in the S<sub>2</sub> subsite of the HIV protease. We have investigated the position of the urethane oxygen on the bicyclic ligand in inhibitor **35g**. This has resulted in a substantial loss of protease inhibitory activity. Furthermore, we have examined the potency enhancing effect of the Tp-THF ligand with various hydroxyethylsulfonamide isosteres to give inhibitors **36** and **37**. The 4-methoxysulfonamide derivative **35a** appears to be the most potent inhibitor in

**Table 1.** Enzymatic Inhibitory and Antiviral Activity of Compounds 35a–g, 36, and 37<sup>b</sup>

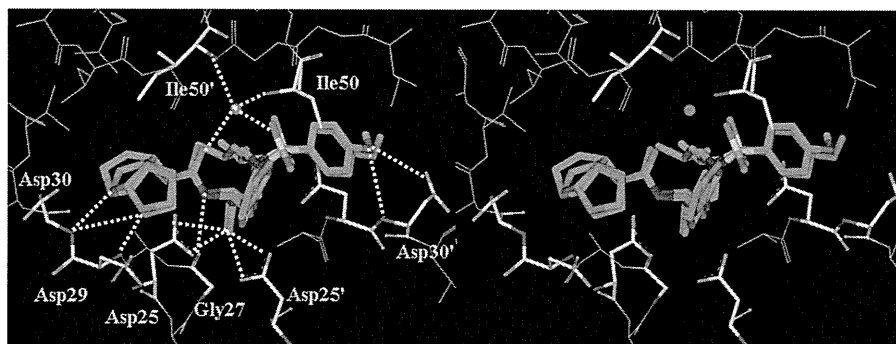
Entry	Inhibitor	$K_i$ (nM)	$IC_{50}$ ( $\mu$ M) <sup>a</sup>
1	 35a	0.0027	0.0005
2	 35b	0.068	0.019
3	 35c	0.005	0.008
4	 35d	1.43	--
5	 35e	9	>1 $\mu$ M
6	 35f	5.3	>1 $\mu$ M
7	 35g	0.11	--
8	 36	0.010	0.0065
9	 37	0.085	0.0045

<sup>a</sup> Values are the mean of at least two experiments. <sup>b</sup> Human T-lymphoid (MT-2) cells ( $2 \times 10^3$ ) were exposed to 100 TCID<sub>50</sub> of HIV-1<sub>LA1</sub> and cultured in the presence of each PI, and  $IC_{50}$  values were determined using the MTT assay. The  $IC_{50}$  values of amprenavir (APV), saquinavir (SQV), and indinavir (IDV) were 0.03, 0.015, and 0.03  $\mu$ M, respectively.

**Table 2.** Comparison of the Antiviral Activity of **35a** and Other PIs against Multidrug Resistant Clinical Isolates in PHA-PBMs Cells<sup>a</sup>

virus	EC <sub>50</sub> (μM)			
	<b>35a</b>	ATV	LPV	DRV ( <b>1a</b> )
HIV-1 <sub>ERS104pre</sub> (X4)	0.0019 ± 0.0015	0.0027 ± 0.0006	0.031 ± 0.004	0.004 ± 0.001
HIV-1 <sub>MDR/B</sub> (X4)	0.0145 ± 0.0001 (8)	0.470 ± 0.007 (174)	> 1 (> 32)	0.034 ± 0.008 (9)
HIV-1 <sub>MDR/C</sub> (X4)	0.0037 ± 0.0018 (2)	0.039 ± 0.003 (14)	0.437 ± 0.004 (14)	0.009 ± 0.005 (2)
HIV-1 <sub>MDR/G</sub> (X4)	0.0026 ± 0.0004 (1)	0.019 ± 0.008 (7)	0.181 ± 0.023 (6)	0.026 ± 0.009 (7)
HIV-1 <sub>MDR/TM</sub> (X4)	0.0275 ± 0.0055 (14)	0.075 ± 0.003 (28)	0.423 ± 0.082 (14)	0.022 ± 0.015 (6)
HIV-1 <sub>MDR/MM</sub> (R5)	0.0050 ± 0.0023 (3)	0.205 ± 0.024 (76)	0.762 ± 0.115 (25)	0.017 ± 0.005 (4)
HIV-1 <sub>MDR/JSL</sub> (R5)	0.0275 ± 0.0009 (14)	0.293 ± 0.099 (109)	> 1 (> 32)	0.023 ± 0.005 (6)

<sup>a</sup>The amino acid substitutions identified in the protease-encoding region of HIV-1<sub>ERS104pre</sub>, HIV-1<sub>B</sub>, HIV-1<sub>C</sub>, HIV-1<sub>G</sub>, HIV-1<sub>TM</sub>, HIV-1<sub>MM</sub>, HIV-1<sub>JSL</sub> compared to the consensus type B sequence cited from the Los Alamos database include L63P; L10I, K14R, L33I, M36I, M46I, F53I, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L; L10I, I15V, K20R, L24I, M36I, M46L, I54V, I62V, L63P, K70Q, V82A, L89M; L10I, V11I, T12E, I15V, L19I, R41K, M46L, L63P, A71T, V82A, L90M; L10I, K14R, R41K, M46L, I54V, L63P, A71V, V82A, L90M; I93L, L10I, K43T, M46L, I54V, L63P, A71V, V82A, L90M, Q92K; and L10I, L24I, I33F, E35D, M36I, N37S, M46L, I54V, R57K, I62V, L63P, A71V, G73S, V82A, respectively. HIV-1<sub>ERS104pre</sub> served as a source of wild-type HIV-1. The EC<sub>50</sub> values were determined by using PHA-PBMs as target cells, and the inhibition of p24 Gag protein production by each drug was used as an end point. The numbers in parentheses represent the fold changes of EC<sub>50</sub> values for each isolate compared to the EC<sub>50</sub> values for wild-type HIV-1<sub>ERS104pre</sub>. All assays were conducted in duplicate, and the data shown represent mean values (±1 standard deviations) derived from the results of two or three independent experiments.

**Figure 2.** Stereoview of inhibitor **35a** modeled into the active site of HIV-1 protease and superimposed on the X-ray crystal structure of **1b** (PDB code 3I7E).

the series comparable to inhibitor **2**. However, the 4-amino derivative **36** exhibited very comparable enzyme inhibitory and antiviral potency similar to **1a**.

We have examined inhibitor **35a** for its activity against a panel of multidrug-resistant HIV-1 variants and compared it with that of other clinically available PIs including **1a**. The results are shown in Table 2. All inhibitors showed high antiviral activity against an HIV-1 clinical strain isolated from a drug-naive patient (wild-type).<sup>19</sup> Compound **35a** displayed the most potent activity with an EC<sub>50</sub> of 1.9 nM. When tested against multidrug-resistant HIV-1 variants, compound **35a** retained impressively high activity to all variants with EC<sub>50</sub> values ranging from 2.6 to 27.5 nM. In contrast, other inhibitors, except **1a**, exhibited substantial loss of activity. Interestingly, **1a** and **35a** showed similar fold-change of EC<sub>50</sub> against most multidrug-resistant HIV strains. The results indicated that **35a** is highly active against multidrug-resistant HIV-1 variants. This inhibitor outperformed the clinically available PIs with exceedingly high antiviral activity and compared well with **1a**, which currently stands as the leading PI for the treatment of drug-resistant HIV infection.

In order to obtain molecular insights into the enzyme–inhibitor interactions of **35a** in the protease active site, an active model of **35a** was created. A stereoview of the overlaid structure of **35a** with the X-ray structure of inhibitor **1b**-bound HIV-1 protease is shown in Figure 2. Inhibitor **35a** was modeled starting from the X-ray crystal structure of **1b**. The conformation of **35a** was optimized using the MMFF94 force

field,<sup>37</sup> as implemented in Molecular Operating Environment (version 2009.10, Chemical Computing Group, Montreal, Canada). The modeled structure maintains the important binding interactions (hydroxyl group with Asp25 and Asp25' carboxylates; cyclic ether oxygens with Asp29 and Asp30 backbone NH bonds; methoxy oxygen with the Asp30' backbone NH bond; carbonyl oxygen and sulfonamide oxygen with a water molecule binding to Ile50 and Ile50') that are observed in the crystal structure of **1b**-bound HIV-1 protease.

## Conclusions

We have reported the structure-based design of novel HIV-1 protease inhibitors incorporating a stereochemically defined 4-hexahydrofurofuranol-derived urethanes as the P2-ligand. The inhibitors were designed to make extensive interactions including hydrogen bonding with the protein backbone of the HIV-1 protease active site. The synthesis of (3*a**S*,4*S*,7*a**R*)-hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol [(–)-**7**, Tp-THF] was carried out in optically active form using (3*a**R*,6*a**S*)-3,3*a*,6,6*a*-tetrahydro-2*H*-cyclopenta[*b*]furan-2-one as the starting material. Inhibitor **35a** has shown excellent enzyme inhibitory activity and antiviral potency comparable to that of approved PI **1a**. Furthermore, it has shown excellent activity against multi-PI-resistant variants, superior to other FDA approved inhibitors examined. The data are comparable to those of **1a**. We have carried out detailed structure–activity studies that indicated that the stereochemistry of the Tp-THF ligand and position of its oxygens are critical to the ligand's high enzyme affinity.

An active model of **35a** was created based upon the X-ray crystal structure of **1b**-bound HIV-1 protease. The overlaid structures revealed that both oxygens of the Tp-THF ligand can interact with the Asp29 and Asp30 backbone NHs, similar to the bis-THF ligand oxygens. Furthermore, the extra methylene unit in the Tp-THF ligand appears to fill in the hydrophobic pocket in the S2-site more effectively compared to the bis-THF in **1a**. The design of an inhibitor targeting the protein backbone may serve as an important guide to combat drug resistance. Further design and chemical modifications are currently underway.

## Experimental Section

**General Experimental Methods.** All anhydrous solvents were obtained according to the following procedures: diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone under argon; toluene, methanol, acetonitrile, and dichloromethane were distilled from calcium hydride; benzene was distilled from sodium. Other solvents were used without purification. All moisture-sensitive reactions were carried out in flame-dried flasks under argon atmosphere. Reactions were monitored by thin layer chromatography (TLC) using Silicycle 60A-F254 silica gel precoated plates. Flash column chromatography was performed using Silicycle 230–400 mesh silica gel. Yields refer to chromatographically and spectroscopically pure compounds. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Varian Inova-300 (300 and 75 MHz), Bruker Avance ARX-400 (400 and 100 MHz), or DRX-500 (500 and 125 MHz). High and low resolution mass spectra were carried out by the Mass Spectroscopy Center at Purdue University. The purity of all test compounds was determined by HRMS and HPLC analysis in the different solvent systems. All test compounds showed  $\geq 95\%$  purity.

**(1*S*,2*R*)-2-[1-(*tert*-Butyldimethylsilyloxy)cyclopent-3-en-2-yl]ethyl Acetate (**5**).** To a stirred suspension of lithium aluminum hydride (93 mg, 2.45 mmol) in dry  $\text{Et}_2\text{O}$  (6 mL) was added dropwise a solution of (–)-(1*S*,5*R*)-2-oxabicyclo[3.3.0]oct-6-en-3-one (**4**) (150 mg, 1.19 mmol) in  $\text{Et}_2\text{O}$  (4 mL + 1 mL rinse) at 0 °C under argon. The reaction mixture was vigorously stirred at this temperature for 1.5 h. Water (0.1 mL) was then carefully added followed by addition of 3 M NaOH (0.1 mL) and then water (0.3 mL). The solution was stirred until formation of a white precipitate was complete. EtOAc (3 mL) and then  $\text{Na}_2\text{SO}_4$  were added, and the resulting suspension was filtered out. The amorphous solid was washed several times with EtOAc (5  $\times$  5 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated in vacuo. The crude oil was purified by flash chromatography on silica gel using hexanes/EtOAc (1:1) as the eluent to give the resulting diol (145 mg, 95%) as a colorless oil. TLC:  $R_f$  = 0.28 (hexanes/EtOAc = 1:2).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  5.74 (m, 1H), 5.56 (m, 1H), 4.48 (dt,  $J$  = 2.4, 6.6 Hz, 1H), 3.84 (m, 1H), 3.71 (ddd,  $J$  = 3.6, 8.7, 10.0 Hz, 1H), 2.75 (m, 1H), 2.67 (m, 1H), 2.36 (d,  $J$  = 17.1 Hz, 1H), 1.98–1.75 (m, 1H).

To a stirred solution of the diol (76 mg, 0.59 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was added 2,4,6-collidine (1.2 mmol, 155  $\mu\text{L}$ ) followed by acetyl chloride (50  $\mu\text{L}$ , 0.71 mmol) at –78 °C under argon. The resulting solution was stirred at this temperature for 5 h at which point additional acetyl chloride (0.25  $\mu\text{L}$ , 0.24 mmol) was added. The solution was stirred for 2 h, and then saturated aqueous  $\text{NaHCO}_3$  solution was added. The two layers were separated, and the aqueous layer was washed with  $\text{CH}_2\text{Cl}_2$  (3  $\times$  5 mL). The combined organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated in vacuo. The crude oil was purified by flash chromatography on silica gel using hexanes/EtOAc (6:1, then 4:1) as the eluent to give the monoacetate (88 mg, 87%) as a colorless oil. TLC:  $R_f$  = 0.26 (hexanes/EtOAc = 2:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  5.80–5.72 (m, 1H), 5.64–5.58 (m, 1H), 4.40 (dt,  $J$  = 2.4, 5.6 Hz, 1H), 4.20 (t,  $J$  = 7.2 Hz, 2H), 2.74–2.56 (m, 2H), 2.33 (d,

$J$  = 17.1 Hz, 1H), 2.06 (s, 3H), 2.04–1.88 (m, 1H), 1.87–1.73 (m, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  171.1, 132.4, 128.4, 72.7, 63.9, 47.2, 42.1, 26.8, 21.0. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_9\text{H}_{15}\text{O}_3$  171.1021; found 171.1020.

To a stirred solution of the above acetate (54 mg, 0.32 mmol) and 2,6-lutidine (74  $\mu\text{L}$ , 0.63 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (125 mg, 108  $\mu\text{L}$ ) at –78 °C under argon. The mixture was stirred for 10 min, at which point reaction completion was observed. Saturated aqueous  $\text{NaHCO}_3$  solution (1 mL) and additional  $\text{CH}_2\text{Cl}_2$  (2 mL) were added. The two layers were separated, and the aqueous layer was further extracted with  $\text{CH}_2\text{Cl}_2$  (2  $\times$  2 mL). The combined organic layer was washed with brine, dried ( $\text{MgSO}_4$ ), filtered, and concentrated under reduced pressure. The crude oil was purified by column chromatography on silica gel using hexanes/EtOAc (20:1) as the eluent to afford silylated product **5** (90 mg, > 99%) as a colorless oil. TLC:  $R_f$  = 0.68 (hexanes/EtOAc = 3:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  5.68 (s, 2H), 4.45 (dt,  $J$  = 5.1, 6.3 Hz, 1H), 4.14 (t,  $J$  = 6.9 Hz, 2H), 2.67–2.55 (m, 1H), 2.47 (dd,  $J$  = 6.9, 15.4 Hz, 1H), 2.23 (dd,  $J$  = 4.8, 15.4 Hz, 1H), 2.04 (s, 3H), 2.01–1.85 (m, 1H), 1.72–1.56 (m, 1H), 0.88 (s, 9H), 0.06 (s, 6H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  171.2, 132.7, 128.4, 73.6, 63.8, 45.9, 41.0, 27.4, 25.8, 21.0, 18.1, –4.6, –5.0.

**(4*S*,4*aS*,7*aR*)-4-(*tert*-Butyldimethylsilyloxy)hexahydrofuro[2,3-*b*]pyrane (**6**).** To a stirred solution of **5** (76 mg, 0.27 mmol) in MeOH (2 mL) was added  $\text{K}_2\text{CO}_3$  (37 mg, 0.27 mmol). The solution was stirred at 23 °C for 2 h. Then saturated aqueous  $\text{NH}_4\text{Cl}$  solution (2 mL) was added to the mixture. EtOAc was added, and the two layers were separated. The aqueous layer was extracted with EtOAc (4  $\times$  3 mL). The combined organic layer was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated under reduced pressure. The resulting oil was purified by flash chromatography on silica gel using hexanes/EtOAc (7:1) as the eluent to give the corresponding alcohol (64 mg, 98%) as a colorless oil. This intermediate was used immediately for the subsequent reaction. TLC:  $R_f$  = 0.29 (hexanes/EtOAc = 5:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  5.72.5.62 (m, 2H), 4.52 (dt,  $J$  = 6.0, 6.9 Hz, 1H), 3.74–3.60 (m, 2H), 2.80–2.68 (m, 1H), 2.49 (ddt,  $J$  = 1.8, 7.2, 16.3 Hz, 1H), 2.34–2.29 (m, 1H), 2.06 (br s, 1H), 1.90–1.62 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  132.9, 128.3, 74.0, 61.1, 46.5, 40.6, 31.2, 25.8, 18.2, –4.7, –5.0.

A stream of ozonized oxygen was bubbled through a solution of the above alcohol (63.8 mg, 0.26 mmol) in  $\text{CH}_2\text{Cl}_2$  (15 mL) at –78 °C until the blue color persisted (5 min). After the solution was flushed with nitrogen,  $\text{Me}_2\text{S}$  (0.5 mL) was added. The solution was warmed to 0 °C and stirred over a 2 h period following which anhydrous  $\text{Na}_2\text{SO}_4$  was added. The solution was left at room temperature overnight and then filtered and concentrated in vacuo. The resulting solid was quickly passed through a short column of silica gel using hexanes/EtOAc (3:1) as the eluent to afford the hemiacetal (99 mg) as a white-solid mixture of isomers which was submitted directly to the next step. TLC:  $R_f$  = 0.26 (hexanes/EtOAc = 3:1). To an ice-cold solution of the crude diacetal (~0.25 mmol) and  $\text{Et}_3\text{SiH}$  (0.16 mL, 1.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) under argon, was slowly added  $\text{BF}_3\text{-Et}_2\text{O}$  (60  $\mu\text{L}$ , 0.5 mmol). The mixture was stirred at 0 °C for 10 min. Saturated aqueous  $\text{NaHCO}_3$  solution (2 mL) and additional  $\text{CH}_2\text{Cl}_2$  were added. The two phases were separated and the aqueous layer was further extracted with  $\text{CH}_2\text{Cl}_2$  (3  $\times$  2 mL). The combined organic layer was washed with brine, dried ( $\text{MgSO}_4$ ), filtered, and concentrated in vacuo. The crude oil was purified by column chromatography on silica gel using hexanes/EtOAc (7:1) as the eluent to give bicyclic acetal **6** (38 mg, 55% 3 steps) as an amorphous solid. TLC:  $R_f$  = 0.50 (hexanes/EtOAc = 3:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  4.95 (d,  $J$  = 3.4 Hz, 1H), 4.24–4.08 (m, 2H), 3.92 (dt,  $J$  = 8.1, 9.1 Hz, 1H), 3.85 (ddd,  $J$  = 2.0, 4.5, 12.2 Hz, 1H), 3.30 (dt,  $J$  = 2.0, 12.3 Hz, 1H), 2.39 (m, 1H), 2.07 (tt,  $J$  = 9.4, 12.0 Hz, 1H), 1.91–1.66 (m, 2H), 1.58–1.48 (m, 1H), 0.89 (s, 9H), 0.07 (s, 3H), 0.067 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  101.2, 68.4, 67.8, 61.1, 47.2, 30.3, 25.7, 22.4, 18.2, –4.6, –4.8.

**(3*aS*,4*S*,7*aR*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol [(–)-**7**].** Bicyclic compound **6** (36 mg, 0.139 mmol) was dissolved in

THF (1 mL), and tetrabutylammonium fluoride (1 M solution THF, 0.21 mL, 0.21 mmol) was added to the solution. The mixture was stirred for 2 h at 23 °C. Saturated aqueous NH<sub>4</sub>Cl solution was added (2 mL), followed by EtOAc (2 mL). The two phases were separated, and the aqueous layer was further extracted with EtOAc (4 × 3 mL). The combined organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The resulting compound was purified by flash chromatography on silica gel using hexanes/EtOAc (1:2 then 1:3) as the eluent to afford pure alcohol (–)-**7** (19 mg, 94%) as an amorphous solid. TLC: *R<sub>f</sub>* = 0.15 (hexanes/EtOAc = 1:3). [α]<sub>D</sub><sup>23</sup> –29.6 (*c* 1.06, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 4.99 (d, *J* = 2.7 Hz, 1H), 4.25–4.16 (m, 2H), 3.96 (q, *J* = 7.5 Hz, 1H), 3.90 (ddd, *J* = 2.4, 4.8, 12.3 Hz, 1H), 3.34 (td, *J* = 3.0, 11.7 Hz, 1H), 2.58–2.45 (m, 1H), 2.14–1.98 (m, 1H), 1.96–1.82 (m, 1H), 1.80–1.62 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 101.4, 68.4, 67.5, 61.0, 46.3, 29.4, 21.8. HRMS-Cl (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>9</sub>H<sub>15</sub>O<sub>3</sub> 127.0759; found 127.0757.

**(3aR,4R,7aS)-Hexahydro-2H-furo[2,3-*b*]pyran-4-ol [(+)-**7**]**. Cyclopentenediol **8** was prepared as described previously.<sup>27b</sup> The same synthetic sequence was applied on the diol as for the synthesis of (–)-**7**. Ligand (+)-**7** was obtained in high enantiomeric purity [99% ee, [α]<sub>D</sub><sup>23</sup> +22.3 (*c* 0.22, CHCl<sub>3</sub>)].

**2-Ethoxy-2,3,6,7-tetrahydrobenzofuran-4(5H)-one (10)**. To a stirred solution of 2-diazo-1,3-cyclohexanedione (300 mg, 2.17 mmol) in freshly distilled ethyl vinyl ether (5 mL) was added [Rh<sub>2</sub>(OAc)<sub>4</sub>] (10 mg, 0.02 mmol). The mixture was stirred at room temperature for 5 h, after which the reaction was diluted with Et<sub>2</sub>O and a few drops of pyridine were added. A red precipitate formed. The solution was filtered on a short pad of silica, flushing with Et<sub>2</sub>O/THF (4:1) as eluent. After evaporation, the residue was purified by column chromatography on silica gel using hexanes/CH<sub>2</sub>Cl<sub>2</sub>/THF (8:1:1) as the eluent to furnish benzofuranone derivative **17** (347 mg, 88%). TLC: *R<sub>f</sub>* = 0.29 (hexanes/EtOAc = 1:1). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.72 (dd, *J* = 3.3, 7.4 Hz, 1H), 3.88 (m, 1H), 3.62 (m, 1H), 2.92 (ddt, *J* = 2.2, 7.4, 15.8 Hz, 1H), 2.70–2.62 (m, 1H), 2.52–2.37 (m, 2H), 2.33 (t, *J* = 6.5 Hz, 2H), 2.12–1.95 (m, 2H), 1.24 (t, *J* = 7.1 Hz, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 Hz) δ 195.2, 175.7, 112.3, 108.5, 65.0, 36.3, 32.7, 23.8, 21.5, 14.9.

**2-Ethoxyhexahydrobenzofuran-4(2H)-one (11)**. To a solution of the ketone **10** (140 mg, 0.77 mmol) in EtOAc (9 mL) was added 5% Pd/C (128 mg, 60 μmol), and the mixture was stirred under H<sub>2</sub> (1 atm) for 1.5 h at room temperature. The mixture was then filtered on Celite and the pad washed with EtOAc. Evaporation of the solvent furnished the corresponding crude ketone **11** as an essentially pure mixture of diastereoisomers (130 mg, dr = 9:1). The ketone was directly submitted to the next step without purification. TLC major isomer: *R<sub>f</sub>* = 0.35 (hexanes/EtOAc = 2:1).

**cis-Octahydrobenzofuran-4-ol [(±)-**12**]**. A solution of ketone **11** (130 mg, ca. 0.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was cooled to –78 °C under Ar. L-Selectride (1 M solution, 0.9 mL, 0.9 mmol) was slowly added to the solution over 5 min and the reaction mixture was stirred for 1.5 h at –78 °C. Upon complete conversion, Et<sub>3</sub>SiH (0.6 mL, 437 mg, 3.7 mmol) was added followed by dropwise addition of TMSOTf (380 μL, 466 mg, 2.1 mmol). The solution was stirred for 2.5 h while slowly warming to 0 °C. The reaction was quenched by addition of saturated aqueous NaHCO<sub>3</sub> solution (5 mL). The two phases were separated, and the aqueous phase was extracted with Et<sub>2</sub>O (5×). The combined organic layer was washed with brine, dried (MgSO<sub>4</sub>), and evaporated under vacuum. The residue was purified by column chromatography on silica gel using hexanes/EtOAc (3:1 to 2:1) as the eluent to yield the desired alcohol (±)-**12** (78 mg, 71% over two steps) as a colorless oil. TLC: *R<sub>f</sub>* = 0.25 (hexanes/EtOAc = 1:2). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 4.01 (dt, *J* = 4.6, 8.8 Hz, 1H), 3.88–3.82 (m, 2H), 3.78 (dt, *J* = 7.1, 8.7 Hz, 1H), 2.31 (m, 1H), 2.12–1.90 (m, 2H), 1.74–1.50 (m, 5H), 1.32–1.22 (m, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 Hz) δ 77.6, 69.1, 66.7, 43.2, 30.2, 26.9, 25.9, 16.2.

**(3aS,4S,7aR)-Octahydrobenzofuran-4-ol [(–)-**12**]**. Racemic alcohol **12** (70 mg, 0.5 mmol) was dissolved in THF (5 mL), and vinyl acetate (120 μL, 1.25 mmol) was added. Amano lipase PS-30 (30 mg) was added, and the resulting suspension was stirred at 15–17 °C. After 48 h, 30 mg of additional enzyme was added and the mixture was left for additional 48 h until ~54% conversion was reached (NMR and GC). The resulting suspension was diluted with Et<sub>2</sub>O and filtered on Celite and the filter cake rinsed with Et<sub>2</sub>O. After evaporation of the remaining solvent, the residue was purified by column chromatography using hexanes/EtOAc (5:1, 3:1, then 2:1) as the eluent to yield acetyl furanol **13** (38 mg, 41%) and the desired enantioenriched (–)-hexahydrobenzofuranol (–)-**12** (24 mg, 35%). The enantiomeric excess of the 2,4-dinitrobenzoate derivative of (–)-**12** was determined to be 98.8% ee by chiral HPLC: column ChiralPak IA, hexane/isopropanol (90/10 to 50/50, 40 min), 1 mL/min, 35 °C, λ = 254 nm, *t<sub>R</sub>* major = 16.54 min, *t<sub>R</sub>* minor = 37.1 min.

**2-[3-(tert-Butyldimethylsilyloxy)-1-hydroxypropyl]cyclopentanone (15)**. A solution of lithium diisopropylamide (14 mmol), freshly prepared by adding *n*-BuLi (1.6 M solution in hexanes, 8.75 mL, 14 mmol) to diisopropylamine (1.97 mL, 1.42 g, 14 mmol) in THF (30 mL) at 0 °C under argon followed by stirring for 30 min, was cooled to –78 °C, and cyclopentanone **14** (1.12 mL, 1.07 g, 12.7 mmol) in THF (5 mL) was added dropwise over 10 min. After being stirred at –78 °C for 1.5 h, 3-*tert*-butyldimethylsilyloxypropionaldehyde (1.55 g, 8.2 mmol) in THF (20 mL) was added dropwise over 5 min. The mixture was stirred for an additional 2 h, and the reaction was quenched by addition of saturated aqueous NH<sub>4</sub>Cl solution (10 mL). Following dilution with Et<sub>2</sub>O, the two phases were separated, and the aqueous phase was extracted with Et<sub>2</sub>O (2×). The combined organic phase was washed with brine, dried (MgSO<sub>4</sub>), filtered, and evaporated. The residue was quickly purified by column chromatography on silica gel using hexanes/EtOAc (20:1 to 10:1) as the eluent to give **15** as a 3:1 mixture of diastereoisomers (2.13 g, 95%). Light yellow oil. TLC: *R<sub>f</sub>* = 0.37 and 0.23 (hexanes/EtOAc = 5:1). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 4.27 (dt, *J* = 3.1, 9.3 Hz, 0.3H), 4.10 (s, 1H), 3.91 (m, 1H), 3.87 (m, 0.3H), 3.85–3.75 (m, 2.6H), 2.38–2.30 (m, 6.5H), 1.80–1.56 (m, 5.2H), 0.88 (brs, 12H), 0.06 (s, 2H), 0.05 (s, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 222.8, 220.4, 70.4, 70.2, 62.6, 60.5, 54.5, 53.9, 39.1, 38.7, 37.0, 36.6, 26.4, 25.9, 25.8, 23.5, 20.7, 20.5, 18.2, –5.5, –5.6. HRMS-Cl (*m/z*): [M – OH]<sup>+</sup> calcd for C<sub>14</sub>H<sub>27</sub>O<sub>2</sub>Si 255.1780; found 255.1785.

**2,3,6,7-Tetrahydrocyclopenta[*b*]pyran-4(5H)-one (16)**. To a solution of DMSO (425 μL, 468 mg, 6 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was added (CF<sub>3</sub>CO)<sub>2</sub>O (406 μL, 609 mg, 2.9 mmol) dropwise at –78 °C under argon. The resulting mixture was stirred at that temperature for 45 min. Then a precooled solution of ketone **15** (272 mg, 1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was added. The reaction mixture was stirred at –78 °C for 30 min, then at –15 °C for 15 min and cooled back to –78 °C. Et<sub>3</sub>N (1.25 mL, 911 mg, 9 mmol) was added, and the mixture was stirred at –78 °C for 45 min. The reaction was quenched by addition of saturated aqueous NH<sub>4</sub>Cl solution and the mixture warmed to room temperature. The two phases were separated and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×) and then EtOAc (1×). The combined organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography using hexanes/EtOAc (20:1, then 15:1 with a few drops of acetic acid) as the eluent to give the corresponding diketone (221 mg, 82%) as a light orange oil. TLC: *R<sub>f</sub>* = 0.37 (hexanes/EtOAc = 10:1). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 12.7 (br s, 1H), 3.90 (t, *J* = 6.2 Hz, 0.66H), 3.89 (t, *J* = 6.5 Hz, 2H), 3.46 (t, *J* = 7.8 Hz, 0.33H), 2.86 (dt, *J* = 3.0, 6.2 Hz, 0.66H), 2.58 (t, *J* = 7.2 Hz, 2H), 2.45 (t, *J* = 6.5 Hz, 2H), 2.40 (t, *J* = 7.9 Hz, 2H), 2.31–2.19 (m, 0.66H), 2.10–1.97 (m, 0.66H), 1.95–1.82 (m, 2H), 0.86 (s, 9H), 0.86 (s, 3H), 0.04 (s, 1H), 0.03 (s, 1H), 0.03 (s, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 212.9, 206.1, 203.6, 175.4, 110.9, 62.4, 59.6, 58.5, 45.6, 38.7, 37.8, 37.0, 25.7, 25.6, 25.0, 20.6, 20.3, 18.1, –5.6. HRMS-Cl (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>26</sub>O<sub>3</sub>Si 271.1729; found 271.1733.

Diketone (54 mg, 0.2 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and cooled to 0 °C under argon. Trifluoroacetic acid (90  $\mu\text{L}$ , 134 mg, 1.2 mmol) was then added dropwise. The mixture was stirred at 0 °C for 30 min and then warmed to room temperature and stirred for 4 h. As completion was reached, solid  $\text{NaHCO}_3$  (~150 mg) was then added and the mixture diluted with EtOAc. After being stirred for 10 min, the suspension was filtered on a small Celite pad. The solvent was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using hexanes/EtOAc (4:1) as the eluent to furnish  $\alpha,\beta$ -unsaturated ketone **16** (26 mg, 94%) as a colorless oil. TLC:  $R_f = 0.23$  (hexanes/EtOAc = 3:1).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  4.49 (t,  $J = 6.9$  Hz, 2H), 2.59–2.45 (m, 6H), 1.89 (m, 2H).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  189.6, 178.5, 114.5, 69.5, 35.4, 32.6, 25.6, 19.0.

**Octahydrocyclopenta[*b*]pyran-4-ol [(±)-18]**. A solution of  $\alpha,\beta$ -unsaturated ketone **16** (109 mg, 0.79 mmol) in EtOAc (6 mL) was loaded with 10% Pd/C (50 mg, 0.047 mmol) and carefully placed under  $\text{H}_2$  (1 atm). The mixture was stirred at room temperature for 12 h. The suspension was then filtered over a Celite pad, the pad washed with EtOAc, and the resulting solution evaporated under reduced pressure. The essentially pure ketone (81 mg) was directly carried out to the next step without further purification. TLC:  $R_f = 0.37$  (hexanes/EtOAc = 3:1).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  4.22–4.15 (m, 2H), 3.69 (td,  $J = 2.8, 12.0$  Hz, 1H), 2.71 (ddd,  $J = 7.2, 12.3, 15.7$  Hz, 1H), 2.48 (dt,  $J = 4.0, 9.0$  Hz, 1H), 2.23 (ddt,  $J = 1.4, 2.8, 15.7$  Hz, 1H), 2.00–1.80 (m, 5H), 1.71–1.63 (m, 1H).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  210.2, 82.8, 65.9, 55.1, 38.5, 33.3, 28.4, 22.8.

The ketone was diluted in  $\text{CH}_2\text{Cl}_2$  (5 mL) under argon and cooled to –78 °C. L-Selectride (1 M solution in THF, 0.80 mL, 0.8 mmol) was added dropwise, and the resulting mixture was stirred at this temperature for 2 h. Hydrogen peroxide (30% aqueous solution, 3 mL) and 3 N NaOH aqueous solution were added, and the mixture was warmed to 23 °C and stirred for 5 h. The phases were separated and the aqueous phase extracted with  $\text{CH}_2\text{Cl}_2$  (4 $\times$ ). The combined organic phase was washed with brine, dried ( $\text{Mg}_2\text{SO}_4$ ), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using hexanes/EtOAc (4:1, then 1.5:1) as the eluent to yield *cis*-bicyclic alcohol (±)-**18** (77 mg, 68% two steps) as a colorless oil. TLC:  $R_f = 0.13$  (hexanes/EtOAc = 2:1).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  4.11 (dt,  $J = 5.6, 11.1$  Hz, 1H), 3.91 (ddd,  $J = 2.0, 4.5, 11.7$  Hz, 1H), 3.84–3.81 (m, 1H), 3.33 (dt,  $J = 2.3, 11.9$  Hz, 1H), 2.17–2.08 (m, 1H), 1.92–1.81 (m, 1H), 1.79–1.55 (m, 7H).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  80.5, 68.3, 65.4, 47.0, 32.6, 29.7, 21.6, 21.3.

**(4*S*,4*aS*,7*aS*)-Octahydrocyclopenta[*b*]pyran-4-ol [(–)-18]**. Racemic alcohol (±)-**18** (68 mg, 0.48 mmol) was dissolved in THF (5 mL), and vinyl acetate (225  $\mu\text{L}$ , 2.4 mmol) was added. Amano lipase PS-30 (30 mg) was added, and the resulting suspension was stirred at 15–20 °C. The mixture was left stirring for > 48 h until around 50% conversion was reached (as seen by NMR). The resulting suspension was diluted with  $\text{Et}_2\text{O}$  and filtered on Celite, and the filter cake was rinsed with  $\text{Et}_2\text{O}$ . After evaporation of the remaining solvent, the residue was purified by column chromatography using hexanes/EtOAc (5:1, 3:1, then 1.5:1) to yield the desired enantio enriched pyranol (–)-**18** (25 mg, 37%).  $[\alpha]_D^{20} -47.5$  ( $c$  1.32,  $\text{CHCl}_3$ ). An enantiopurity of 94.1% ee for the alcohol was measured by chiral HPLC analysis of the corresponding activated carbonate **31d**: column ChiralPak IA, 0.7 mL/min, hexanes/IPA (98:2 to 85:15, from 0 to 45 min),  $\lambda = 210$  nm,  $T = 30$  °C,  $t_R$  minor = 22.4 min,  $t_R$  major = 23.3 min.

(±)-**endo,cis-Bicyclo[4.3.0]nonan-2-ol [(±)-19]**. Enone **17**<sup>33</sup> (106 mg, 0.77 mol) was dissolved in THF (10 mL), and the flask was purged with argon. Pd/C 10% (60 mg, 0.06 mmol) was added to the solution, and the resulting suspension was stirred under hydrogen (1 atm). TLC monitoring first shows isomerization of the enone, through migration of the olefin to the internal position,

followed by slow formation of the reduced *cis*-product. After 12 h, the solution was filtered on a pad of Celite and the solvent removed in vacuo. The residue was purified by flash column chromatography on silica gel using hexanes/EtOAc (30:1 to 10:1) to give the reduced ketone (98 mg, 92%). TLC:  $R_f = 0.65$  (hexanes/EtOAc = 5:1).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  2.62–2.54 (m, 1H), 2.48–2.38 (m, 1H), 2.38–2.23 (m, 2H), 2.08–1.98 (m, 1H), 1.94–1.30 (m, 9H).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  214.6, 53.1, 42.9, 39.6, 31.0, 27.2, 26.6, 23.8, 23.0. A solution of the ketone (135 mg, 0.98 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was cooled to –78 °C under argon. L-Selectride (1 M solution THF, 1.2 mL) was added dropwise to the solution, and the reaction mixture was stirred at –78 °C for 1 h. Hydrogen peroxide solution (30% solution, 1.5 mL) and then NaOH (3 M solution, 1.5 mL) were added, and the mixture was warmed to 23 °C and stirred for 1 h. After dilution with water (2 mL) and then addition of  $\text{Na}_2\text{SO}_3$  saturated aqueous solution (3 mL), the aqueous phase was successively extracted with  $\text{CH}_2\text{Cl}_2$  (4 $\times$ ). The combined organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and evaporated in vacuo. The residue was purified by column chromatography on silica gel using hexanes/EtOAc (6:1) to yield racemic alcohol (±)-**19** (92 mg, 66%) as a colorless oil. TLC:  $R_f = 0.25$  (hexanes/EtOAc = 5:1).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.96 (m, 1H), 2.26–2.17 (m, 1H), 1.93 (m, 1H), 1.79–1.53 (m, 7H), 1.47–1.15 (5 H), 0.96 (dq,  $J = 3.3, 13.0$  Hz, 1H).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  71.6, 46.4, 40.1, 31.5, 29.5, 27.0, 23.9, 21.4, 21.2. HRMS-EI ( $m/z$ ):  $[\text{M} - \text{OH}]^-$  calcd for  $\text{C}_9\text{H}_{15}$  122.1096, found 122.1097.

**(1*R*,2*S*,6*R*)-Bicyclo[4.3.0]nonan-2-ol [(–)-19]**. Racemic **19** (86 mg, 0.62 mmol) was dissolved in THF (5 mL), and vinyl acetate (0.5 mL) was added. Amano lipase PS-30 (60 mg) was added, and the resulting suspension was stirred at 23 °C until 50% conversion was reached (NMR) in ~6 h. The resulting suspension was diluted with  $\text{Et}_2\text{O}$  and filtered on Celite, and the filter cake was rinsed with  $\text{Et}_2\text{O}$ . After evaporation of the remaining solvent, the residue was purified by column chromatography using hexanes/EtOAc (8:1, 6:1, then 4:1) to yield acetate **21** and the desired enantioenriched (–)-indanol (–)-**19** (38.5 mg, 45% yield).  $[\alpha]_D^{20} -28.3^\circ$  ( $c$  1.02,  $\text{CHCl}_3$ ), ( $[\alpha]_D^{20}$  lit.  $-27.2^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ )).<sup>38</sup> The enantiomeric excess of the 2,4-dinitrobenzoate derivative was determined to be 89.9% ee by chiral HPLC, column ChiralPak IA, hexane/isopropanol (100/0 to 90/10, 15 min; 90/10 to 80/20, 15 min), 1 mL/min,  $t_R$  minor = 16.58 min,  $t_R$  major = 19.5 min.

**3-[(4-Iodotetrahydrofuran-3-yl)oxy]propan-1-ol (23)**. To a solution of freshly distilled 2,5-dihydrofuran (700 mg, 0.740 mL, 10 mmol), in a mixture of dry 1,3-propanediol/dimethoxyethane (1:1, 5 mL) at 0 °C under argon was successively added  $\text{NH}_4\text{OAc}$  (77 mg, 1 mmol), followed by *N*-iodosuccinimide (11 mmol, 2.47 g). The mixture was warmed to 23 °C and stirred for 12 h protected from light. The reaction was quenched by addition of saturated aqueous  $\text{Na}_2\text{SO}_3$  and then diluted with water. The mixture was extracted with  $\text{Et}_2\text{O}$ /EtOAc (1:1). The combined organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using hexanes/EtOAc (4:1, 3:1, then 2.5:1) to give iodo alcohol **23** (1.2 g, 45%) as a pale yellow oil. TLC:  $R_f = 0.3$  (hexanes/EtOAc = 1:1).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  4.33 (m, 1H), 4.29–4.19 (m, 3H), 4.04 (dd,  $J = 2.2, 9.8$  Hz, 1H), 3.79 (dd,  $J = 1.5, 9.8$  Hz, 1H), 3.76–3.69 (m, 3H), 3.60 (m, 1H), 1.81 (m, 2H).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  88.2, 76.1, 71.8, 67.9, 60.6, 32.3, 23.4.

**3-[(4-Iodotetrahydrofuran-3-yl)oxy]propanal (24)**. Oxalyl chloride (580 mg, 392  $\mu\text{L}$ , 4.6 mmol) was diluted in  $\text{CH}_2\text{Cl}_2$  (12 mL) under argon, and the solution was cooled to –78 °C. Dry DMSO (715 mg, 650  $\mu\text{L}$ , 9.15 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was added to the cold solution dropwise, and the mixture was stirred for 30 min. A solution of alcohol **23** (500 mg, 1.83 mmol) in  $\text{CH}_2\text{Cl}_2$  (4 mL) was then added slowly, and the mixture was kept stirring for an additional hour at –78 °C.  $\text{Et}_3\text{N}$  (1.3 g, 1.8 mL,



12.8 mmol) was then introduced. The white suspension was stirred at  $-78\text{ }^{\circ}\text{C}$  for 20 min and slowly warmed to room temperature. A 0.5 M phosphate buffer solution pH 5.5 (20 mL) was added. The two phases were separated, and the resulting aqueous phase was extracted with  $\text{Et}_2\text{O}$  ( $4\times$ ). The combined organic phase was dried ( $\text{MgSO}_4$ ), filtered, and evaporated. The residue was purified by flash column chromatography using hexanes/ $\text{EtOAc}$  (6:1 to 4:1) to yield the desired aldehyde **24** (433 mg, 86%) as a light yellow oil. TLC:  $R_f = 0.76$  (hexanes/ $\text{EtOAc} = 1:1$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.77 (t,  $J = 1.3$  Hz, 1H), 4.35 (m, 1H), 4.30–4.19 (m, 3H), 4.04 (dd,  $J = 2.3$ , 9.8 Hz, 1H), 3.92 (ddd,  $J = 5.3$ , 6.7, 9.5 Hz, 1H), 3.77 (dd,  $J = 1.7$ , 10.1 Hz, 1H), 3.75 (ddd,  $J = 5.2$ , 6.2, 9.5 Hz, 1H), 2.69 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  200.1, 88.3, 76.1, 71.8, 63.1, 43.7, 23.3.

**Hexahydro-2H-furo[3,4-*b*]pyran-4-ol [(±)-25]**. To a solution of aldehyde **24** (100 mg, 0.37 mmol) in DME (10 mL) was successively added indium (60 mg, 0.55 mmol),  $\text{CuI}$  (48 mg, 0.25 mmol), and a catalytic amount of iodine (10 mg, 0.037 mmol). After the suspension was stirred for 5 min, water (4 mL) was added and the mixture was stirred at room temperature for 4 h. The suspension was filtered on a Celite pad, washing the pad with THF. The solvent was reduced under vacuum and the resulting aqueous phase acidified with 1 M HCl and saturated with NaCl. The aqueous phase was extracted with  $\text{EtOAc}$ , and the combined organic phase was dried over  $\text{MgSO}_4$ . After filtration and evaporation, the crude was purified by flash column chromatography on silica gel using hexanes/ $\text{EtOAc}$  (1:1 to 1:5) to provide the bicyclic alcohol (±)-**25** (25 mg, 47%) as a mixture of diastereoisomers. TLC:  $R_f = 0.28$  ( $\text{EtOAc}$  100%). Pyridinium chlorochromate (74 mg, 0.346 mmol) was added to a suspension of flame-dried 4 Å molecular sieves in  $\text{CH}_2\text{Cl}_2$  (2 mL) at room temperature under argon. A solution of the above alcohol (25 mg, 0.173 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.5 mL) was transferred to the suspension at  $0\text{ }^{\circ}\text{C}$ , and the solution was stirred for 1 h at  $0\text{ }^{\circ}\text{C}$ . The reaction was quenched by addition of isopropanol, and the mixture was filtered on a silica pad, flushing with  $\text{Et}_2\text{O}$ . After evaporation of the solvent, the corresponding ketone thus obtained was used directly in the next step. TLC:  $R_f = 0.45$  (hexanes/ $\text{EtOAc} = 1:1$ ). The ketone was redissolved in  $\text{EtOH}$  (1.5 mL). The solution was cooled to  $-20\text{ }^{\circ}\text{C}$ , and  $\text{NaBH}_4$  (25 mg, 0.66 mmol) was added at once. After being stirred at this temperature for 30 min, the reaction was quenched by addition of saturated aqueous  $\text{NH}_4\text{Cl}$  solution (1.5 mL). The solution was extracted with  $\text{EtOAc}$  and the combined organic phase dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and evaporated. The corresponding racemic alcohol (±)-**25** was purified by flash column chromatography using hexanes/ $\text{EtOAc}$  (1:1 to 1:5) as the eluent. Colorless oil (12 mg, 50% two steps). TLC:  $R_f = 0.25$  (100%  $\text{EtOAc}$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  4.26 (m, 1H), 4.05 (t,  $J = 3.0$  Hz, 1H), 4.04–3.95 (m, 3H), 3.94–3.85 (m, 2H), 3.40 (dt,  $J = 2.5$ , 11.8 Hz, 1H), 2.60 (m, 1H), 1.94 (d,  $J = 4.0$  Hz, 1H), 1.80 (ddt,  $J = 4.6$ , 11.5, 12.5 Hz, 1H), 1.74 (m, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  78.3, 74.5, 67.1, 66.4, 65.0, 45.5, 30.0.

To a solution of racemic (±)-**25** (10 mg, 0.07 mmol) in dry THF (1 mL) under an argon atmosphere was added vinyl acetate (60 mg, 65  $\mu\text{L}$ , 0.7 mmol) followed by addition of immobilized Amano Lipase PS-30 (10 mg) on Celite-545. The mixture was stirred at  $15\text{--}20\text{ }^{\circ}\text{C}$  for 2 days until  $> 50\%$  conversion could be observed by NMR of aliquots. The resulting suspension was diluted in  $\text{Et}_2\text{O}$  and filtered on a small Celite pad. The solvents were evaporated and the residue was purified by flash chromatography using hexanes/ $\text{EtOAc}$  (1:1 to 1:5) as the eluent to give enantiomeric alcohol **25** (4.6 mg, 46%) as a colorless oil. An enantiopurity of  $> 99.5\%$  ee for the alcohol was measured by analysis of the corresponding activated carbonate **31f** on chiral HPLC (column ChiralPak IC, hexane/isopropanol 52:48, 1 mL/min,  $\lambda = 215\text{ nm}$ ,  $T = 24\text{ }^{\circ}\text{C}$ ,  $t_{\text{R}}$  minor = 14.4 min,  $t_{\text{R}}$  major = 15.5 min).

**(3a*R*,6a*R*)-Tetrahydrofuro[2,3-*b*]furan-3(2*H*)-one (28)**. Enantiomerically pure (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-ol (bis-THF) **27** (85 mg, 0.65 mmol) was diluted in dry  $\text{CH}_2\text{Cl}_2$  (6 mL) under argon. The solution was cooled to  $0\text{ }^{\circ}\text{C}$ , and anhydrous  $\text{Na}_2\text{HPO}_4$  (52 mg, 0.36 mol) was added. Dess–Martin periodinane (360 mg, 0.85 mmol) was added at once at  $0\text{ }^{\circ}\text{C}$  and the resulting suspension warmed to  $23\text{ }^{\circ}\text{C}$  and stirred for 3 h. The reaction was then quenched by successive addition of saturated aqueous  $\text{NaHCO}_3$  and saturated aqueous  $\text{Na}_2\text{SO}_3$  solutions (1.5 + 1.5 mL). The phases were separated, and the aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$  and then  $\text{EtOAc}$ . The combined organic phases were dried ( $\text{Na}_2\text{SO}_4$ ), filtered on a small pad of silica gel, and evaporated to dryness. The residue was purified by column chromatography on silica gel using hexanes/ $\text{EtOAc}$  (3:1) to furnish ketone **28** (73 mg, 87%) as a white crystalline solid. TLC:  $R_f = 0.57$  (hexanes/ $\text{EtOAc} = 1:1$ ). Spectral data corresponded to those previously reported in the literature.<sup>35</sup>

**(3a*S*,7a*R*)-Tetrahydro-2*H*-furo[2,3-*b*]pyran-5(3*H*)-one (29)**.  $\text{AlMe}_3$  (25% w/w hexanes, 250  $\mu\text{L}$ , 0.6 mmol) was diluted in dry  $\text{CH}_2\text{Cl}_2$  (5 mL) under argon, and the solution was cooled to  $-78\text{ }^{\circ}\text{C}$ . A solution of ketone **28** (64 mg, 0.5 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (5 mL) was slowly added dropwise. After 10 min,  $\text{TMSCHN}_2$  (2 M solution in  $\text{Et}_2\text{O}$ , 275  $\mu\text{L}$ , 0.55 mmol) was added. The mixture was stirred for 2 h while warming to  $-30\text{ }^{\circ}\text{C}$ . Saturated Rochelle's salt solution (5 mL) was added, and the mixture was stirred for 1 h. The phases were separated. The aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$ , and the combined organic phase was dried ( $\text{MgSO}_4$ ). The solution was filtered on a small silica gel pad, flushing with  $\text{Et}_2\text{O}$ , and the collected organic phase was evaporated. A crude mixture of the desired ketone along with  $\alpha$ -silylated derivatives and isomers was then obtained. The mixture was redissolved in THF (5 mL).  $\text{AcOH}$  (6 drops) and TBAF (0.5 mL, 0.5 mmol) were successively added. The resulting mixture was stirred at  $23\text{ }^{\circ}\text{C}$  for 3 h and evaporated to dryness. The residue was purified by flash column chromatography on silica gel using hexanes/ $\text{EtOAc}$  (5:1) as the eluent to give ketone **29** (45 mg, 63%). TLC:  $R_f = 0.35$  (hexanes/ $\text{EtOAc} = 2:1$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.49 (d,  $J = 6.8$  Hz, 1H), 4.11 (d,  $J = 18.2$  Hz, 1H), 4.10 (m, 1H), 3.92 (d,  $J = 18.2$  Hz, 1H), 3.74 (dt,  $J = 6.5$ , 8.9 Hz, 1H), 2.85 (m, 1H), 2.71 (d,  $J = 6.3$ , 15.6 Hz, 1H), 2.48 (d,  $J = 3.9$ , 15.6 Hz, 1H), 2.15 (m, 1H), 1.55 (ddt,  $J = 7.7$ , 8.9, 12.7 Hz, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  210.7, 100.9, 67.5, 67.1, 39.2, 36.2, 31.3.

**(3a*S*,5*R*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-5-ol (30)**. A solution of ketone **29** (25 mg, 0.173 mmol) dissolved in  $\text{CH}_2\text{Cl}_2$  (5 mL) was cooled to  $-78\text{ }^{\circ}\text{C}$  under argon. L-Selectride (1 M in THF, 200  $\mu\text{L}$ , 0.2 mmol) was added dropwise. The solution was stirred at this temperature for 3 h and quenched by addition of saturated aqueous  $\text{NH}_4\text{Cl}$  solution. The aqueous phase was extracted with  $\text{EtOAc}$ . The combined organic extract was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and evaporated. The crude was purified by column chromatography on silica gel using hexanes/ $\text{EtOAc}$  (2:1, 1:1, then 1:2) to yield alcohol **30** as a 5:1 mixture of diastereoisomers (18 mg, cis major). The stereoisomers were separated in the subsequent synthesis of the mixed activated carbonate **31g**. TLC:  $R_f = 0.25$  (hexanes/ $\text{EtOAc} = 1:2$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  5.08 (d,  $J = 3.8$  Hz, 0.2H), 5.05 (d,  $J = 3.3$  Hz, 1H), 4.16–4.11 (m, 1.2H), 3.95–3.84 (m, 1.6H), 3.81–3.70 (m, 2H), 3.63 (m, 1H), 3.27 (dd,  $J = 7.9$ , 11.2 Hz, 0.2H), 2.35–1.70 (m, 6H).

**(3a*S*,4*S*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (4-Nitrophenyl) Carbonate (31a)**. Furopyranol ligand (–)-**7** (9 mg, 0.063 mmol) was diluted in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) under argon. The solution was cooled to  $0\text{ }^{\circ}\text{C}$ , and dry pyridine (17  $\mu\text{L}$ ,  $\sim 0.21$  mmol) was added. 4-Nitrophenyl chloroformate (24 mg, 0.12 mmol) was added at once to the solution, upon which a white precipitate formed. The mixture was stirred for 2 h while warming to room temperature. Upon completion, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel using hexanes/ $\text{EtOAc}$  (6:1, then 3:1) as the eluent to give the corresponding

activated carbonate **31a** (18 mg, >99%). TLC:  $R_f$  = 0.25 (hexanes/EtOAc = 3:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  8.29 (d,  $J$  = 8.7 Hz, 2H), 7.39 (d,  $J$  = 8.7 Hz, 2H), 5.30–5.19 (m, 1H), 5.07 (d,  $J$  = 2.7 Hz, 1H), 4.28 (dt,  $J$  = 3 Hz, 1H), 4.04–3.95 (m, 2H), 3.47–3.37 (m, 1H), 2.80–2.68 (m, 1H), 2.30–2.10 (m, 1H), 2.05–1.90 (m, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  155.3, 151.7, 145.4, 125.3, 121.7, 101.1, 75.4, 68.5, 60.5, 43.2, 25.8, 22.5.

**(3aR,4R,7aS)-Hexahydro-2H-furo[2,3-*b*]pyran-4-yl (4-Nitrophenyl) Carbonate (31b)**. The title compound was obtained from (+)-**7** as described for (–)-**7** in 86% yield after purification by column chromatography on silica gel using hexanes/EtOAc (6:1, then 3:1). Spectral data were consistent with those recorded for **31a**.

**(3aR,4S,7aR)-Octahydrobenzofuran-4-yl (4-Nitrophenyl) Carbonate (31c)**. The title compound was obtained from (–)-**12** as described for (–)-**7** in 83% yield after purification by column chromatography on silica gel using hexanes/EtOAc (8:1 to 6:1). TLC:  $R_f$  = 0.7 (hexanes/EtOAc = 3:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.28 (d,  $J$  = 9.2 Hz, 2H), 7.39 (d,  $J$  = 9.2 Hz, 2H), 5.07 (m, 1H), 4.13–4.05 (m, 2H), 3.90 (q,  $J$  = 8.2 Hz, 1H), 2.72 (m, 1H), 2.10–2.00 (m, 2H), 1.90–1.68 (m, 4H), 1.55–1.45 (m, 1H), 1.34–1.23 (m, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  155.4, 151.9, 145.2, 125.2, 121.7, 77.7, 77.1, 66.5, 41.2, 27.0, 26.2, 25.4, 18.0.

**((4S,4aR,7aS)-Octahydrocyclopenta[*b*]pyran-4-yl) (4-Nitrophenyl) Carbonate (31d)**. The title compound was obtained from (–)-**18** as described for (–)-**7** in 85% yield after purification by column chromatography on silica gel using hexanes/ $\text{CH}_2\text{Cl}_2$ /THF (4:1:0 then 4:1:0.1) as the eluent. TLC:  $R_f$  = 0.31 (hexanes/EtOAc = 1:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.28 (d,  $J$  = 9.1 Hz, 2H), 7.38 (d,  $J$  = 9.1 Hz, 2H), 5.21 (m, 1H), 4.00 (ddd,  $J$  = 1.8, 4.7, 12.0 Hz, 1H), 3.93 (dt,  $J$  = 2.5, 2.7 Hz, 1H), 3.43 (dt,  $J$  = 2.1, 12.0 Hz, 1H), 2.36 (m, 1H), 2.04–1.82 (m, 4H), 1.82–1.62 (m, 4H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  155.5, 151.9, 145.3, 125.3, 121.8, 80.7, 77.3, 65.0, 43.7, 32.6, 26.3, 22.3, 21.7.

**(3aR,4S,7aR)-Octahydro-1H-inden-4-yl (4-Nitrophenyl) Carbonate (31e)**. The title compound was obtained from (–)-**19** as described for (–)-**7** in 90% yield after purification by column chromatography on silica gel using hexanes/EtOAc (20:1 to 10:1) as the eluent.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.27 (d,  $J$  = 9.1 Hz, 2H), 7.38 (d,  $J$  = 9.1 Hz, 2H), 5.05 (m, 1H), 2.41 (m, 1H), 2.05 (m, 1H), 1.98–1.24 (m, 11H), 1.05 (dq,  $J$  = 3.4, 12.7 Hz, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  155.7, 151.9, 145.2, 125.2, 121.8, 80.7, 42.8, 40.2, 31.3, 26.6, 25.7, 23.4, 22.4, 21.3.

**(4S,4aS,7aR)-Hexahydro-2H-furo[3,4-*b*]pyran-4-yl (4-Nitrophenyl) Carbonate (31f)**. The title was obtained from (–)-**25** as described for (–)-**7** in >99% yield following column chromatography purification on silica gel using hexanes/EtOAc (3:1, then 2:1) as the eluent.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.29 (d,  $J$  = 9.1 Hz, 2H), 7.38 (d,  $J$  = 9.1 Hz, 2H), 5.32 (m, 1H), 4.20–3.88 (m, 6H), 3.50 (m, 1H), 2.81 (m, 1H), 2.10–1.90 (m, 2H).

**[(3aS,5R,7aR)-Hexahydro-2H-furo[2,3-*b*]pyran-5-yl] (4-Nitrophenyl) Carbonate (31g)**. The title compound was obtained from **30** as described for (–)-**7** in 70% yield. Purification and separation from the 5-*epi* diastereoisomer were performed following flash column chromatography on silica gel using hexanes/EtOAc (3:1, 2:1, then 1:1) as the eluent. Amorphous solid (70% from a 5:1 mixture of diastereoisomers). TLC:  $R_f$  = 0.16 (hexanes/EtOAc = 2:1).  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ , 800 MHz)  $\delta$  7.64 (d,  $J$  = 9.0 Hz, 2H), 6.69 (d,  $J$  = 9.0 Hz, 2H), 4.76 (d,  $J$  = 3.6 Hz, 1H), 4.35 (m, 1H), 4.02 (dt,  $J$  = 3.8, 8.6 Hz, 1H), 3.94 (dt,  $J$  = 2.8, 13.0 Hz, 1H), 3.60 (q,  $J$  = 8.0 Hz, 1H), 3.12 (dd,  $J$  = 2.0, 13.0 Hz), 2.04 (m, 1H), 1.67 (dq,  $J$  = 3.1, 15.1 Hz, 1H), 1.50 (m, 1H), 1.46–1.38 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{C}_6\text{D}_6$ , 200 MHz)  $\delta$  154.9, 151.9, 145.2, 124.9, 121.2, 100.7, 72.0, 67.4, 63.8, 35.9, 27.9, 27.3.

**(3aS,4S,7aR)-Hexahydro-2H-furo[2,3-*b*]pyran-4-yl-(2S,3R)-4-(*N*-isobutyl-4-methoxyphenyl sulfonamido)-3-hydroxy-1-phenylbutan-2-yl Carbamate (35a)**. Sulfonamide isostere **32** (42 mg, 0.08 mmol) was dissolved in a 30% TFA solution in  $\text{CH}_2\text{Cl}_2$  (3 mL), the solution was stirred at 23 °C for 2 h after which the

solvent was evaporated under reduced pressure. The corresponding Boc-deprotected intermediate (0.08 mmol) was then diluted in dry acetonitrile (0.8 mL) at 0 °C under argon and  $\text{Et}_3\text{N}$  (0.3 mL, 0.2 mmol) was added. A solution of activated carbonate **31a** (18.6 mg, 0.06 mmol) in acetonitrile or THF (0.5 mL) was then added to the mixture. The reaction was stirred at 23 °C until completion was reached (2–3 days). The solution was then evaporated *in vacuo* and the resulting residue purified by flash chromatography on silica gel using hexanes/EtOAc (2:1 then 1:1) as the eluent to afford the inhibitor **35a** as an amorphous solid (19.8 mg, 55%). TLC  $R_f$  = 0.35 (hexanes/EtOAc = 1:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.71 (d,  $J$  = 8.9 Hz, 2H), 7.33–7.17 (m, 5H), 6.97 (d,  $J$  = 8.9 Hz, 2H), 5.05–4.90 (m, 1H), 4.93 (d,  $J$  = 3.6 Hz, 1H), 4.84 (d,  $J$  = 8.4 Hz, 1H), 4.15 (dt,  $J$  = 2.4, 9.0 Hz, 1H), 3.87 (s, 3H), 3.98–3.76 (m, 4H), 3.31 (t,  $J$  = 11.7 Hz, 1H), 3.22–2.90 (m, 4H), 2.90–2.78 (m, 2H), 2.48–2.32 (m, 1H), 1.96–1.25 (m, 5H), 0.92 (d,  $J$  = 6.6 Hz, 3H), 0.87 (d,  $J$  = 6.6 Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  163.1, 155.5, 137.6, 129.8, 129.4, 128.4, 126.5, 114.3, 101.1, 72.9, 70.2, 68.5, 60.9, 58.9, 55.7, 54.9, 53.8, 43.5, 35.6, 27.3, 26.2, 22.3, 20.2, 19.9. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{29}\text{H}_{40}\text{N}_2\text{O}_8\text{NaS}$  599.2403, found 599.2406.

**(3aS,4S,7aR)-Hexahydro-2H-furo[2,3-*b*]pyran-4-yl (2S,3R)-4-(4-Amino-*N*-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl Carbamate (36)**. The title compound was obtained from **31a** and sulfonamide isostere **33** as described for inhibitor **35a**, in 64% yield following purification by flash chromatography using  $\text{CHCl}_3$ /2% MeOH as the eluent. TLC:  $R_f$  = 0.45 (hexanes/EtOAc = 1:3).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.55 (d,  $J$  = 8.7 Hz, 2H), 7.32–7.16 (m, 5H), 6.67 (d,  $J$  = 8.7 Hz, 2H), 4.97 (m, 1H), 4.93 (d,  $J$  = 3.4 Hz, 1H), 4.85 (d,  $J$  = 8.7 Hz, 1H), 4.20–4.11 (m, 3H), 3.92–3.80 (m, 5H), 3.31 (dt,  $J$  = 2.2, 11.9 Hz, 1H), 3.15 (dd,  $J$  = 8.1, 15.2 Hz, 1H), 3.05 (dd,  $J$  = 4.2, 14.1 Hz, 1H), 3.01–2.80 (m, 3H), 2.75 (dd,  $J$  = 6.6, 13.4 Hz, 1H), 2.40 (m, 1H), 1.97–1.60 (m, 4H), 1.46 (m, 1H), 0.92 (d,  $J$  = 6.6 Hz, 3H), 0.87 (d,  $J$  = 6.6 Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  155.5, 150.7, 137.7, 129.5, 129.5, 128.4, 126.5, 126.2, 114.1, 101.1, 72.8, 70.1, 68.5, 60.8, 58.9, 54.8, 53.8, 43.4, 35.5, 27.3, 26.2, 22.2, 20.2, 19.9. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{28}\text{H}_{39}\text{N}_3\text{O}_7\text{NaS}$  584.2406; found 584.2402.

**(3aS,4S,7aR)-Hexahydro-2H-furo[2,3-*b*]pyran-4-yl (2S,3R)-3-Hydroxy-4-(4-(hydroxymethyl)-*N*-isobutylphenylsulfonamido)-1-phenylbutan-2-yl Carbamate (37)**. The title compound was obtained from **31a** and sulfonamide isostere **34** as described for inhibitor **35a** in 72% yield following purification by flash chromatography on silica gel using  $\text{CHCl}_3$ /2% MeOH as the eluent. Amorphous solid. TLC:  $R_f$  = 0.23 (hexanes/EtOAc = 1:2).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.76 (d,  $J$  = 8.1 Hz, 2H), 7.52 (d,  $J$  = 8.1 Hz, 2H), 7.32–7.17 (m, 5H), 4.96 (m, 1H), 4.93 (d,  $J$  = 3.2 Hz, 1H), 4.85 (d,  $J$  = 8.5 Hz, 1H), 4.80 (s, 2H), 4.15 (t,  $J$  = 8.5 Hz, 1H), 3.92–3.80 (m, 4H), 3.70 (s, 1H), 3.31 (t,  $J$  = 11.6 Hz, 1H), 3.16 (dd,  $J$  = 8.0, 15.0 Hz, 1H), 3.10–2.95 (m, 3H), 2.88–2.76 (m, 2H), 2.41 (m, 1H), 2.04 (m, 1H), 1.95–1.78 (m, 2H), 1.76–1.56 (m, 2H), 1.47 (m, 1H), 0.93 (d,  $J$  = 6.6 Hz, 3H), 0.88 (d,  $J$  = 6.6 Hz, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  155.6, 146.2, 137.6, 137.1, 129.4, 128.5, 127.6, 127.1, 126.5, 101.1, 72.8, 70.2, 68.4, 64.2, 60.8, 58.8, 54.9, 53.7, 43.4, 35.5, 27.3, 26.2, 22.2, 20.1, 19.9. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{29}\text{H}_{40}\text{N}_2\text{O}_8\text{NaS}$  599.2403, found 599.2414.

**(3aR,4R,7aS)-Hexahydro-2H-furo[2,3-*b*]pyran-4-yl ((2S,3R)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (35b)**. The title compound was obtained from **31b** and sulfonamide isostere **32** in 65% yield as described for inhibitor **35a**, following purification by column chromatography on silica gel using hexanes/EtOAc (3:1, then 1.5:1) as the eluent. White amorphous solid. TLC:  $R_f$  = 0.44 (hexanes/EtOAc = 1:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.70 (d,  $J$  = 8.9 Hz, 2H), 7.31–7.26 (m, 2H), 7.25–7.20 (m, 3H), 6.98 (d,  $J$  = 8.9 Hz, 2H), 5.00 (m, 1H), 4.97 (d,  $J$  = 2.7 Hz, 1H), 4.88 (d,  $J$  = 8.0 Hz, 1H), 4.17 (t,  $J$  = 7.7 Hz, 1H), 3.99–3.72 (m, 6H), 3.87 (s, 3H), 3.31 (dt,  $J$  = 1.9, 12.0 Hz, 1H), 3.13 (dd,  $J$  = 8.4, 15.0 Hz, 1H), 3.08–2.84 (m, 4H), 2.79 (dd,  $J$  = 6.7, 13.4 Hz, 1H), 2.53 (m, 1H), 2.00 (m, 1H), 1.83 (m, 1H), 1.73



(m, 1H), 1.68–1.54 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  163.1, 155.7, 137.7, 129.8, 129.5, 128.5, 126.5, 114.3, 101.2, 72.6, 70.2, 68.4, 60.8, 58.7, 55.6, 55.1, 53.7, 43.6, 35.3, 27.3, 26.2, 22.5, 20.1, 19.9. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{29}\text{H}_{40}\text{N}_2\text{O}_8\text{NaS}$  599.2403, found 599.2407.

**(3a*R*,4*S*,7a*R*)-Octahydrobenzofuran-4-yl ((2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl Carbamate (35c).** The title compound was obtained from **31c** and sulfonamide isostere **32** in 75% yield as described for inhibitor **35a**, following purification by column chromatography on silica gel using hexanes/EtOAc (3:1, then 2.5:1) as the eluent. TLC:  $R_f = 0.39$  (hexanes/EtOAc = 1:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.72 (d,  $J = 8.9$  Hz, 2H), 7.311–7.16 (m, 5H), 6.98 (d,  $J = 8.9$  Hz, 2H), 4.83 (m, 2H), 3.95–3.75 (m, 5H), 3.87 (s, 3H), 3.68 (q,  $J = 8.1$  Hz, 1H), 3.14 (dd,  $J = 8.4, 15.2$  Hz, 1H), 3.08 (dd,  $J = 4.1, 14.1$  Hz, 1H), 3.05–2.99 (m, 1H), 2.96 (dd,  $J = 8.4, 13.4$  Hz, 1H), 2.87–2.75 (m, 2H), 2.35 (m, 1H), 1.83 (m, 1H), 1.70–1.40 (m, 7H), 1.20 (m, 1H), 0.92 (d,  $J = 6.6$  Hz, 3H), 0.87 (d,  $J = 6.6$  Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  163.0, 156.1, 137.7, 129.7, 129.5, 129.4, 128.4, 126.4, 114.3, 73.0, 71.8, 66.6, 58.8, 55.6, 54.7, 53.7, 41.2, 35.6, 27.3, 27.2, 27.0, 25.7, 20.1, 19.9, 17.7. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{30}\text{H}_{42}\text{N}_2\text{O}_7\text{NaS}$  597.2610, found 597.2621.

**(4*S*,4a*R*,7a*S*)-Octahydrocyclopenta[*b*]pyran-4-yl ((2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (35d).** The title compound was obtained from **31d** and sulfonamide isostere **32** in 81% yield as described for inhibitor **35a**, following purification by column chromatography on silica gel using hexanes/EtOAc (3:1, then 2.5:1) as the eluent. TLC:  $R_f = 0.58$  (hexanes/EtOAc = 1:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.70 (d,  $J = 8.9$  Hz, 2H), 7.30–7.17 (m, 5H), 6.96 (d,  $J = 8.9$  Hz, 2H), 4.94 (m, 1H), 4.81 (d,  $J = 8.1$  Hz, 1H), 3.86 (s, 3H), 3.90–3.76 (m, 4H), 3.33 (t,  $J = 11.9$  Hz, 1H), 3.13 (dd, AB,  $J = 8.3, 15.0$  Hz, 1H), 3.08–2.91 (m, 3H), 2.85 (m, 1H), 2.79 (dd,  $J = 6.8, 13.5$  Hz, 1H), 2.04 (m, 1H), 1.81 (m, 2H), 1.76–1.64 (m, 3H), 1.64–1.49 (m, 3H), 0.90 (d,  $J = 6.6$  Hz, 3H), 0.86 (d,  $J = 6.6$  Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  163.0, 156.0, 137.7, 129.8, 129.4, 128.4, 126.4, 114.3, 80.5, 72.7, 71.7, 65.2, 58.7, 55.6, 54.8, 53.7, 44.1, 35.6, 32.5, 27.2, 26.6, 22.0, 21.6, 20.1, 19.8. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{30}\text{H}_{42}\text{N}_2\text{O}_7\text{S}$  597.2610, found 597.2612.

**(3a*R*,4*S*,7a*R*)-Octahydro-1*H*-inden-4-yl-(2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl Carbamate (35e).** The title compound was obtained from **31e** and sulfonamide isostere **32** as described for inhibitor **35a**. Following preliminary purification by flash chromatography using hexanes/ $\text{CH}_2\text{Cl}_2$ /THF (8:1:1) as the eluent, the inhibitor was obtained as a mixture of unseparable isomeric compounds. Compound **35e** was derivatized into the corresponding *N,O*-isopropylidene compound by treatment of **35e** (20 mg) with 2,2-dimethoxypropane (0.1 mL) and a catalytic amount of *p*TSA (1.5 mg) in dry  $\text{CH}_2\text{Cl}_2$  (1 mL) for 8 h at 23 °C. After neutralization with  $\text{Et}_3\text{N}$ , the organic phase was evaporated to dryness. Following a quick silica gel column (hexanes/EtOAc = 8:1), the resulting inhibitor was purified by HPLC: preparative HPLC column Sunfire Prep C18 OBD, 30 mm  $\times$  100 mm, eluent MeOH/ $\text{H}_2\text{O}$  85:15 (30 min) and then 90:10 (15 min), flow rate 15 mL  $\cdot$  min $^{-1}$ ,  $t_R = 42$  min. The isopropylidene derivative was then obtained as a colorless oil (24 mg). The product was then taken into MeOH (2 mL). *p*TSA  $\cdot$   $\text{H}_2\text{O}$  (36  $\mu\text{mol}$ , 1.5 mg) was added, and the resulting solution was refluxed for 6 h. After neutralization with a few drops of  $\text{Et}_3\text{N}$ , the solution was evaporated and the residue purified by column chromatography on silica gel using hexanes/ $\text{CH}_2\text{Cl}_2$ /THF (8:1:1) to give inhibitor **35e** (15 mg, 43% from **31e**). TLC:  $R_f = 0.35$  (hexanes//EtOAc = 5:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.71 (d,  $J = 8.9$  Hz, 2H), 7.32–7.18 (m, 5H), 6.97 (d,  $J = 8.9$  Hz, 2H), 4.79 (m, 1H), 4.70 (d,  $J = 8.1$  Hz, 1H), 3.90 (m, 1H), 3.87 (s, 3H), 3.81 (m, 1H), 3.18–3.02 (m, 3H), 2.98–2.82 (m, 2H), 2.78 (dd,  $J = 6.6, 13.2$  Hz, 1H), 2.10 (m, 1H), 1.90 (m, 1H), 1.82 (m, 1H), 1.74–1.19 (m, 11H),

0.95 (m, 1H), 0.90 (d,  $J = 6.6$  Hz, 3H), 0.86 (d,  $J = 6.6$  Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  163.0, 156.4, 137.7, 129.9, 129.5, 129.4, 128.5, 126.4, 114.3, 74.9, 72.8, 58.8, 55.6, 54.8, 53.8, 43.1, 39.9, 35.7, 31.3, 27.2, 26.9, 26.1, 23.5, 22.2, 21.3, 20.1, 19.9. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{31}\text{H}_{44}\text{N}_2\text{O}_6\text{NaS}$  595.2818, found 595.2816.

**(4*S*,4a*S*,7a*R*)-Hexahydro-2*H*-furo[3,4-*b*]pyran-4-yl ((2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (35f).** The title compound was obtained from **31f** and sulfonamide isostere **32** in 75% yield as described for inhibitor **35a**, following purification by column chromatography using hexanes/EtOAc (3:1, then 2.5:1) as the eluent. TLC:  $R_f = 0.24$  (hexanes/EtOAc = 1:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 800 MHz)  $\delta$  7.70 (d,  $J = 8.8$  Hz, 2H), 7.30 (m, 2H), 7.24–7.20 (m, 3H), 6.97 (d,  $J = 8.8$  Hz, 2H), 5.05 (m, 1H), 4.83 (d,  $J = 8.5$  Hz, 1H), 4.03 (t,  $J = 3.2$  Hz, 1H), 3.96 (m, 1H), 3.87 (s, 3H), 3.87 (s, 3H), 3.88–3.81 (m, 5H), 3.62 (t,  $J = 8.3$  Hz, 1H), 3.39 (t,  $J = 11.5$  Hz, 1H), 3.14 (dd,  $J = 8.4, 15.0$  Hz, 1H), 3.02 (dd,  $J = 4.0, 14.1$  Hz, 1H), 2.99–2.94 (m, 2H), 2.84 (dd,  $J = 8.7, 14.1$  Hz, 1H), 2.77 (dd,  $J = 6.6, 13.4$  Hz, 1H), 2.51 (m, 1H), 1.81 (m, 1H), 1.78 (dq,  $J = 4.5, 12.4$  Hz, 1H), 1.71 (dd,  $J = 5.4, 12.4$  Hz, 1H), 0.91 (d,  $J = 6.6$  Hz, 3H), 0.87 (d,  $J = 6.6$  Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 200 MHz)  $\delta$  163.0, 155.5, 137.5, 129.6, 129.45, 129.38, 128.5, 126.6, 114.3, 78.4, 74.4, 72.6, 70.0, 66.1, 64.9, 58.8, 55.6, 54.9, 53.7, 42.7, 35.4, 27.2, 26.9, 20.1, 19.8. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{29}\text{H}_{40}\text{N}_2\text{O}_8\text{S}$  599.2403, found 599.2397.

**(3a*S*,5*R*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-5-yl ((2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (35g).** The title compound was obtained from **31g** and sulfonamide isostere **32** in 86% yield as described for inhibitor **35a**, following purification by column chromatography on silica gel using hexanes/EtOAc (gradient 3:1 to 1.5:1) as the eluent. TLC:  $R_f = 0.33$  (hexanes/EtOAc = 1:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.72 (d,  $J = 8.9$  Hz, 2H), 7.32–7.26 (m, 2H), 7.25–7.17 (m, 3H), 6.98 (d,  $J = 8.9$  Hz, 2H), 4.98 (d,  $J = 3.5$  Hz, 1H), 4.89 (d,  $J = 8.7$  Hz, 1H), 4.54 (m, 1H), 4.11 (dt,  $J = 3.5, 8.3$  Hz, 1H), 3.87 (s, 3H), 3.90–3.77 (m, 4H), 3.74 (m, 1H), 3.56 (d,  $J = 12.7$  Hz, 1H), 3.12 (dd,  $J = 8.5, 15.1$  Hz, 1H), 3.09–2.91 (m, 3H), 2.84 (dd,  $J = 8.5, 14.1$  Hz, 1H), 2.79 (dd,  $J = 6.8, 13.4$  Hz, 1H), 2.08 (m, 1H), 2.04–1.93 (m, 2H), 1.90–1.76 (m, 3H), 0.91 (d,  $J = 6.6$  Hz, 3H), 0.87 (d,  $J = 6.6$  Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  163.4, 155.7, 137.6, 129.7, 129.5, 128.5, 126.5, 114.4, 101.0, 72.5, 68.0, 67.1, 65.4, 58.8, 55.6, 54.9, 53.8, 36.2, 35.8, 28.3, 27.8, 27.2, 20.1, 19.9. HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{29}\text{H}_{40}\text{N}_2\text{O}_8\text{NaS}$  599.2403, found 599.2397.

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**Supporting Information Available:** HPLC and HRMS data of inhibitors **35a–g**, **36**, and **37**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## Probing Multidrug-Resistance and Protein–Ligand Interactions with Oxatricyclic Designed Ligands in HIV-1 Protease Inhibitors

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The introduction of HIV-1 protease inhibitors (PIs) into highly active antiretroviral therapy (HAART) in 1996 had a critical impact in decreasing HIV-related morbidity and mortality.<sup>[1]</sup> Indeed, HAART regimens with PIs initially suppressed HIV-1 replication in patients to undetectable HIV-1 RNA levels in plasma.<sup>[2]</sup> However, one of the serious shortcomings of current treatments is the rapid emergence of drug-resistant HIV strains.<sup>[3]</sup> There is an urgent need for improved PIs for the treatment of the increasing number of treatment-experienced patients harboring multidrug-resistant HIV-1 strains.<sup>[4,5]</sup>

We recently reported a number of PIs that were developed by incorporating structure-based designed novel nonpeptide ligands/scaffolds that target the HIV-1 protease substrate binding site.<sup>[6–17]</sup> One of the PIs, darunavir (**1**, DRV, Figure 1), was first approved for HIV/AIDS patients harboring drug-resistant HIV who do not respond to other antiretroviral drugs.<sup>[11]</sup> Recently, DRV has received full approval for all HIV/AIDS patients, including children infected with HIV-1.<sup>[12]</sup> DRV has a stereochemically defined fused *bis*-tetrahydrofuran (*bis*-THF) moiety as the P2 ligand.<sup>[7,8]</sup> The X-ray crystal structures of the complexes of both **1**- and **2**-bound HIV-1 protease revealed extensive protein–ligand hydrogen bonding interactions involving the backbone of HIV-1 protease throughout the active site.<sup>[13,14]</sup> In particular, both oxygen atoms of the P2 *bis*-THF ligand are involved in hydrogen bonding with Asp29 and Asp30 backbone NH groups.<sup>[15]</sup> In addition, the bicyclic ligand appears to fill the hydrophobic pocket at the S2 subsite. The P2 *bis*-THF ligand is responsible for the superior drug-resistance properties of both DRV (**1**) and TMC-126 (**2**). In our preliminary investigation, we chose to incorporate *para*-methoxysulfonamide as the P2' ligand owing to its marked hydrogen bonding interaction in the S2' site. To combat drug resistance, our inhibitor design strategies focused on maximizing inhibitor interactions with

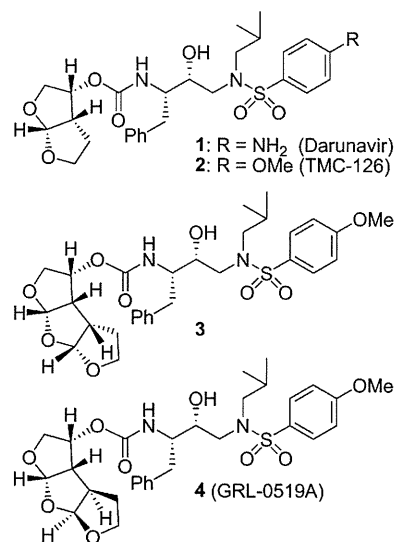


Figure 1. Structure of darunavir (DRV, **1**) and protease inhibitors **2–4**.

the HIV-1 protease active site, particularly by promoting extensive hydrogen bond interactions with the protein backbone atoms.<sup>[15]</sup> We have recently shown that the enhancement of 'backbone binding' leads to the design of PIs that maintain full potency against a panel of multidrug-resistant HIV-1 variants.<sup>[17]</sup> Based on our examination of the protein–ligand crystal structure of DRV-bound HIV-1 protease, we have further speculated that the incorporation of another tetrahydrofuran ring on the *bis*-THF ligand would provide additional ligand–binding site interactions. Particularly, it appears that the ligand oxygen atoms can effectively maintain backbone hydrogen bonding with Asp29 and Asp30, and can fill the hydrophobic pocket effectively. This in turn could further improve drug-resistance properties of the PIs. Such oxatricyclic ligands could have a variety of possible stereochemical motifs, including *syn-syn-syn* (SSS-type) and *syn-anti-syn* (SAS-type) isomers. Our X-ray crystal structure-based preliminary modeling suggested that the SAS-type ligand-based inhibitor **4** would make enhanced interactions in the S2 subsite relative to the SSS-derived inhibitor **3** (Figure 1).

The synthesis of the oxatricyclic *tris*-THF ligand with an SSS configuration was carried out as shown in Scheme 1. Optically active *bis*-THF derivatives **6** and **7** were conveniently prepared in multi-gram quantities from dihydrofuran **5** in a three-step sequence followed by lipase-catalyzed optical resolution as described previously.<sup>[18,19]</sup> Optically active alcohol **7** was treated with triphenylphosphine and iodine in the presence of imidazole to provide the corresponding iodide. Treatment of the re-

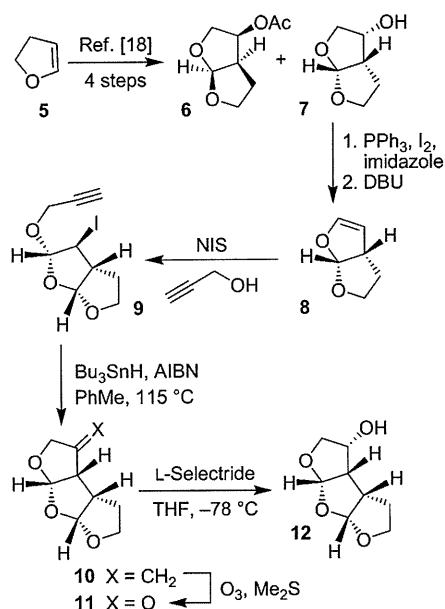
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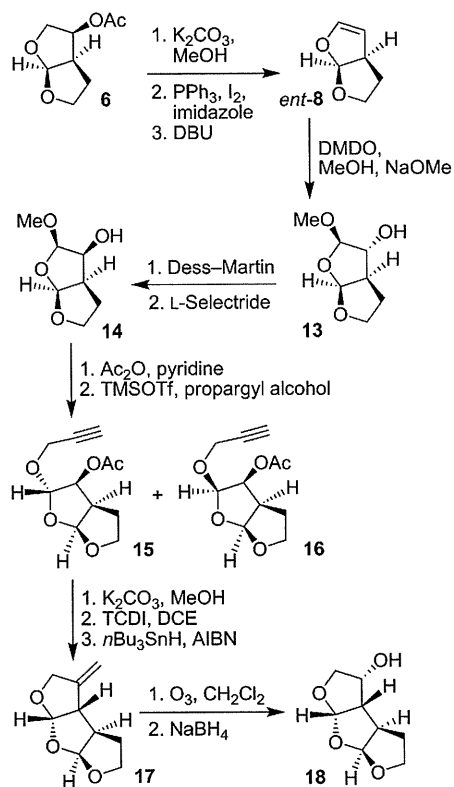
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Scheme 1. Synthesis of the *syn-syn-syn* tris-THF ligand **12**.

sulting iodide with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) furnished volatile bicyclic enol ether **8** with *N*-iodosuccinimide (NIS) and propargyl alcohol in dichloromethane at 0–23 °C afforded iodoether **9** in 58% yield over three steps.<sup>[18]</sup> Radical cyclization of **9** with tri(*n*-butyl)tin hydride in the presence of azobisisobutyronitrile (AIBN) in toluene at reflux provided tricyclic olefin **10**. The relative stereochemistry of the tricyclic core was established by extensive NMR experiments (COSY, NOESY). Ozonolysis of the olefin, followed by *L*-selectride reduction of the resulting ketone furnished *endo*-alcohol **12** as a single isomer (by <sup>1</sup>H NMR analysis).

The synthesis of *tris*-THF ligand with a *SAS* ring fused system is shown in Scheme 2. Optically active (3*S*,3*aR*,6*aS*)-*bis*-THF alcohol *ent*-**7** was readily obtained by saponification of acetate derivative **6**. It was converted into bicyclic enol ether *ent*-**8** as described above. This enol ether was exposed to acetone-free dimethyldioxirane (DMDO)<sup>[20]</sup> in dichloromethane at –78 °C to provide the corresponding epoxide. Treatment of the resulting epoxide with a catalytic amount of sodium methoxide (10%) in methanol provided epoxide-opened product **13** as a single diastereomer.<sup>[21]</sup> The depicted stereochemistry of **13** was fully supported by extensive NMR studies. Dess–Martin oxidation<sup>[22]</sup> of **13** followed by *L*-selectride reduction of the resulting ketone provided *endo*-alcohol **14** as a single isomer in 68% yield over two steps. To append the third oxacyclic ring, alcohol **14** was first acylated, and the resulting acetate was treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) and propargyl alcohol, which afforded acetals **15** and **16** as a 4:1 mixture of diastereomers. The isomers could not be separated at this stage; however, removal of the acetate provided the corresponding alcohols, which were readily separated by column chromatography. The major diastereomeric alcohol was converted into tricyclic olefin **17** in a two-step sequence involving: 1) reaction of alcohol with thiocarbonyldiimidazole (TCDI) in dichloroethane (DCE) to provide the corresponding



Scheme 2. Synthesis of the *syn-anti-syn* tris-THF alcohol **18**.

thiocarbamate, and 2) treatment of the resulting thiocarbamate with tri(*n*-butyl)tin hydride in the presence of AIBN in toluene at reflux to provide olefin **17** in 75% yield over two steps.<sup>[23]</sup> The stereochemistry of olefin **17** was established by <sup>1</sup>H NMR spectroscopy. Olefin **17** was converted into *endo*-alcohol **18** by ozonolysis of the olefin followed by sodium borohydride reduction of the resulting ketone to furnish **18** as a single isomer. Mosher ester analysis<sup>[24]</sup> revealed high optical purity (99% *ee*).

The synthesis of *tris*-THF-containing PIs is shown in Scheme 3. Optically active *tris*-THF ligand alcohols **12** and **18** were converted into the corresponding *para*-nitrophenyl carbonates **19** and **20** by treatment with *para*-nitrophenyl chloroformate and *N*-methylmorpholine (NMM). Treatment of amine **21**,<sup>[25]</sup> synthesized previously, with activated carbonate **19** or **20** in the presence of diisopropylethylamine (DIPEA) provided PIs **3** and **4** in 65% yield.

Inhibitor **3** shows potent inhibitory activity ( $K_i = 60 \mu\text{M}$ ). In comparison, inhibitor **4** (GRL-0519A, Figure 1) has a  $K_i$  value of 5.9  $\mu\text{M}$ , a 10-fold improvement over **3**. Both PIs **3** ( $\text{IC}_{50} = 3.5 \text{ nM}$ ) and **4** ( $\text{IC}_{50} = 1.8 \text{ nM}$ ) also showed excellent antiviral activity against the wild-type HIV-1<sub>LAV</sub>. PI **4** (GRL-0519A) was the most potent against a laboratory HIV-1 strain, HIV-1<sub>LAV</sub>, with an  $\text{EC}_{50}$  value of 1.8 nM and a favorable cytotoxicity profile ( $\text{CC}_{50} = 44.6 \mu\text{M}$ ) as examined by using MT-2 cells as target cells, producing a selectivity index ( $\text{CC}_{50}/\text{EC}_{50}$ ) of 24778.

We next examined GRL-0519A against a variety of primary HIV-1 strains, which were isolated from patients with AIDS who had failed a number of anti-HIV therapeutic regimens after re-