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Highly Potent HIV-1 Protease Inhibitors with Novel Tricyclic P2 Ligands: Design, Synthesis, and Protein−Ligand X-ray Studies

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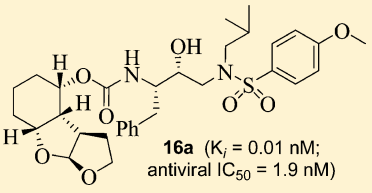
Supporting Information

ABSTRACT: The design, synthesis, and biological evaluation of a series of HIV-1 protease inhibitors incorporating stereochemically defined fused tricyclic P2 ligands are described. Various substituent effects were investigated to maximize the ligand-binding site interactions in the protease active site. Inhibitors **16a** and **16f** showed excellent enzyme inhibitory and antiviral activity, although the incorporation of sulfone functionality resulted in a decrease in potency. Both inhibitors **16a** and **16f** maintained activity against a panel of multidrug resistant HIV-1 variants. A high-resolution X-ray crystal structure of **16a**-bound HIV-1 protease revealed important molecular insights into the ligand-binding site interactions, which may account for the inhibitor's potent antiviral activity and excellent resistance profiles.



INTRODUCTION

HIV-1 protease inhibitors (PIs) are critical components of current antiretroviral therapies. However, the rapid emergence of drug-resistance severely compromises the clinical benefits of PIs.[1−3] In our continuing efforts to address issues of drug resistance, our inhibitor design strategy focuses on maximizing active-site interactions with the protease, particularly by promoting extensive hydrogen-bonding interactions with the backbone atoms throughout the active site.[4−6] Recently, our structure-based design targeting the protein backbone led to the discovery of exceedingly potent HIV-1 PI **1** (K_i = 5.9 pM, IC_{50} = 1.8 nM, Figure 1).[7,8] This inhibitor has shown a marked potency against a range of multidrug-resistant HIV-1 variants.[9] We determined that the syn-anti-syn-fused tricyclic ether (P2 ligand) in **1** is responsible for its enhanced broad-range potency compared to the related FDA approved inhibitor darunavir (DRV) (**2**).[4,10]

Our X-ray structural studies of **1**-bound HIV-1 protease revealed the formation of an extensive hydrogen-bonding network between the inhibitor and the active site.[7,8] Particularly, the P2 ligand is involved in strong hydrogen-bonding interactions with the backbone amides of conserved residues Asp 29 and Asp 30 in the S2 subsite. The tricyclic P2 ligand also appeared to fit nicely in the hydrophobic pocket formed by the surrounding side chains of the Ile47, Val32, Ile84, Leu76, and Ile50′ residues. This molecular insight has now led us to investigate a range of P2 ligands designed on the basis of the tricyclic platform in inhibitor **1**. In particular, we have been interested in investigating the syn-anti-syn tricyclic

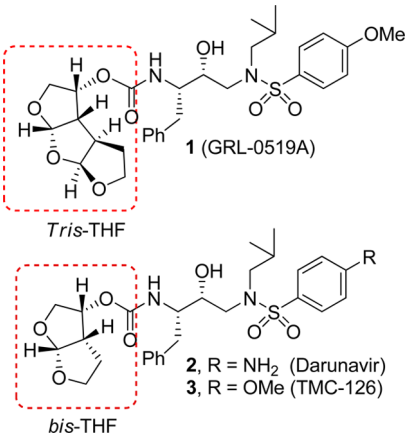


Figure 1. Structures of protease inhibitors **1**−**3**.

structural motif with functionalities that can interact with the conserved backbone and residues in the S2 subsite. We also planned to develop efficient methods for synthesizing these ligands rapidly using cycloaddition-based strategies. Herein, we report the design, synthesis, and biological evaluation of a series of novel HIV-1 PIs incorporating syn-anti-syn-fused tricyclic P2 ligands. Two of these inhibitors exhibited very potent antiviral activity against a panel of multidrug-resistant HIV-1 variants. A protein−ligand X-ray crystal structure of one of these inhibitors

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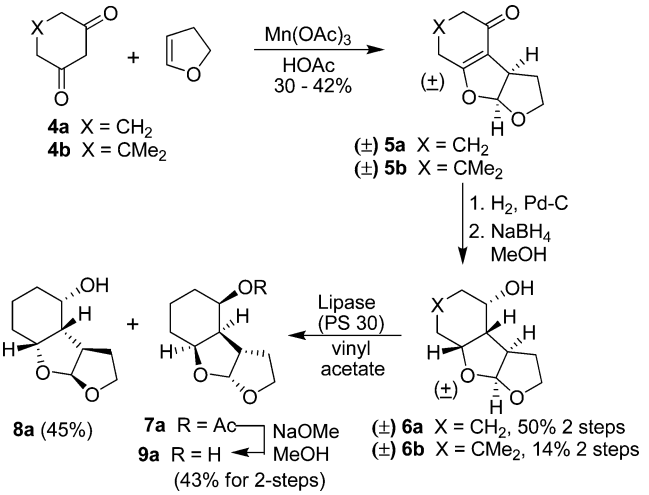
provided important molecular insights into the ligand-binding site interactions.

CHEMISTRY

For the rapid synthesis of tricyclic P2 ligands, we planned to explore the feasibility of $Mn(OAc)_3$-based annulation of readily available 1,3-diketone derivatives and cyclic enol ethers such as dihydrofuran and dihydropyran. Similar annulation reactions have been shown to provide good yields of bicyclic derivatives efficiently.[11] The synthesis of our substituted fused tricyclic P2 ligands is shown in Scheme 1. Reactions of 1,3-diketones **4a,b**

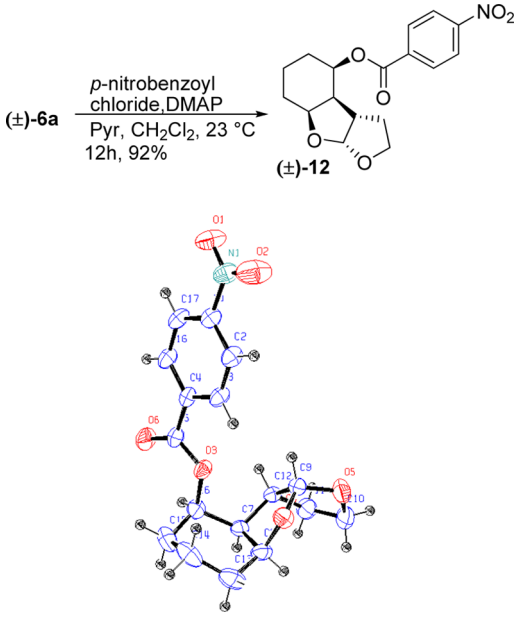
Scheme 1. Synthesis of Tricyclic Ligands



with dihydrofuran in the presence of $Mn(OAc)_3 \cdot 2H_2O$ in glacial acetic acid at 60 °C furnished the corresponding tricyclic derivatives **5a** (30% yield) and **5b** (42% yield). Enone **5a** was exposed to hydrogenation over 10% Pd−C in MeOH at 65 psi hydrogen pressure to give the corresponding ketone. The reduction of the resulting ketone with $NaBH_4$ in MeOH provided racemic endo alcohol **6a** in 50% yield in two steps. The syn-anti-syn relative ring stereochemistry of **6a** was supported by ^{1}H NMR NOESY experiments and was further confirmed by the X-ray structure of corresponding *p*-nitrobenzoate derivative **12** (Scheme 2).[12] The observed selectivity of the hydrogenation of **5a** presumably resulted from the directing effect by the terminal THF ring oxygen.[13] Further investigation is ongoing to determine the origin of the syn-anti-syn relative ring stereochemistry, and the details will be reported in due course. Hydrogenation of **5b** proceeded sluggishly to provide the corresponding ketone (17% yield). Subsequent $NaBH_4$ reduction (81% yield) afforded racemic alcohol **6b**.

Racemic alcohol **6a** was subjected to enzymatic resolution utilizing lipase PS-30 in vinyl acetate at 23 °C for 18 h.[14,15] The protocol provided optically active acetate derivative **7a** (45% yield) and alcohol **8a** (45% yield). Acetate **7a** was converted to the alcohol **9a** in 89% yield by transesterificaton using NaOMe in MeOH. Alcohol **8a** was converted to the corresponding Mosher ester, and ^{19}F NMR analysis revealed its optical purity to be 98% ee.[16] The absolute stereochemistry of alcohol **8a** was predicted on the basis of the Kazlauskas model as well as the optical resolution of structurally related bis-THF alcohols.[17] Ultimately, it was confirmed through X-ray analysis of the related oxygen-containing tricyclic derivative (Scheme 4). After

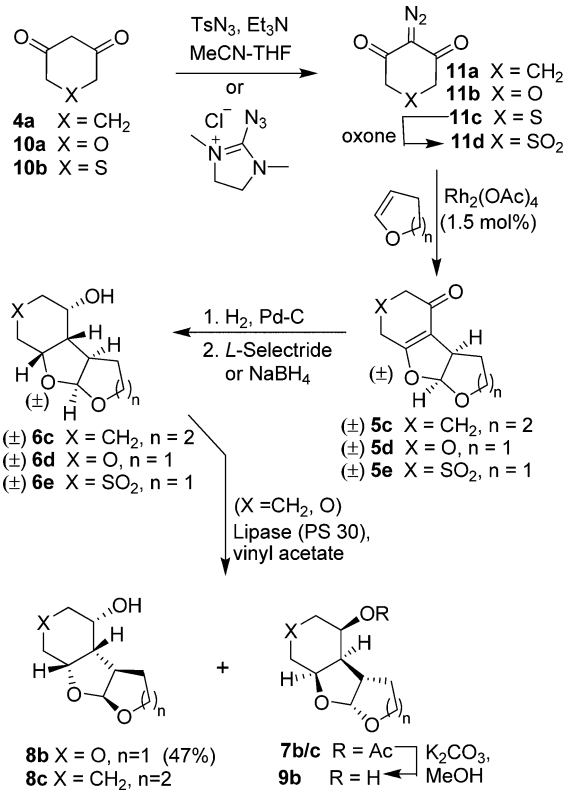
Scheme 2. Synthesis of Benzoate Ester 12 and Its Structure in an ORTEP Diagram



several unsuccessful attempts at resolving racemic alcohol **6b**, we decided to move forward with this ligand as a racemate.

We were also interested in evaluating the importance and effect of replacing the terminal furan in **8a** with a pyran ring. To this end, known diazo compound **11a**[18] was reacted with rhodium diacetate in 2,3-dihydro-4*H*-pyran to obtain intermediate **5c** in 77% yield, as shown in Scheme 3. In an effort to

Scheme 3. Synthesis of Heteroatom-Substituted Tricyclic P2 Ligands



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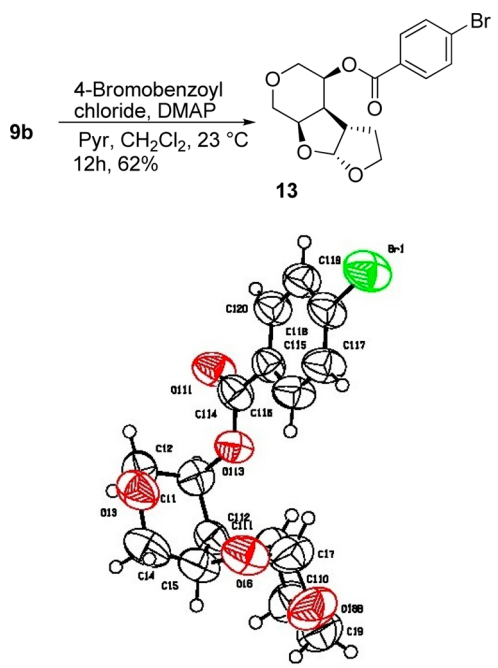
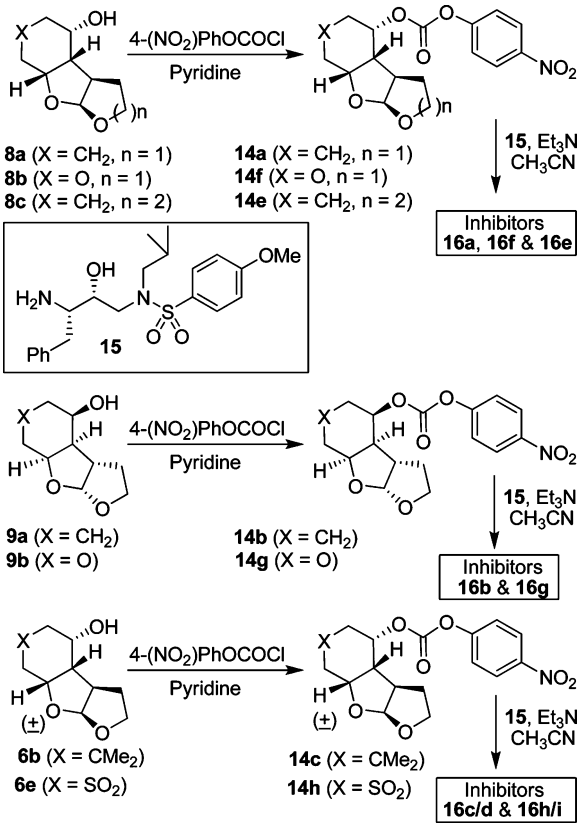
promote further polar interaction in the active site, we planned to incorporate a heteroatom within the cyclohexyl ring of the tricyclic ligand. Corresponding oxygen- and sulfur-containing 1,3-diketones **10a** and **10b** were synthesized on the basis of literature procedures.[19,20] However, the $Mn(OAc)_3$-based annulation of diketones **10a** and **10b** did not provide the desired enone. We then devised an alternate strategy. The synthesis of heteroatom-substituted tricyclic ligands is shown in Scheme 3. Diketone **10a** was converted to diazo derivative **11b** by treating the diketone with tosyl azide in the presence of Et_3N. This diazo transfer reaction also proceeded well for **11c** (68% yield) using procedures developed by Kitamura and co-workers.[21] Sulfide **11c** was conveniently oxidized to sulfone **11d** in 82% yield using oxone.[22,23] Diazo compounds **11b** and **11d** were subjected to rhodium-catalyzed carbenoid cycloaddition with dihydrofuran using $Rh(OAc)_2$ (1.5 mol %) to afford fused heterocyclic compounds **5d** and **5e** in 67% and 48% yields, respectively.[24,25] The catalytic hydrogenation of enones **5d** and **5e** using 10% Pd–C in MeOH at 1 atm furnished the corresponding syn-anti-syn ketone. The reduction of the resulting ketones with L-selectride yielded racemic alcohols **6d** and **6e** in 23% and 28% yields over 2 steps, respectively. Enzymatic resolution of racemic alcohol **6d** provided optically pure alcohol **8b** in 47% yield and acetate **7b** in 48% yield.[14,15] Saponification of **7b** provided alcohol **9b** in 71% yield. Similarly, racemic alcohol **6c** was converted to optically active alcohols **8c** and acetate **7c**. After several unsuccessful attempts at enzymatic resolution, alcohol **6e** (X = SO_2) was carried through as a racemic mixture. The syn-anti-syn relative stereochemistry of **9b** was supported by ^1H NMR NOESY experiments. Ultimately, our determination of the X-ray structure of *p*-bromobenzoate **13**[12] confirmed the syn-anti-syn relative stereochemistry, as shown in Scheme 4.

The preparation of various *para*-nitrophenyl carbonates **14a–c** and **14e–h** is shown in Scheme 5. Various ligand alcohols were reacted with *para*-nitrophenyl chloroformate and pyridine in CH_2Cl_2 to provide mixed carbonates **14a–c** and

Scheme 4. ORTEP Diagram of syn-anti-syn Compound 13



Scheme 5. Synthesis of Inhibitors 16a–i



14e–h in good to excellent yields (70–97% yields).[26] The syntheses of HIV-1 protease inhibitors **16a,b** and **16e–g** were carried out by treatment of optically active amine **15** in the presence of Et_3N with carbonates obtained from optically active alcohols **8a–c** and **9a,b**. These inhibitors were obtained in 50–82% yields. For the syntheses of inhibitors **16c,d,h,i**, the corresponding carbonates derived from racemic alcohols **6b** and **6e** were reacted with optically active amine **15** in the presence of Et_3N to provide the corresponding mixture of diastereomeric inhibitors. Separation of these inhibitors by HPLC using a reverse-phase analytical column provided pure inhibitors **16c,d,h,i.** Stereochemical assignment of the respective inhibitors with diastereomeric P2 ligands was made on the basis of the comparison of the HPLC retension time of the diastereomeric inhibitors as well as the comparison of the ^1H NMR data of inhibitors **16a,b** and **16e–g** synthesized using optically pure ligands.

RESULTS AND DISCUSSION

Inhibitors **16a–i** were initially evaluated in enzyme-inhibitory assays using the protocol reported by Toth and Marshall.[27] On the basis of the exhibited enzyme-inhibitory potency, selected inhibitors were further evaluated in antiviral assays. The results are shown in Table 1. Our depicted synthesis route allowed us to prepare both enantiomers of the tricyclic ligands. We have prepared and evaluated the effect of enantiomeric pure ligands with a syn-anti-syn ring stereochemistry. 4(*S*)-Cyclohexyl ligand-derived inhibitor **16a** exhibited very impressive enzyme and antiviral potency (K_i = 10 pM, antiviral IC_{50} = 1.9 nM) over inhibitor **16b** (K_i = 0.45 nM, antiviral IC_{50} = 240 nM), which has a 4(*R*)-configuration. This result is consistent with our previous finding with DRV and tricyclic P2 ligand in

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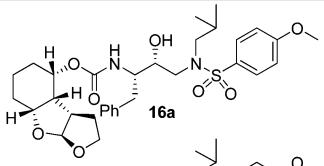
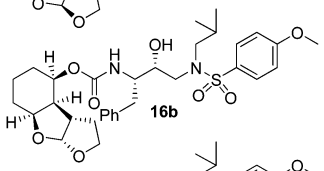
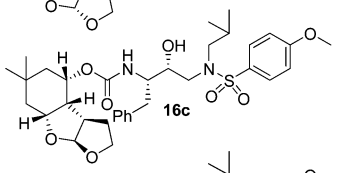
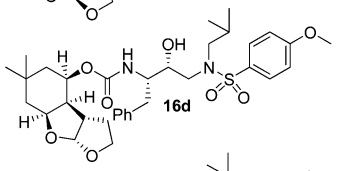
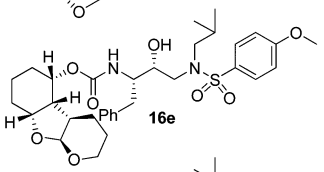
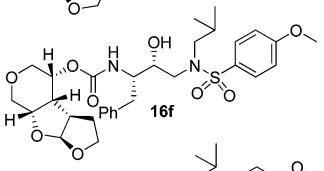
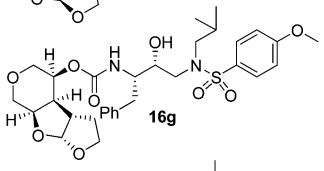
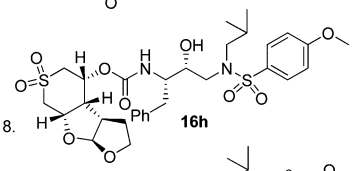
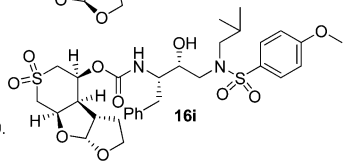
Table 1. Structures and Potency of HIV-1 Protease Inhibitors 16a−i

Entry	Inhibitor	K_i (nM)	IC_{50} (μM)[a,b]
1.	 **16a**	0.01	0.0019
2.	 **16b**	0.45	0.24
3.	 **16c**	8.8	nt
4.	 **16d**	139	nt
5.	 **16e**	0.41	0.048
6.	 **16f**	0.021	0.0045
7.	 **16g**	3.1	0.26
8.	 **16h**	0.6	>1
9.	 **16i**	10.5	>1

[a]The numbers are the mean values of at least two experiments. [b]Human T-lymphoid (MT-2) cells (2×10^3) were exposed to 100 $TCID_{50}$ of HIV-1LAI and were cultured in the presence of each PI. IC_{50} values were determined using the MTT assay. The IC_{50} values of amprenavir (APV), saquinavir (SQV), indinavir (IDV), and darunavir (DRV) were 30, 15, 30, and 3 nM, respectively. Nt, not tested.

inhibitor **1**. Incorporation of a gem-dimethyl group at the C2 position resulted in inhibitors **16c** and **16d** that showed a drastic loss of enzyme affinity, possibly because of steric repulsion between the dimethyl and side-chain residues within the S2 subsite of the HIV protease. We have also investigated the effects of the 6−5−6 fused ring system over the 6−5−5 ring system. As shown, replacement of the tetrahydrofuran ring in **16a** with a tetrahydropyran ring resulted in inhibitor **16e** (K_i = 0.41 nM, antiviral IC_{50} = 48 nM, entry 5), which showed a significant reduction in both enzyme inhibitory and antiviral activity over **16a**. We have explored the substitution of the C3-methylene group with an oxygen atom or with a sulfone functionality. Interestingly, pyran (oxygen)-substituted inhibitor **16f** (K_i = 21 pM, antiviral IC_{50} = 4.5 nM, entry 6) with a 4(*R*)-configuration displayed comparable enzyme inhibitory and antiviral potency to that of **16a**. Consistent with the cyclohexyl derivatives, the corresponding enantiomerically pure ligand in inhibitor **16g** showed a substantial reduction in potency. Further substitution of the C2 methylene with a polar sulfone functionality resulted in a drastic reduction in enzymatic activity and antiviral potency (inhibitors **16h**,**i**, entries 8 and 9).

Because of the potent enzyme inhibitory and antiviral proprieties of inhibitors **16a** and **16f**, we selected these inhibitors for further evaluation against a panel of multidrug-resistant (MDR) HIV-1 variants. The antiviral activities of these inhibitors were compared to clinically available PIs, DRV, and amprenavir (APV),[4,28] and the results are shown in Table 2. Both inhibitors **16a** and **16f** exhibited low-nanomolar EC_{50} values against the wild type.

HIV-$1_{ERS104pre}$ is a laboratory strain isolated from a drug-naïve patient.[28] Inhibitor **16a** had the most potent activity (EC_{50} = 3.6 nM), which was similar to that of DRV and nearly 10-fold better than that of APV. Interestingly, inhibitor **16f**, with its cyclohexane ring replaced with a 3-tetrahydropyran ring, showed a 2-fold reduction in antiviral potency compared to inhibitor **16a**.

Inhibitor **16a** was tested against a panel of multidrug-resistant HIV-1 strains, and the EC_{50} of **16a** remained in the low-nanomolar value range (8−16 nM), with fold-changes in its activity being similar to those observed for DRV.[4,28] In contrast, inhibitor **16f** displayed 4- and 6-fold reductions in antiviral activity against viral strains C and G compared to **16a**. Although inhibitors **16a** and **16f** both displayed a superior profile compared to another approved PI, APV, overall, inhibitor **16a** maintained impressive potency against all tested multidrug-resistant HIV-1 strains. It compared favorably with DRV, a leading PI for the treatment of multidrug-resistant HIV infection.[10]

To gain molecular insights into the ligand-binding site interactions responsible for the potent activity and excellent resistant profile of **16a**, we have determined the X-ray crystal structure of the HIV wild-type protease cocrystallized with **16a**, as described for DRV.[29] The structure was refined at a 1.29 Å resolution to an *R* factor of 0.14. The structure comprises the protease dimer and the inhibitor bound in two orientations related by a twofold rotation with 55/45% relative occupancies. The protease dimer is similar to that in the protease−DRV complex with an rmsd of 0.11 Å on all Cα atoms.[30] Inhibitor **16a**'s binding elements are similar to those of inhibitor **1**.[7,8] The inhibitor makes extensive interactions in the HIV-1 protease active site and most notably displays favorable polar interactions, including hydrogen bonds and weaker C−H···O and C−H···π interactions, as shown in Figure 2.

It is bound in the active-site cavity through a series of hydrogen-bond interactions and weaker CH···O interactions with the main-chain atoms of the HIV-1 protease. The inhibitor hydroxyl group interacts with all four carboxylate oxygen atoms of the catalytic Asp25 and Asp25′, with interatomic distances of 2.6−3.2 Å. A tetracoordinated water (not labeled in the figure) mediates hydrogen bonds with both NH atoms of the flap

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Table 2. Comparison of the Antiviral Activity of 16a, 16f, APV, and DRV against Multidrug-Resistant HIV-1 Variants

	$EC_{50} \pm SD$, (μM) (fold change)[a,b]			
virus	**16a**	**16f**	APV	DRV
HIV-1_{104pre}(wt)	0.0036 ± 0.0004	0.0083 ± 0.0021	0.028 ± 0.006	0.0037 ± 0.0007
HIV-$1_{MDR/C}$	0.008 ± 0.005 (2)	0.0329 ± 0.0030 (4)	0.325 ± 0.055 (12)	0.010 ± 0.002 (3)
HIV-$1_{MDR/G}$	0.012 ± 0.009 (3)	0.0795 ± 0.0018 (10)	0.426 ± 0.012 (16)	0.019 ± 0.005 (5)
HIV-$1_{MDR/TM}$	0.016 ± 0.001 (4)		0.448 ± 0.050 (16)	0.024 ± 0.008 (6)

[a]The amino acid substitutions identified in the protease-encoding region of HIV-$1_{ERS104pre}$ (wild type), HIV-$1_{MDR/C}$, HIV-$1_{MDR/G}$, and HIV-$1_{MDR/TM}$ compared to the consensus type B sequence cited from the Los Alamos database include L63P; L10I, I15V, K20R, L24I, M36I, M46L, I54V, I62V, L63P, K70Q, V82A, L89M; L10I, V11I, T12E, I15V, L19I, R41K, M46L, L63P, A71T, V82A, L90M; and L10I, K14R, R41K, M46L, I54V, L63P, A71V, V82A, L90M, I93L, respectively. HIV-$1_{ERS104pre}$ served as a source of wild-type HIV-1. [b]The EC_{50} values were determined using PHA-PBMCs 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 the EC_{50} values for each isolate compared to the EC_{50} values for wild-type HIV-$1_{ERS104pre}$. 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.

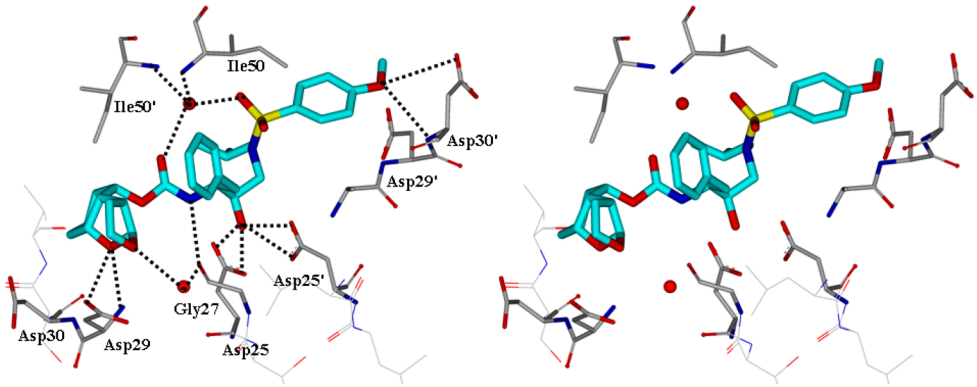

Figure 2. Stereoview of the X-ray structure of inhibitor **16a**-bound HIV-1 protease (PDB code: 4KB9). All strong hydrogen-bonding interactions are shown as dotted lines.

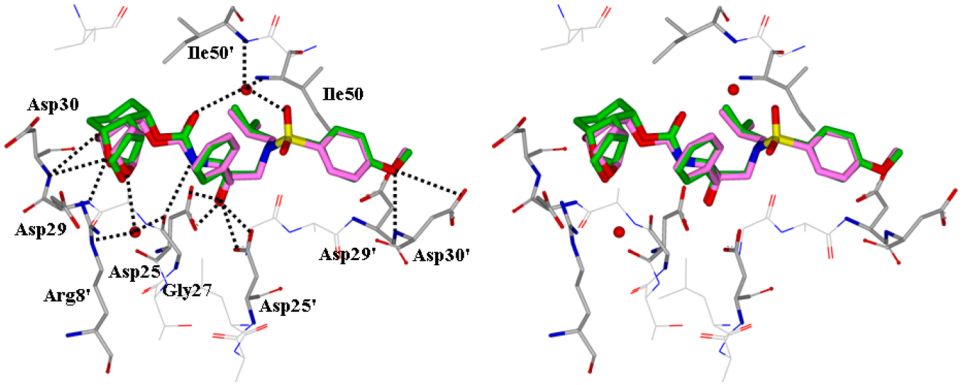

Figure 3. Stereoview of the overlay X-ray structures of inhibitor **16a** (green)-bound HIV-1 protease (PDB code: 4KB9) and **1** (magenta)-bound HIV-1 protease (PDB code: 3OK9). All strong hydrogen bonding interactions of inhibitor **1** are shown as dotted lines.

residues Ile50/50′ as well as the inhibitor's urethane carbonyl and one of the sulfonamide (SO_2) oxygens. The oxygen atom (OMe) of sulfonamide isostere in the P2′ position forms a hydrogen bond with the amide NH of Asp30′ at 3.3 Å. The hydrogen bond between the inhibitor urethane amide and the carbonyl oxygen atom of Gly27 is 3.3 Å in length. The cyclohexane ring appeared to fill in the hydrophobic pocket surrounding the Ile47, Val32, Ile84, Leu76, and Ile50′ residues. The tetrahydrofuran ring oxygen forms a hydrogen bond with the backbone amide NH of Asp29. The carboxylate group of Asp29 also forms a hydrogen bond with the ring oxygen of the first tetrahydrofuran ring. The protease-inhibitor interaction is further stabilized by the water-mediated hydrogen bond of the Gly27 carbonyl oxygen with the second tetrahydrofuran oxygen of the P2 group. An ovarlay of the X-ray structures of **16a**-bound HIV-1 protease and inhibitor **1**-bound HIV-1 protease is shown in Figure 3. The binding elements of inhibitor **16a** are similar to inhibitor **1** with a tris-THF P2 ligand, with the exception of cyclohexane replacing the first tetrahydrofuran ring in the P2 ligand. It appears that the first THF ring oxygen in inhibitor **1** is involved in hydrogen bonding with the backbone amide NH of Asp30 in the S2 subsite. This hydrogen-bond interaction is absent in inhibitor **16a**, where the cyclohexyl ring appeared to fill in the S2 subsite. As shown in Table 2, the resistance profile of **16a** can be compared favorably with DRV. However, it should be noted that inhibitor **1** displayed significantly improved antiviral potency over DRV against a variety of multidrug-resistant clinical HIV-1 strains.[7] The

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additional backbone interactions of tris-THF ligand in inhibitor **1** may be responsible for the improved resistance profile of inhibitor **1** over DRV.

CONCLUSIONS

We have designed a number of syn-anti-syn-fused tricyclic derivatives as P2 ligands in the S2 subsite. The P2 ligands were first synthesized stereoselectively in racemic form. The enzymatic resolution of these racemic alcohols provided rapid access to optically active ligand alcohols. Various substituents at the C2-methylene position were investigated to enhance interaction in the active site. The synthesis of the ligands was carried out using either $Mn(OAc)_3$-based annulation or a rhodium carbenoid cycloaddition reaction as the key step. Inhibitor **16a**, with a stereochemically defined fused cyclohexyl hexahydrofurofuran derivative, displayed remarkable enzyme inhibitory and antiviral potency. Inhibitor **16a** has also shown excellent activity against multi-PI-resistant variants compared to other FDA approved inhibitors. A protein−ligand X-ray structure of **16a**-bound HIV-1 protease was determined at a 1.29 Å resolution. The inhibitor appeared to make extensive interactions throughout the active site. Of particular interest, the cyclohexane ring appeared to nicely pack the hydrophobic pocket, and the first tetrahydrofuran oxygen forms a strong hydrogen bond with the backbone amide NH of Asp29. Also, the second tetrahydrofuran ring oxygen forms a water-mediated hydrogen bond with the Gly27 carbonyl oxygen and with a carboxylate oxygen atom of Asp29. These extensive interactions with the HIV-1 protease active site may be responsible for inhibitor **16a**′s potent antiviral activity and drug-resistance profiles. Further design and optimization of inhibitors utilizing this molecular insight are in progress.

EXPERIMENTAL SECTION

General Methods. All anhydrous solvents were obtained according to the following procedures: diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone under argon and dichloromethane from calcium hydride. All other solvents were reagent grade. All moisture-sensitive reactions were carried out in a flame-dried flask under a nitrogen atmosphere. Column chromatography was performed with Whatman 240−400 mesh silica gel under low pressure at 3−5 psi. Thin-layer chromatography was carried out with E. Merck silica gel 60-F-254 plates. Yields refer to chromatographically and spectroscopically pure compounds. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova-300 (300 and 75 MHz, respectively), Bruker Avance ARX-400 (400 and 100 MHz), and Bruker Avance 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. All test compounds showed ≥95% purity.

*3,3a,5,6,7,8a-Hexahydrofuro[2,3-b]benzofuran-4(2H)-one (**5a**).* Manganese(III) acetate (5.74 g, 21.4 mmol) was dissolved in 80.0 mL of glacial acetic acid at 60 °C under argon. To this mixture was added cyclohexane-1,3-dione (1 g, 8.92 mmol) and 2,3-dihydrofuran (1.35 mL, 17.8 mmol), and the reaction was stirred for 24 h. The reaction was diluted with water and extracted with dichloromethane (×4). The organic extracts were combined and washed with saturated aqueous sodium bicarbonate. The organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using a hexane/ethyl acetate (1:3) solvent system to furnish the desired ketone (0.47 g, 30% yield). R_f = 0.3 (50% hexanes/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (d, J = 5.9 Hz, 1H), 4.07 (t, J = 8.2 Hz, 1H), 3.70 (t, J = 7.7, 5.8 Hz, 1H), 3.65−3.62 (m, 1H), 2.55−2.37 (m, 2H), 2.32 (dd, J = 7.3, 5.8 Hz, 2H), 2.12−2.05 (m, 1H), 2.05−1.95 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.2, 177.4, 113.6, 112.8, 67.8, 43.8, 36.6, 30.3, 23.6, 21.6.

*6,6-Dimethyl-3,3a,5,6,7,8a-decahydrofuro[2,3-b]benzofuran-4(2H)-one (**5b**).* The titled compound was obtained following the procedure outlined for compound **5a** (42% yield). R_f = 0.40 (50% hexanes/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 (d, J = 5.8 Hz, 1H), 4.02 (t, J = 8.4 Hz, 1H), 3.65 (t, J = 7.7 Hz, 1H), 3.58−3.52 (m, 1H), 2.26 (d, J = 2.7 Hz, 2H), 2.14 (d, J = 6.5 Hz, 2H), 2.04−1.97 (m, 2H), 1.03 (s, 3H), 1.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.3, 176.1, 112.8, 112.0, 67.7, 50.8, 43.5, 37.4, 33.7, 30.2, 28.7, 28.0.

*2,3,3a,6,7,8a-Hexahydrofuro[2,3-b]benzofuran-4(5H)-one (**5c**).* The titled compound was obtained following the procedure outlined for compound **5d** (77% yield). R_f = 0.43 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (d, J = 7.6 Hz, 1H), 3.87−3.71 (m, 2H), 3.15−3.09 (m, 1H), 2.55−2.44 (m, 2H), 2.37−2.29 (m, 2H), 2.07−1.98 (m, 2H), 1.97−1.86 (m, 1H), 1.82−1.74 (m, 1H), 1.71−1.61 (m, 1H), 1.60−1.51 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3, 176.3, 116.0, 106.6, 60.5, 36.5, 35.1, 23.6, 21.5, 20.3, 19.1.

*3,3a,7,8a-Tetrahydro-2H-furo[3′,2′:4,5]furo[2,3-c]pyran-4(5H)-one (**5d**).* To a solution of 2,3-dihydrofuran (6.0 mL) and diazo compound **11b** (300 mg, 0.32 mmol, 1.0eq) was added rhodium(II) diacetate (13.0 mg, 0.03 mmol, 1.5 mol %). The mixture was allowed to stir for 3 h. Upon completion, the reaction was concentrated under vacuum and purified by flash chromatography (15% ethyl acetate/hexanes) to give the desired tricyclic product as a colorless oil (260 mg, 67% yield). R_f = 0.40 (40% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.33 (d, J = 5.8 Hz, 1H), 4.40 (q, J = 16.7 Hz, 2H), 4.10 (t, J = 8.2 Hz, 1H), 4.00 (s, 2H), 3.74 (t, J = 7.6 Hz, 1H), 3.66 (m, 1H), 2.13−1.98 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0, 175.0, 114.6, 111.3, 71.0, 68.1, 62.1, 43.1, 29.9; LRMS-EI (m/z) 182 (M +), LRMS-CI (m/z) 183 (M + H).

*2,3,3a,8a-Tetrahydro-5H-furo[2,3-b]thiopyrano[4,3-d]furan-4(7H)-one 6,6-dioxide (**5e**).* The titled compound was obtained following the procedure outlined for compound **5b** (48% yield). R_f = 0.38 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.33 (d, J = 5.9 Hz, 1H), 4.16−4.07 (m, 2H), 3.95 (d, J = 1.6 Hz, 2H), 3.84−3.77 (m, 1H), 3.69−3.60 (m, 2H), 2.14−2.06 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 166.2, 115.0, 113.9, 68.1, 60.1, 50.8, 44.3, 29.9.

*4-Diazo-2H-pyran-3,5(4H,6H)-dione (**11b**).* Ethyl 2-(2-oxopropoxy) acetate (15.0 g) was dissolved in THF (250 mL) and added dropwise (over 5 h) to a THF (1.0 L) solution of potassium *t*-butoxide (11.5 g, 103 mmol, 1.1 equiv) at 65 °C. Once the addition was complete, the reaction mixture was stirred for 15 min at 65 °C and then concentrated under vacuum to give a brown solid. Ethyl acetate was added to the solid, and 6 N HCl (20.0 mL) was added slowly while vigorously stirring the suspension. The organic layer was separated, dried over magnesium sulfate, and concentrated under vacuum (<20 °C). The crude mixture (~7.0 g, 61.3 mmol, 2.0 equiv) was dissolved in THF and cooled to 0 °C, and triethylamine (10.0 mL, 70.0 mmol, 2.30 equiv) and sulfonyl azide (6.0 g, 30.4 mmol, 1.0 equiv) were added sequentially. The reaction was allowed to stir for 4 h. Upon completion, the reaction was concentrated under vacuum and purified by flash chromatography (20% ethyl acetate/hexanes) to give the desired diazo compound as a light-yellow solid (3.0 g, 23% in two steps). Note that to remove trace amounts of the $TsNH_2$ byproduct, the diazo compound was recrystallized from diethyl ether. R_f = 0.86 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.27 (s, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.6, 71.9.

*4-Diazo-2H-thiopyran-3,5(4H,6H)-dione 1,1-Dioxide (**11d**).* In a dry flask, 2-chloro-1,3-dimethylimidazolinium chloride (890 mg, 5.26 mmol) was dissolved in MeCN (9 mL), and sodium azide (342 mg, 5.23 mmol) was added at 0 °C and stirred for 30 min. A cooled solution of **10b** (500 mg, 4.39 mmol) and triethylmine (1.22 mL, 8.88 mmol) in THF (18 mL) was cannulated to the mixture. The reaction was monitored by TLC until **10b** was consumed, and the reaction was quenched with water and extracted three times with CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum, and the residue was purified by flash chromatography using (6:1) hexanes/ether to obtain **11c** (290 mg, 68% yield) as an off-

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white crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.4, 35.7.

In a flask, diazo compound **11c** (740 mg, 4.74 mmol) was dissolved in a mixture of H_2O (5 mL) in MeOH (30 mL). To the solution was added oxone (5.00 g, 9.50 mmol), and the mixture was stirred for 3 h. Upon completion, the reaction was filtered on Celite, and the pad was washed with ether. Methanol and ether were removed under reduced pressure (<30 °C), and the resulting aqueous mixture was extracted with dichloromethane three times. The combined extracts were combined, dried over Na_2SO_4, and concentrated (<30 °C). The crude residue was purified by flash chromatography using 3:1 hexanes/ether to obtain **11d** (630 mg, 82% yield) R_f = 0.42 (SiO_2, hexanes/EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 60.4.

*Decahydrofuro[2,3-b]benzofuran-4-ol (**6a**).* To a solution of enone **5a** (44.0 mg, 2.22 mmol) in ethanol (15.0 mL) was added 10% Pd/C (44.0 mg). The resulting solution was then placed under 65 psi H_2 gas overnight. Upon completion, the mixture was filtered through a plug of Celite. Evaporation of the solvent and purification of the residue on silica gel using ethyl acetate/hexanes (3:1) as the eluent furnished the corresponding compound as a colorless oil (0.23 g, 52% yield). R_f = 0.26 (50% hexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66 (d, J = 4.9 Hz, 1H), 4.39–4.43 (m, 1H), 3.92 (dd, J = 7.7, 6.1 Hz, 2H), 3.18–3.11 (m, 1H), 3.02 (t, J = 12.0 Hz, 1H), 2.44–2.29 (m, 2H), 2.11–1.79 (m, 4H), 1.75–1.60 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.4, 108.3, 77.3, 68.2, 51.9, 46.8, 40.8, 28.7, 27.8, 17.7.

To a cold solution (0 °C) of the above ketone (230 mg, 1.25 mmol) in methanol (10 mL) was added $NaBH_4$ (57 mg, 1.5 mmol), and the mixture was stirred for 1 h at 0 °C. The reaction was quenched with saturated NH_4Cl, and the methanol was removed under vacuum. The aqueous layer was washed with ethyl acetate. The organic layers were combined, dried over Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography using an ethyl acetate/hexane (1:1) solvent system to afford the corresponding alcohol (217 mg, 95% yield). R_f = 0.18 (hexane/ethyl acetate 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.69 (d, J = 5.3 Hz, 1H), 4.24–4.17 (m, 1H), 3.97 (q, J = 7.4 Hz, 1H), 3.89–3.78 (m, 2H), 3.01–2.94 (m, 1H), 2.20–2.10 (m, 1H), 2.00 (q, J = 4.5 Hz, 1H), 1.95–1.85 (m, 2H), 1.81–1.70 (m, 2H), 1.69–1.55 (m, 2H), 1.52–1.45 (m, 1H), 1.37–1.28 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.9, 76.7, 65.2, 63.2, 43.9, 40.4, 31.4, 27.8, 21.4, 20.5, 15.6; LRMS-EI (m/z) 185 (M + H).

*6,6-Dimethyloctahydrofuro[2,3-b]benzofuran-4(2H)-ol (**6b**).* The target compound was obtained following the procedures outlined for compound **6a** (17% yield). R_f = 0.53 (50% hexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (d, J = 5.3 Hz, 1H), 4.66 (t, J = 6.0 Hz, 1H), 3.92–3.85 (m, 2H), 3.20–3.05 (m, 2H), 2.25–2.22 (m, 1H), 2.22–2.09 (m, 1H), 2.03–1.95 (m, 1H), 1.81 (dd, J = 15.0, 4.7 Hz, 2H), 1.77–1.68 (m, 1H), 1.02 (s, 3H), 0.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.0, 107.8, 79.9, 68.3, 57.1, 53.3, 42.9, 39.7, 35.4, 31.7, 31.0, 27.6.

The target alcohol was obtained following the procedure outlined for compound **6a** (step 2) (81% yield). R_f = 0.2 (50% hexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70 (d, J = 5.2 Hz, 1H), 4.39–4.22 (m, 2H), 4.13–4.06 (m, 1H), 3.93–3.83 (m, 1H), 2.96–2.81 (m, 2H), 2.22–2.12 (m, 2H), 2.08–2.00 (m, 1H), 1.70 (ddd, J = 13.2, 6.6, 1.9 Hz, 1H), 1.59 (ddd, J = 13.0, 6.1, 2.0 Hz, 1H), 1.42 (t, J = 12.5 Hz, 1H), 1.30 (t, J = 12.5 Hz, 1H), 0.97 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.9, 77.7, 68.6, 67.3, 45.2, 44.9, 44.0, 42.5, 32.8, 31.4, 27.8, 25.0; LRMS-CI (m/z) 213.9 (M + H).

*(4aS,9aR)-Octahydro-2H-pyrano[2,3-b]benzofuran-5(3H)-ol (**6c**).* The target compound was obtained following the procedure outlined for compound **6a** (62% yield). R_f = 0.45 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.03 (d, J = 3.7 Hz, 1H), 4.46–4.20 (m, 1H), 3.86–3.81 (m, 1H), 3.55–3.49 (m, 1H), 2.82 (t, J = 8.5 Hz, 1H), 2.46–2.41 (m, 1H), 2.39 (t, J = 6.5 Hz, 1H), 2.34–2.29 (m, 1H), 2.24–2.11 (m, 1H), 1.96–1.71(m, 4H), 1.69 –1.61 (m, 1H), 1.52–1.42 (m, 1H), 1.42–1.32 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.0, 100.6, 76.2, 63.0, 50.9, 41.0, 40.4, 28.9, 22.1, 17.0.

The target alcohol was obtained following the procedure outlined for compound **6a** (step 2) (80% yield). R_f = 0.3 (40% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.05 (d, J = 4.8 Hz, 1H), 4.07–4.00 (m, 2H), 3.92–3.89 (m, 1H), 3.65–3.59 (m, 1H), 2.37–2.30 (m, 1H), 2.22–2.18 (m, 1H), 2.02–1.91 (m, 3H), 1.88–1.74 (m, 4H), 1.51–1.38 (m, 2H), 1.30–1.21 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.9, 77.3, 65.2, 63.2, 43.9, 40.2, 31.4, 27.8, 21.3, 20.5, 15.6.

*Octahydro-2H-furo[3′,2′:4,5]furo[2,3-c]pyran-4-ol (**6d**).* Compound **5d** (260 mg, 1.4 mmol, 1.0 equiv) was treated with 10% Pd/C (35.0 mg) in methanol in the presence of hydrogen at 1 atm. The reaction was stirred for 12 h. Upon completion, the reaction was filtered through a plug of Celite, concentrated under vacuum, and purified by flash chromatography (gradient of 10–25% ethyl acetate/hexanes) to obtain the desired compound as a colorless oil (126 mg, 6:1 inseparable mixture of diastereomers, 48% yield). R_f = 0.3 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (d, J = 5.1 Hz, 1H, minor), 5.68 (d, J = 4.5 Hz, 1H, major), 4.62 (dt, J = 6.6, 3.2 Hz, 1H, minor), 4.39 (dt, J = 8.1, 3.3 Hz, 1H, major), 4.14 (d, J = 16.7 Hz, 1H, major), 4.07 (s, 1H, major), 4.04–4.00 (m, 2H, major), 3.97 (s, 2H, major), 3.95–3.87 (m, 2H, major), 3.70 (dd, J = 12.8, 3.2 Hz, 1H, major), 3.43–3.38 (m, 1H, minor), 3.27–3.19 (m, 1H), 3.19–3.12 (m, 1H), 2.77 (dd, J = 6.5, 2.2 Hz, 1H, minor), 2.24–2.20 (m, 1H, minor), 1.90 (q, J = 6.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.8 (major), 109.1 (minor), 108.8 (major), 75.2 (major), 75.0 (major), 73.9 (minor), 69.0 (major), 68.2 (major), 55.2 (minor), 49.6 (major), 46.9 (major), 44.6 (minor), 31.6 (minor), 27.2 (major); LRMS-CI (m/z) 185.1 (M + H).

The ketone obtained above (120 mg, 0.65 mmol, 1.0 equiv) was dissolved in THF (5.0 mL) and cooled to −78 °C. L-Selectride (0.78 mL, 0.78 mmol, 1.2 equiv) was added dropwise, and the reaction was allowed to stir for 2 h. Upon completion, the reaction was quenched with saturated NH_4Cl (2.0 mL) and warmed to room temperature. The reaction mixture was diluted with ethyl acetate (5.0 mL) and extracted two more times. The organic layers were combined, washed with brine, and dried over Mg_2SO_4, and the residue was purified by flash chromatography to obtain desired compound **6d** (60 mg, 48% yield) as a colorless oil. R_f = 0.3 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (d, J = 5.2 Hz, 1H), 4.16 (d, J = 13.4 Hz, 1H), 3.91 (s, 1H), 3.92–3.84 (m, 2H), 3.83–3.78 (m, 1H), 3.65–3.55 (m, 1H), 3.53 (d, J = 13.3 Hz, 1H), 3.32 (d, J = 11.8 Hz, 1H), 3.07–3.65 (q, J = 5.2 Hz, 1H), 2.54 (d, J = 11.7 Hz, 1H), 2.21–2.15 (m, 1H), 2.06 (t, J = 4.4 Hz, 1H), 1.69–1.63 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.5, 74.1, 70.5, 68.3, 67.2, 65.3, 46.8, 46.0, 30.4.

*4-Hydroxyoctahydro-5H-furo[2,3-b]thiopyrano[4,3-d]furan 6,6-dioxide (**6e**).* The target compound was obtained following the procedure outlined for compound **6d** (28% yield, 2 steps). R_f = 0.1 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (d, J = 5.5 Hz, 1H), 4.52–4.39 (m, 1H), 4.15–4.05 (m, 2H), 3.92–3.83 (m, 1H), 3.68 (dt, J = 12.9, 3.7 Hz, 1H), 3.37 (dt, J = 14.6, 3.2 Hz, 1H), 3.24–3.18 (m, 3H), 3.08–3.02 (m, 1H), 2.01–1.95 (m, 1H), 1.73 (dd, J = 12.8, 4.7 Hz, 1H), 1.48 (td, J = 10.6, 2.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.0, 70.4, 67.1, 62.7, 57.5, 56.4, 52.3, 43.1, 30.1.

*(S)-Alcohol (**8a**).* Alcohol (**6a**) (20.0 mg, 0.11 mmol) was dissolved in THF (1.0 mL) under argon. Lipase Amano PS-30 (25.0 mg) and vinyl acetate (0.18 mL, 1.91 mmol) were subsequently added at room temperature. The reaction was stirred until completion (50:50 by ^{1}H NMR), filtered through a plug of Celite, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using ethyl acetate/hexanes (1:2) to yield the corresponding alcohol and acetate. (*S*)-alcohol **8a** (9 mg, 45% yield). R_f = 0.13 (50% ethyl acetate/hexanes); $[\alpha]_D^{23}$ +9.6 (*c* 1.0, $CHCl_3$). (*R*)-acetate **7a** (11 mg, 45% yield). R_f = 0.40 (50% ethyl acetate/hexanes); $[\alpha]_{23}^{D}$ −1.69 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.67 (d, J = 5.3 Hz, 1H), 5.10–5.06 (m, 1H), 4.22 (q, J = 5.1 Hz, 1H), 3.93–3.80 (m, 3H), 2.77–2.68 (m, 1H), 2.21 (q, J = 5.1 Hz 1H), 2.14–2.06 (m, 1H), 2.05 (s, 3H), 1.75–1.60 (m, 4H), 1.57 −1.46 (m, 1H), 1.29–1.18 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 108.9, 76.1, 70.2, 67.3, 46.3, 44.9, 31.3, 27.6, 27.5, 21.2, 15.7; LRMS-CI (m/z) 229.1 (M + H).

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*(R)-Alcohol (**9a**).* The titled compound was obtained from compound **7a** following the procedure for **9b** (89% yield). R_f = 0.13 (50% ethyl acetate/hexanes); $[\alpha]_D^{23}$ −9.7 (*c* 1.0, $CHCl_3$).

*(R)-Alcohol (**8b**).* To a solution of racemic alcohol **6d** (60.0 mg, 0.32 mmol, 1.0 equiv) in THF (10 mL) was added vinyl acetate (0.60 mL, 6.44 mmol, 20.0 equiv) and lipase PS-30 immobilized on Celite (120 mg total). The reaction was stirred for 20 h at 23 °C and monitored by NMR (1:1 mixture of alcohol and acetate after 20 h). Upon completion, the reaction was filtered through a plug of Celite, concentrated under vacuum, and purified by flash chromatography. (*R*)-alcohol **8b** was obtained as a colorless oil (28 mg, 47% yield). R_f = 0.24 (80% ethyl acetate/hexanes); $[\alpha]_D^{23}$ +12.1 (*c* 0.8, $CHCl_3$).

*(S)-Acetate (**7b**).* (35 mg, 48% yield, white solid). R_f = 0.32 (80% ethyl acetate/hexanes); $[\alpha]_D^{23}$ −27.3 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.77 (d, *J* = 5.0 Hz, 1H), 5.0 (d, *J* = 4.9 Hz, 1H), 4.17 (d, *J* = 13.1 Hz, 1H), 4.07 (dt, *J* = 4.7, 2.3 Hz, 1H), 3.97−3.88 (m, 2H), 3.84 (d, *J* = 13.2 Hz, 1H), 3.58 (d, *J* = 13.3 Hz, 1H), 3.41 (d, *J* = 12.8 Hz, 1H), 2.76−2.72 (m, 1H), 2.36 (t, *J* = 4.9 Hz, 1H), 2.24−2.18 (m, 1H), 2.13 (s, 3H), 1.72−1.67 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 109.5, 73.2, 67.9, 67.7, 67.3, 66.7, 45.6, 44.3, 30.5, 21.2. (*S*)-Alcohol **9b**: To a cold (0 °C) methanol solution of (*S*)-acetate **7b** was added a 1.0 M solution of NaOMe/MeOH (1.0 mL). The solution was stirred for 15 min and quenched with saturated NH_4Cl. The reaction was concentrated under vacuum to remove the methanol and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, concentrated, and chromatographed (70% ethyl acetate/hexanes) to give the desired alcohol as a colorless oil (20 mg, 71% yield). $[\alpha]_{23}^{D}$ −16.2 (*c* 1.0, $CHCl_3$).

*(S)-Alcohol (**8c**) and (R)-Acetate (**7c**).* The titled compound was obtained by enzymatic resolution of alcohol **6c** using the procedure described for (*R*)-alcohol **8b** and (*S*)-acetate **7b**. (*S*)−Alcohol **8c** (38% yield). $[\alpha]_D^{23}$ −4.33 (*c* 1.1, $CHCl_3$). (*R*)-Acetate **7c** (39% yield). $[\alpha]_{23}^{D}$ +7.04 (*c* 1.4, $CHCl_3$).

*(3aR,3bR,4S,7aR,8aS)-Decahydrofuro[2,3-b]benzofuran-4-yl (4-Nitrophenyl) Carbonate (**14a**).* To a solution of alcohol **8a** (7.0 mg, 0.04 mmol) in dichloromethane (1.0 mL) under argon atmosphere was added 4-nitrophenyl chloroformate (11.0 mg, 0.06 mmol), and the solution was cooled to 0 °C followed by the addition of pyridine (12.2 μL, 0.15 mmol). The reaction was warmed to room temperature and stirred for 3 h (70% yield). R_f = 0.63 (50% hexanes/ethyl acetate); $[\alpha]_D^{23}$ +11.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, *J* = 9.2 Hz, 2H), 7.39 (d, *J* = 9.2 Hz, 2H), 5.78 (d, *J* = 5.2 Hz, 1H), 5.11−5.03 (m, 1H), 4.29 (dd, *J* = 4.9, 9.9 Hz, 1H), 4.00−3.85 (m, 2H), 2.95−2.83 (m, 1H), 2.35 (dd, *J* = 5.0, 9.6 Hz, 1H), 2.26−2.11 (m, 1H), 1.98−1.62 (m, 6H), 1.37−1.28 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 152.1, 145.3, 130.6, 123.7, 108.9, 75.6, 71.7, 67.6, 46.7, 45.4, 31.3, 27.9, 27.2, 15.6; LRMS-APCI (*m*/*z*) 350.2 $(M + H)^+$.

*(3aS,3bS,4R,7aS,8aR)-Decahydrofuro[2,3-b]benzofuran-4-yl (4-Nitrophenyl) Carbonate (**14b**).* The titled compound was obtained following the procedure outlined for compound **14a** (84% yield). R_f = 0.60 (50% ethyl acetate/hexanes); $[\alpha]_D^{23}$ −11.4 (*c* 1.0, $CHCl_3$).

*6,6-Dimethyldecahydrofuro[2,3-b]benzofuran-4-yl (4-Nitrophenyl) Carbonate (**14c**).* The titled compound was obtained following the procedure outlined above compound **14a** (97% yield). R_f = 0.68 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 9.2 Hz, 2H), 7.37 (d, *J* = 9.2 Hz, 2H), 5.77 (d, *J* = 5.3 Hz, 1H), 5.21 (dt, *J* = 11.4, 5.5 Hz, 1H), 4.50−4.37 (m, 1H), 3.97 (dd, *J* = 7.4, 6.4 Hz, 1H), 3.90−3.86 (m, 1H), 3.01−2.98 (m, 1H), 2.62−2.59 (m, 1H), 2.08−2.04 (m, 1H), 1.97−1.86 (m, 1H), 1.79−1.69 (m, 2H), 1.67−1.55 (m, 1H), 1.22−1.12 (m, 1H), 1.03 (s, 3H), 0.93 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 151.9, 145.4, 125.3, 121.7, 108.4, 76.0, 66.5, 44.6, 42.8, 40.6, 38.3, 32.4, 32.2, 30.9, 25.0; LRMS-APCI (*m*/*z*) 378.1 $(M + H)^+$.

*(4aR,4bR,5S,8aR,9aS)-Decahydro-2H-pyrano[2,3-b]benzofuran-5-yl (4-Nitrophenyl) Carbonate (**14e**).* The titled compound was obtained following the procedure outlined above compound **14a** (95% yield). R_f = 0.23 (30% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CHCl_3$) δ 8.28 (d, *J* = 9.2 Hz, 2H), 7.38 (d, *J* = 8.8 Hz, 2H), 5.30 (d, *J* = 3.9 Hz, 1H), 5.07−5.01 (m, 1H), 4.23−4.16 (m, 1H), 3.84 (td, *J* = 11.3, 3.0 Hz, 1H), 3.72 (ddt, *J* = 11.0, 4.2, 2.0 Hz, 1H), 3.04 (q, *J* = 8.2 Hz, 1H), 2.18−2.10 (m, 2H), 2.06−2.02 (m, 2H), 1.94−1.82 (m, 2H), 1.75−1.54 (m, 4H), 1.37−1.24 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 151.9, 145.4, 125.3, 121.8, 101.1, 78.3, 74.6, 61.1, 41.1, 37.1, 30.8, 28.2, 23.9, 23.3, 19.5

*4-Nitrophenyl ((3aR,3bR,4R,7aS,8aS)-Octahydro-5H-furo-[3′,2′:4,5]furo[2,3-c]pyran-4-yl) Carbonate (**14f**).* The titled compound was obtained following the procedure outlined for compound **14a** (72% yield). R_f = 0.14 (60% ethyl acetate/hexanes); $[\alpha]_D^{23}$ +39.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.9 Hz, 2H), 7.40 (d, *J* = 9.0 Hz, 2H), 5.87 (d, *J* = 5.0 Hz, 1H), 4.89 (dt, *J* = 4.9, 2.2 Hz, 1H), 4.25 (d, *J* = 13.1 Hz, 1H), 4.16−4.12 (m, 2H), 3.97−4.01 (m, 1H), 3.88−3.93 (m, 1H), 3.64 (dd, *J* = 13.2, 2.4 Hz, 1H), 3.49 (dd, *J* = 13.1, 1.0 Hz, 1H), 2.92 (dt, *J* = 10.2, 5.0 Hz, 1H), 2.46 (t, *J* = 4.9 Hz, 1H), 2.21−2.31 (m, 1H), 1.71−1.77 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.2, 152.2, 145.3, 125.3, 121.6, 109.5, 73.1, 72.5, 68.0, 67.4, 67.0, 45.8, 44.5, 30.6; LRMS-CI (*m*/*z*) 374.3 (M + Na).

*4-Nitrophenyl ((3aS,3bS,4S,7aR,8aR)-Octahydro-5H-furo-[3′,2′:4,5]furo[2,3-c]pyran-4-yl) Carbonate (**14g**).* The titled compound was obtained following the procedure outlined above compound **14a** (97% yield). R_f = 0.14 (60% ethyl acetate/hexanes); $[\alpha]_D^{23}$ −41.4 (*c* 0.95, $CHCl_3$).

*6,6-Dioxidooctahydro-5H-furo[2,3-b]thiopyrano[4,3-d]furan-4-yl (4-Nitrophenyl) Carbonate (**14h**).* The titled compound was obtained following the procedure outlined above compound **14a** (52% yield). R_f = 0.28 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 9.2 Hz, 2H), 7.42 (d, *J* = 9.2 Hz, 2H), 5.79 (d, *J* = 5.5 Hz, 1H), 5.33−5.31 (m, 1H), 4.25 (td, *J* = 11.6, 3.8 Hz, 1H), 4.14−4.11 (m, 1H), 3.94−3.88 (m, 1H), 3.82−3.71 (m, 2H), 3.34−3.23 (m, 2H), 3.17−3.10 (m, 1H), 2.97−2.91 (m, 1H), 2.07−1.97 (m, 1H), 1.84−1.72 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.9, 151.6, 145.7, 125.4, 121.7, 108.8, 70.7, 69.0, 67.0, 57.7, 53.5, 50.2, 43.0, 30.2.

*(3aS,3bR,4S,7aR,8aS)-Decahydrofuro[2,3-b]benzofuran-4-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16a**).* To a solution of activated alcohol **14a** (1.0 equiv) and isostere **15** (1.0 equiv) in acetonitrile was added triethylamine (5.0 equiv). The reaction was allowed to stir until the consumption of the activated alcohol. The reaction mixture was concentrated under vacuum and purified by flash column chromatography to provide inhibitor **16a** (63% yield). R_f = 0.3 (50% hexanes/ethyl acetate); $[\alpha]_D^{23}$ +7.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 3H), 7.21 (dd, *J* = 12.4, 6.9 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 5.41 (d, *J* = 5.1 Hz, 1H), 4.96−4.85 (m, 1H), 4.81 (d, *J* = 8.9 Hz, 1H), 4.23−4.08 (m, 1H), 3.88 (s, 3H), 3.87−3.72 (m, 4H), 3.17 (dd, *J* = 15.3, 8.4 Hz, 1H), 3.11−2.99 (m, 2H), 2.99−2.92 (m, 1H), 2.79 (td, *J* = 14.4, 13.8, 8.2 Hz, 2H), 2.36−2.34 (m, 1H), 2.06 (d, *J* = 5.1 Hz, 1H), 1.99−1.87 (m, 1H), 1.83 (dd, *J* = 13.8, 7.0 Hz, 1H), 1.79−1.71 (m, 1H), 1.68−1.53 (m, 3H), 1.48−1.36 (m, 2H), 1.22 (bs, 2H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 156.0, 137.6, 129.7, 129.4, 129.3, 128.4, 126.4, 114.3, 108.9, 75.7, 73.1, 70.1, 67.5, 58.7, 55.5, 54.7, 53.7, 46.6, 44.8, 35.6, 31.1, 29.6, 28.0, 27.3, 27.1, 20.1, 19.8, 15.2; LRMS-ESI (*m*/*z*) 617.8 $[M + H]^+$; HRMS-ESI (*m*/*z*) $[M + H]^+$ calcd for ($C_{32}H_{44}N_2O_8S$), 617.2896; found, 617.2892.

*(3aS,3bS,4R,7aS,8aR)-Decahydrofuro[2,3-b]benzofuran-4-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16b**).* The indicated inhibitor was obtained following the general procedure outlined above for inhibitor **16a** (52% yield). R_f = 0.31 (50% hexanes/ethyl acetate).; $[\alpha]_D^{23}$ +8.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.5 Hz, 2H), 7.39−7.13 (m, 5H), 7.07−6.88 (m, 2H), 5.57 (d, *J* = 4.7 Hz, 1H), 4.92 (d, *J* = 8.4 Hz, 1H), 4.86 (s, 1H), 4.18 (s, 1H), 3.98−3.70 (m, 7H), 3.22−2.90 (m, 4H), 2.90−2.72 (m, 2H), 2.66 (s, 1H), 2.13 (s, 1H), 2.08−2.01 (m, 1H), 1.82 (dt, *J* = 15.5, 7.8 Hz, 3H), 1.59 (d, *J* = 9.4 Hz, 3H), 1.49−1.41 (m, 1H), 1.36−1.39 (m, 1H), 1.22−1.14 (m, 1H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 156.1, 137.7, 129.8, 129.4, 129.3, 128.4, 126.4, 114.3, 108.9, 75.8, 72.6, 70.4, 67.4, 58.7, 55.5, 55.2, 53.6, 46.7,

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45.1, 35.4, 31.2, 27.8, 27.3, 27.2, 20.1, 19.8, 15.2; LRMS-ESI (m/z) 617.80 (M + H)$^+$. HRMS-ESI (m/z) [M + Na]$^+$ calcd for ($C_{32}H_{44}N_2O_8SNa$), 639.2716; found, 639.2719.

*(3aS,3bR,4S,7aR,8aS)-6,6-Dimethyldecahydrofuro[2,3-b]-benzofuran-4-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16c**).* The indicated inhibitor was obtained following the general procedure outlined above for inhibitor **16a**. The product was obtained as a mixture of diastereomers (**16c**/**16d**) (61% yield, **16c**/**16d**). R_f = 0.33, (50% hexanes/ethyl acetate). **16c**/**16d** were separated by HPLC with column YMC-Pack-ODS-A (250 × 10 mm) and a solvent/flow rate of 2.5 mL/min (CH_3CN/H_2O, 65:35). Retention time: **16c** = 18.31 min and **16d** = 18.99 min. For **16c**: $[\alpha]_D^{23}$ −19.0 (c 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.9 Hz, 2H), 7.28 (s, 2H), 7.25−7.18 (m, 3H), 6.99 (d, J = 8.9 Hz, 2H), 5.57 (d, J = 5.0 Hz, 1H), 5.01−4.94 (m, 1H), 4.82 (d, J = 8.9 Hz, 1H), 4.26−4.15 (m, 1H), 4.05 (q, J = 8.1 Hz, 2H), 3.94 (s, 1H), 3.88 (s, 4H), 3.83 (s, 1H), 3.24−3.10 (m, 2H), 3.01−2.95 (m, 1H), 2.87 (d, J = 8.3 Hz, 2H), 2.80 (d, J = 13.2 Hz, 2H), 2.15−2.05 (m, 1H), 2.03−1.90 (m, 2H), 1.89−1.80 (m, 2H), 1.75−1.55 (m, 3H) 0.97−0.94 (m, 6H), 0.90−0.86 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.0, 155.7, 137.7, 129.6, 129.4, 129.2, 128.4, 127.7, 126.5, 114.3, 113.8, 109.7, 73.1, 71.0, 68.4, 58.8, 55.6, 54.7, 53.7, 44.6, 42.4, 41.3, 40.7, 35.6, 32.6, 31.2, 27.7, 27.2, 24.6, 20.1, 19.8. HRMS-ESI (m/z) [M + H]$^+$ calcd for ($C_{34}H_{48}N_2O_8S$), 645.3209; found, 645.3210.

*3aS,3bS,4R,7aS,8aR)-6,6-Dimethyldecahydrofuro[2,3-b]-benzofuran-4-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16d**).* $[\alpha]_D^{23}$ +21.5 (c 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, J = 8.3 Hz, 2H), 7.43−7.13 (m, 5H), 6.98 (d, J = 8.6 Hz, 2H), 5.69 (s, 1H), 5.10−4.98 (m, 1H), 4.93 (d, J = 8.3 Hz, 1H), 4.27 (s, 1H), 4.09 (d, J = 6.5 Hz, 1H), 3.87 (s, 6H), 3.64 (s, 1H), 3.13 −3.08 (m, 1H), 3.02 (d, J = 14.3 Hz, 2H), 2.96−2.91 (m, 2H), 2.79 (dd, J = 13.4, 6.7 Hz, 1H), 2.68 (s, 1H), 2.08−2.05 (m, 1H), 1.90−1.54 (m, 1H), 1.86−1.80 (m, 1H), 1.75−1.71 (m, 1H), 1.64−1.61 (s, 1H), 1.55−1.51 (s, 1H), 0.95 (s, 3H), 0.91−0.86 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1, 156.13, 137.7, 129.7, 129.4, 128.5, 126.5, 114.3, 113.9, 109.8, 72.6, 71.1, 68.5, 58.8, 55.6, 55.2, 53.6, 45.3, 42.4, 41.3, 35.1, 32.7, 31.9, 31.3, 27.8, 27.3, 24.8, 22.7, 20.1, 19.9; HRMS-ESI (m/z) [M + Na]$^+$ calcd for ($C_{34}H_{48}N_2O_8SNa$), 667.3209; found, 667.3040.

*(4aR,4bR,5S,8aR,9aS)-Decahydro-2H-pyrano[2,3-b]benzofuran-5-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16e**).* The indicated inhibitor was obtained following the general procedure outlined above for inhibitor **16a** (80% yield). R_f = 0.31 (ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 8.9 Hz, 2H), 7.32−7.19 (m, 5H), 6.98 (d, J = 8.9 Hz, 2H), 5.12 (d, J = 3.9 Hz, 1H), 5.01 (dd, J = 18.6, 4.5 Hz, 1H), 4.96−4.86 (m, 1H), 4.86−4.72 (m, 1H), 4.41−4.30 (m, 1H), 3.87 (s, 3H), 3.86−3.73 (m, 2H), 3.67 (d, J = 12.2 Hz, 1H), 3.41 (t, J = 10.7 Hz, 1H), 3.18−3.07 (m, 1H), 3.07−2.99 (m, 2H), 2.99−2.70 (m, 2H), 2.58 (dd, J = 15.0, 7.4 Hz, 1H), 2.06 (ddd, J = 16.0, 10.5, 6.4 Hz, 1H), 1.88−1.68 (m, 2H), 1.53 (s, 4H), 1.53−1.37 (m, 4H), 1.35−1.16 (m, 2H), 0.91 (d, J = 6.7 Hz, 3H), 0.87 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 155.8, 137.6, 129.5, 129.4, 128.4, 126.5, 114.3, 100.9, 74.5, 73.0, 72.6, 61.0, 58.7, 55.5, 54.7, 53.7, 41.2, 36.6, 35.4, 30.9, 29.6, 28.6, 27.2, 23.8, 23.3, 21.9, 20.1, 19.8; HRMS-ESI (m/z) [M + H]$^+$ calcd for ($C_{33}H_{46}N_2O_8S$), 631.3053; found, 631.3047.

*(3aS,3bR,4R,7aS,8aS)-Octahydro-2H-furo[3′,2′:4,5]furo[2,3-c]-pyran-4-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16f**).* The indicated inhibitor was obtained following the general procedure outlined above for inhibitor **16a** (82% yield). R_f = 0.42 (80% ethyl acetate/hexanes); $[\alpha]_D^{23}$ +12.8 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, d-MeOH) δ 7.79 (d, J = 8.8 Hz, 2H), 7.29−7.26 (m, 4H), 7.22−7.16 (m, 1H), 7.11 (d, J = 8.8 Hz, 2H), 5.51 (d, J = 4.8 Hz, 1H), 4.67 (d, J = 5.2 Hz, 1H), 4.05(d, J = 8.0 Hz, 1H), 3.98 (s, 1H), 3.89 (s, 4H), 3.81−3.84 (m, 3H), 3.71 (d, J = 12.0 Hz, 1H), 3.61 (dd, J = 13.2, 2.0 Hz, 1H), 3.47−3.39 (m, 2H), 3.26−3.23 (m, 1H), 3.11 (dd, J = 13.5, 8.4 Hz, 1H), 2.95 (dd, J = 14.8, 8.1 Hz, 1H), 2.86 (dd, J = 13.5, 6.6 Hz, 1H), 2.63−2.46 (dd, J = 13.6, 2.8 Hz, 1H), 2.32 (t, J = 4.9 Hz, 1H), 2.16−2.09 (m, 1H), 2.08−2.04 (m, 2H), 1.73−1.59 (m, 2H), 0.97 (d, J = 6.4 Hz, 3H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, d-MeOH) δ 164.6, 158.0, 140.2, 131.9, 130.7, 130.6, 129.3, 127.1, 115.4, 110.8, 75.0, 74.4, 69.2, 69.1, 68.2, 68.0, 59.0, 57.2, 56.2, 54.1, 46.8, 45.6, 37.3, 31.2, 28.0, 20.5; HRMS-ESI (m/z) [M + H]$^+$ calcd for ($C_{31}H_{42}N_2O_9S$), 619.2689; found, 619.2679.

*(3aS,3bS,4S,7aR,8aR)-Octahydro-2H-furo[3′,2′:4,5]furo[2,3-c]-pyran-4-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16g**).* The indicated inhibitor was obtained following the general procedure outlined above for inhibitor **16a** (70% yield). R_f = 0.27 (80% ethyl acetate/hexanes); $[\alpha]_D^{23}$ −11.5 (c 1.7, $CHCl_3$); ^{1}H NMR (400 MHz, d-MeOH) δ 7.79 (d, J = 8.8 Hz, 2H), 7.30−7.24 (m, 4H), 7.21−7.17 (m, 1H), 7.11(d, J = 8.8 Hz, 2H), 5.72 (d, J = 5.0 Hz, 1H), 4.70 (d, J = 5.2 Hz, 1H), 4.14−3.97 (m, 2H), 3.90 (s, 4H), 3.76−3.81 (m, 2H), 3.70 (dd, J = 10.7, 7.1 Hz, 1H), 3.61 (dd, J = 13.4, 2.4 Hz, 1H), 3.57−3.43 (m, 2H), 3.26−3.13 (m, 1H), 3.10−2.98 (m, 2H), 2.92 (dd, J = 13.6, 7.1 Hz, 1H), 2.73 (dt, J = 10.0, 4.8 Hz, 1H), 2.65 (dd, J = 13.7, 10.9 Hz, 1H), 2.43 (t, J = 4.9 Hz, 1H), 2.25−2.10 (m, 1H), 2.06−1.97 (m, 2H), 1.86−1.69 (m, 1H), 1.05−0.83 (m, 6H); ^{13}C NMR (100 MHz, d-MeOH) δ 164.5, 158.0, 140.2, 132.3, 130.6, 130.5, 129.2, 127.2, 115.4, 110.8, 79.5, 75.0, 74.0, 69.0, 68.3, 68.1, 58.6, 57.6, 56.2, 53.8, 47.1, 45.5, 37.1, 31.3, 28.0, 20.5; HRMS-ESI (m/z) [M + Na]$^+$ calcd for ($C_{31}H_{42}N_2O_9SNa$), 641.2509; found, 641.2501.

*(3aS,3bR,4R,7aS,8aS)-6,6-Dioxidooctahydro-2H-furo[2,3-b]-thiopyrano[4,3-d]furan-4-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16h**).* The indicated inhibitor was obtained following the general procedure outlined above for inhibitor **16a**. The product was obtained as a mixture of diastereomers (**16h**/**16i**) (61% yield). **16h**/**16i** were separated was separated by chiral HPLC, and titled inhibitor **16h** was determined to be >95%. Column ChiralPak IC, hexanes/IPA (52−48%, 20 min), 2.5 mL/min, 24 °C, retention time **16h** = 6.38 min. R_f = 0.28 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 5.4 Hz, 2H), 7.27−7.25 (m, 5H), 6.99 (d, J = 6.0 Hz, 2H), 5.69 (d, J = 5.5 Hz, 1H), 5.58 (d, J = 5.5 Hz, 1H), 5.43 (d, J = 9.1 Hz, 1H), 5.37 (d, J = 9.3 Hz, 1H), 5.19−5.10 (m, 2H), 4.08−3.98 (m, 1H), 3.95−3.88 (m, 1H), 3.86 (s, 3H), 3.83−3.71 (m, 3H), 3.68−3.49 (m, 1H), 3.40−3.30 (m, 1H), 3.28−3.18 (m, 2H), 3.20−3.05 (m, 2H), 3.05−2.91 (m, 1H), 2.91−2.69 (m, 1H), 2.50 (dd, J = 13.4, 9.7 Hz, 1H), 2.21−2.10 (m, 1H), 1.92−1.87 (m, 1H), 1.83−1.73 (m, 1H), 1.72−1.51 (m, 1H), 0.92 (d, J = 8.8 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 156.4, 143.3, 129.7, 128.5, 128.4, 127.9, 114.4, 109.1, 74.9, 72.6, 71.8, 68.7, 57.7, 55.9, 50.2, 43.0, 41.4, 37.1, 32.0, 29.2, 29.0, 27.3, 27.1, 23.9, 20.1, 19.8;HRMS (m/z) [M + Na]$^+$ calcd for ($C_{31}H_{42}N_2O_{10}S_2Na$), 689.2178; found, 689.2169.

*(3aS,3bS,4S,7aR,8aR)-6,6-Dioxidooctahydro-2H-furo[2,3-b]-thiopyrano[4,3-d]furan-4-yl ((2S,3R)-3-Hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (**16i**).* Retention time = 8.02 min. R_f = 0.28 (50% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 5.1 Hz, 2H), 7.28−7.26 (m, 5H), 6.98 (d, J = 8.3 Hz, 2H), 5.69 (d, J = 5.5 Hz, 1H), 5.58 (d, J = 5.5 Hz, 1H), 5.43 (d, J = 9.1 Hz, 1H), 5.37 (d, J = 9.3 Hz, 1H), 5.19−5.10 (m, 2H), 4.08−3.98 (m, 1H), 3.95−3.88 (m, 1H), 3.86 (s, 3H), 3.83−3.71 (m, 3H), 3.68−3.49 (m, 1H), 3.40−3.30 (m, 1H), 3.28−3.18 (m, 2H), 3.20−3.05 (m, 2H), 3.05−2.91 (m, 1H), 2.91−2.69 (m, 1H), 2.50 (dd, J = 13.4, 9.7 Hz, 1H), 2.21−2.10 (m, 1H), 1.89 (dq, J = 13.9, 7.2, 6.7 Hz, 1H), 1.83−1.73 (m, 1H), 1.72−1.51 (m, 1H), 0.92 (d, J = 4.5 Hz, 3H), 0.87 (d, J = 2.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 156.4, 143.3, 129.7, 128.4, 128.3, 127.9, 113.8, 109.1, 74.9, 72.6, 71.8, 68.5, 57.7, 55.9, 50.2, 43.0, 41.4, 37.2, 32.1, 31.8, 30.9, 29.2, 28.9, 27.3, 27.1, 23.8, 20.1, 19.8; HRMS (m/z) [M + Na]$^+$ calcd for ($C_{31}H_{42}N_2O_{10}S_2Na$), 689.2178; found, 689.2170.

*Determination of the X-ray Structure of the Inhibitor **16a**−HIV-1 Protease Complex.* The HIV-1 protease was expressed and purified as previously described.[29,30] The protease−inhibitor complex was crystallized at room temperature by the hanging-drop vapor-diffusion method with well solutions of 1.15 M ammonium chloride and 0.1 M sodium acetate buffer (pH 5.5). Diffraction data were collected on a single crystal cooled to 90 K at the SER-CAT BM beamline 22,

Advanced Photon Source, Argonne National Laboratory (Chicago, IL), with an X-ray wavelength of 1.0 Å, and the data were processed by HKL-2000[31] with Rmerge of 6.1%. The PR structure was used in molecular replacement by PHASER[32,33] in the CCP4i suite[34,35] and was refined to a 1.29 Å resolution using SHELX-97[36,37] and COOT[38] for manual modification. PRODRG-2[39] was used to construct the inhibitor and the restraints for refinement. Alternative conformations were modeled, anisotropic atomic displacement parameters (B factors) were applied for all atoms including solvent molecules, and hydrogen atoms were added in the final round of the refinement. The final refined solvent structure was composed of 1 sodium ion, 2 chloride ions, 3 acetate ions, 2 glycerol molecules, and 220 water molecules. The crystallographic statistics are listed in Table S1 in the Supporting Information. The coordinates and structure factors of the PR with **16a** complex have been deposited in Protein Data Bank[40] under code 4KB9.

■ ASSOCIATED CONTENT

Supporting Information

HPLC and HRMS data for inhibitors **16a**–**16i**. Crystallographic data collection and refinement statistics for inhibitor **16a**. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The PDB accession code for **16a**-bound HIV-1 protease X-ray structure is 4KB9.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS:

PI, protease inhibitor; APV, amprenavir; DRV, darunavir; SQV, saquinavir; *bis*-THF, *bis*-tetrahydrofuran; MDR, multidrug resistant

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