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

Arun K. Ghosh,*,[†] Cuthbert D. Martyr,[†] Melinda Steffey,[†] Yuan-Fang Wang,[‡] Johnson Agniswamy,[‡] Masayuki Amano,[§] Irene T. Weber,[‡] and Hiroaki Mitsuya[§,||]

[†]Departments of Chemistry and Medicinal Chemistry, Purdue University, West Lafayette, Indiana 47907, United States

[‡]Department of Biology, Molecular Basis of Disease, Georgia State University, Atlanta, Georgia 30303, United States

[§]Departments of Hematology and Infectious Diseases, Kumamoto University Graduate School of Medical and Pharmaceutical Sciences, Kumamoto 860-8556, Japan;

[||]Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, United States

S *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.

23c, $K_i = 2.9$ pM
$ID_{50} = 2.4$ nM
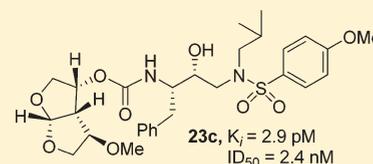
KEYWORDS: HIV-1 protease inhibitors, Darunavir, bis-THF, drug-resistant, design, synthesis, X-ray structure

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.[1] 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.[2] Later, darunavir received an expanded approval for the treatment of all therapy-naïve HIV/AIDS patients, including pediatric patients.[3] Darunavir incorporates a stereochemically defined (3*R*,3a*S*,6a*R*)-bis-tetrahydrofuranyl (bis-THF) urethane as the P2 ligand in a (*R*)-hydroxyethyl sulfonamide isostere.[4] 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.[5] A number of other clinical protease inhibitors (**2**, Brecanavir; **3**, GS-8374) have also incorporated this bis-THF ligand and demonstrated marked antiviral potency against a panel of multidrug-resistant HIV-1 variants.[6,7]

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.[8-12] Quaedflieg and co-workers reported a chiral synthesis that involved a conjugate addition of nitromethane.[13] Other syntheses have been attempted with the use of various catalysts to obtain the desired product.[14] 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**.[11] It was prepared in multigram quantities by Wittig olefination of (*S*)-glyceraldehyde acetonide with (ethoxycarbonylmethylene) triphenylphosphorane, as described previously.[11] The requisite (*S*)-glyceraldehyde can be obtained from ascorbic

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

Scheme 1. Synthesis of Bis-THF Ligand 11

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.[23] 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$_3$ 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[24] gave **16** in 87% yield.

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

We investigated the O-alkylation of **5** using tert-butyl bromo-acetate **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$_2$CO$_3$ in DMF also proceeded with a moderate yield (59%). However, the use of CsOH−H$_2$O and activated molecular sieves, in the presence of tetrabutylammonium iodide in acetonitrile, provided the best results.[22] These conditions afforded the desired O-alkylated product **7** in 85%
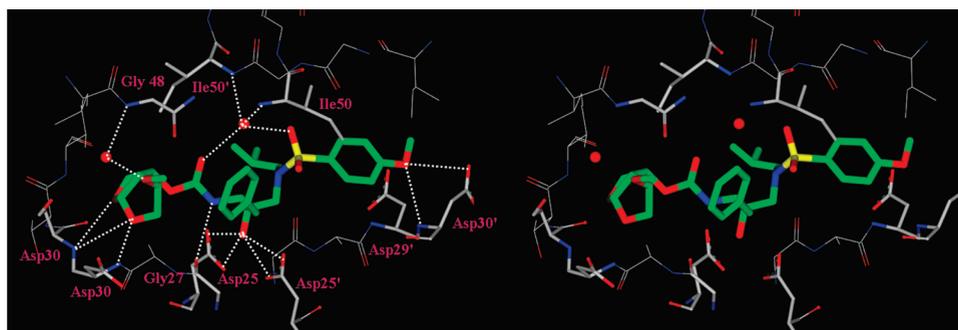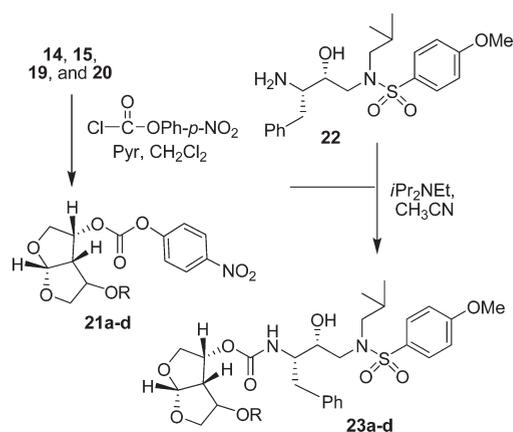
Figure 2. Stereoview of the X-ray structure of inhibitor **23c** (green)-bound HIV-1 protease (pdb code: 3QAA). All strong hydrogen bonding interactions are shown as dotted lines.

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**.[8] 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.[25] 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.[26,27] 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)^{a,b}
1.	**24** (TMC-126)[27]	0.014	1.4
2.	**23a**	0.035	55
3.	**23b**	0.073	335
4.	**23c** (GRL-04410)	0.0029	2.4
5.	**23d**	0.3	---

^{a} Values are means of at least three experiments. ^{b} Human T-lymphoid (MT-2) cells were exposed to 100 $TCID_{50}$ values of HIV-1$_{LAI}$ 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 Cα atoms.[28] The inhibitor interactions in the active site cavity consist of a series of hydrogen bonds and weaker CH···O interactions, as described previously for darunavir and GRL-09865.[28,29] 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···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

300

dx.doi.org/10.1021/ml100289m |*ACS Med. Chem. Lett.* 2011, 2, 298–302

can be altered by sequence polymorphisms and drug resistant mutations.[30−32] 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

ⓢ Supporting Information. Experimental procedures and ^{1}H- and ^{13}C-NMR spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: akghosh@purdue.edu (A.K.G.).

■ ACKNOWLEDGMENT

■ REFERENCES

(1) Ghosh, A. K. Harnessing Nature's Insight: Design of Aspartyl Protease Inhibitors from Treatment of Drug-Resistant HIV to Alzheimer's Disease. *J. Med. Chem.* **2009**, *52*, 2163–2176.

(2) FDA approves Darunavir on June 23, 2006: FDA approved a new HIV treatment for patients who do not respond to existing drugs. Please see http://www.fda.gov/bbs/topics/NEWS/2006/NEW01395.html.

(3) On October 21, 2008, FDA granted traditional approval to Prezista (darunavir), coadministered with ritonavir and with other antiretroviral agents, for the treatment of HIV-1 infection in treatment-experienced adult patients. In addition, a new dosing regimen for treatment-naïve adult patients was approved.

(4) Ghosh, A. K.; Sridhar, P. R.; Kumaragurubaran, N.; Koh, Y.; Weber, I. T.; Mitsuya, H. A Privileged Ligand for Darunavir and a New Generation of HIV-Protease Inhibitors That Combat Drug-Resistance. *ChemMedChem* **2006**, *1*, 939–950.

(5) Ghosh, A. K.; Chapsal, B. D.; Weber, I. T.; Mitsuya, H. Design of HIV Protease Inhibitors Targeting Protein Backbone: An Effective Strategy for Combating Drug Resistance. *Acc. Chem. Res.* **2008**, *41*, 78–86.

(6) Miller, J. F.; Andrews, C. W.; Brieger, M.; Furfine, E. S.; Hale, M. R.; Hanlon, M. H.; Hazen, R. J.; Kaldor, I.; McLean, E. W.; Reynolds, D.; Sammond, D. M.; Spaltenstein, A.; Tung, R.; Turner, E. M.; Xu, R. X.; Sherrill, R. G. Ultra-potent P1 modified arylsulfonamide HIV protease inhibitors: The discovery of GW0385. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1788–1794.

(7) Chilar, T.; He, G.-X.; Kiu, X.; Chen, J.; Hatada, M.; Swaminathan, S.; McDermott, M. J.; Yang, Z.-Y.; Mulato, A. S.; Chen, X.; Leavitt, S. A.; Stray, K. M.; Lee, W. A. Suppression of HIV-1 Protease Inhibitor Resistance by Phosphonate-mediated Solvent Anchoring. *J. Mol. Biol.* **2006**, *363*, 635–647.

(8) Ghosh, A. K.; Martyr, C. D. Darunavir (Prezista): A HIV-1 Protease Inhibitor for Treatment of Multidrug-Resistant HIV; Li, J. J., Johnson, D. S., Eds.; In *Modern Drug Synthesis*; Wiley: Hoboken, New Jersey, 2010; pp 29−44.

(9) Ghosh, A. K.; Chen, Y. Synthesis and Optical Resolution of High Affinity P2-Ligands for HIV-1 Protease Inhibitors. *Tetrahedron Lett.* **1995**, *36*, 505–508.

(10) Ghosh, A. K.; Kincaid, J. F.; Walters, D. E.; Chen, Y.; Chaudhuri, N. C.; Thompson, W. J.; Culberson, C.; Fitzgerald, P. M. D.; Lee, H. Y.; McKee, S. P.; Munson, P. M.; Duong, T. T.; Darke, P. L.; Zugay, J. A.; Schleif, W. A.; Axel, M. G.; Lin, J.; Huff, J. R. Nonpeptidal P2-Ligands for HIV Protease Inhibitors: Structure-Based Design, Synthesis and Biological Evaluations. *J. Med. Chem.* **1996**, *39*, 3278–3290.

(11) Ghosh, A. K.; Leshchenko, S.; Noetzel, M. Stereoselective Photochemical 1,3-Dioxolane Addition to α,β Unsaturated-γ-lactone: Synthesis of Bis-tetrahydrofuranyl Ligand for HIV Protease Inhibitor UIC-94-017 (TMC-114). *J. Org. Chem.* **2004**, *69*, 7822–7829.

(12) Ghosh, A. K.; Li, J.; Perali, R. S. A Stereoselective Anti-aldol Route to (3R,3aS,6aR)-Tetrahydro-2H-furo[2,3-b]furan-3-ol: A Key Ligand for a New Generation of HIV Protease Inhibitors. *Synthesis* **2006**, 3015–3018.

(13) Quaedflieg, P. J. L. M.; Kesteleyn, B. R. R.; Wigerinck, P. B. T. P.; Goyvaerts, N. M. F.; Jan Vijn, R.; Liebregts, C. S. M.; Kooistra, J. H. M. H.; Cusan, C. Stereoselective and Efficient Synthesis of (3R,3aS,6aR)-Hexahydrofuro[2,3-b]furan-3-ol. *Org. Lett.* **2005**, *7*, 5917–5920.

(14) Black, D. M.; Davis, R.; Doan, B. D.; Lovelace, T. C.; Millar, A.; Toczko, J. F.; Xie, S. Highly diastereo- and enantioselective catalytic synthesis of the bis-tetrahydrofuran alcohol of Brecanavir and Darunavir. *Tetrahedron: Asymmetry* **2008**, *19*, 2015–2019.

(15) Andrews, G. C.; Crawford, T. C.; Bacon, B. E. Stereoselective, catalytic reduction of L-ascorbic acid: a convenient synthesis of L-gulono-1,4-lactone. *J. Org. Chem.* **1981**, *46*, 2977–2979.

(16) Hubschwerlen, C.; Specklin, J.-L.; Higelin, J. L-(S)-Glyceraldehyde Acetonide. *Organic Syntheses*; Wiley & Sons: New York, 1998; Coll Vol. No. IX, p 454.

(17) Maloneyhuss, K. E. A Useful Preparation of L-(S)-Glyceraldehyde Acetonide. *Synth. Commun.* **1985**, *15*, 273–277.

(18) Hirth, G.; Walther, W. Synthesis of the (R)- and (S)-Glycerol Acetonides. Determination of the Optical Purity. *Helv. Chim. Acta* **1985**, *68*, 1863–1871.

(19) Al-Hakim, A. H.; Haines, A. H.; Morley, C. Synthesis of 1,2-O-Isopropylidene-L-threitol and its Conversion to (R)-1,2-O-Isopropylideneglycerol. *Synthesis* **1985**, *2*, 207–208.

(20) Mann, J.; Weymouth-Wilson, A. C. Photoinduced-Addition of Methanol to (5S)-(5-O-tert-Butyldimethylsiloxymethyl)Furan-2(5H)-one: (4R,5S)-4-Hydroxymethyl-(5-O-*tert*-Butyldimethyl siloxymethyl) Furan-2(5H)-one. *Organic Syntheses*; Wiley & Sons: New York, 2004; Collect. Vol. No. X, p 152; 1998.

(21) Borcherding, D. R.; et al. Potential inhibitors of S-adenosyl-methionine-dependent methyltransferases. Molecular dissections of neplanocin A as potential inhibitors of S-adenosylhomocysteine hydrolase. *J. Med. Chem.* **1988**, *31*, 1729–1738.

(22) Dueno, E. E.; Chu, F.; Kim, S.-I.; Jung, K. W. Cesium promoted O-alkylation of alcohols for the efficient ether synthesis. *Tetrahedron Lett.* **1999**, *40*, 1843–1846.

(23) Brückner, R.; Priepke, H. Asymmetric Induction in the [2,3]-Wittig Rearrangement by Chiral Substituents in the Allyl Moiety. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 278–280.

(24) Dodge, J. A.; Nissen, J. S.; Presnell, M. A General Procedure for Mitsunobu Inversion of Sterically Hindered Alcohols: Inversion of Menthol. (1S,2S,5R)-5-Methyl-2-(1-Methylethyl)Cyclohexyl 4-Nitrobenzoate. *Organic Syntheses*; Wiley & Sons: New York, 1998; Collect. Vol. No. IX, pp 607−611.

(25) Toth, M. V.; Marshall, G. R. A simple, continuous fluorometric assay for HIV protease. *Int. J. Pept. Protein Res.* **1990**, *36*, 544–550.

(26) Koh, Y.; Nakata, H.; Maeda, K.; Ogata, H.; Bilcer, G.; Devasamudram, T.; Kincaid, J. F.; Boross, P.; Wang, Y.-F.; Tie, Y.; Volarath, P.; Gaddis, L.; Harrison, R. W.; Weber, I. T.; Ghosh, A. K.; Mitsuya, H. A Novel bis-Tetrahydrofuranylurethane-containing Nonpeptide Protease Inhibitor (PI) UIC-94017 (TMC114) Potent Against Multi-PI-Resistant HIV In Vitro. *Antimicrob. Agents Chemother.* **2003**, *47*, 3123–3129.

(27) Yoshimura, K.; Kato, R.; Kavlick, M. F.; Nguyen, A.; Maroun, V.; Maeda, K.; Hussain, K. A.; Ghosh, A. K.; Gulnik, S. V.; Erickson, J. W.; Mitsuya, H. UIC-94003: A Potent Protease Inhibitor (PI) That Inhibits Multi-PI-Resistant HIV-1 Replication in vitro. *J. Virol.* **2002**, *76*, 1349–1358.

(28) Tie, Y.; Boross, P. I.; Wang, Y. F.; Gaddis, L.; Hussain, A. K.; Leshchenko, S.; Ghosh, A. K.; Louis, J. M.; Harrison, R. W.; Weber, I. T. High Resolution Crystal Structures of HIV-1 Protease with a Potent Non-Peptide Inhibitor (UIC-94017) Active Against Multi-Drug-Resistant Clinical Strains. *J. Mol. Biol.* **2004**, *338*, 341–352.

(29) Wang, Y. F.; Tie, Y.; Boross, P. I.; Tozser, J.; Ghosh, A. K.; Harrison, R. W.; Weber, I. T. Potent new antiviral compound shows similar inhibition and structural interactions with drug resistant mutants and wild type HIV-1 protease. *J. Med. Chem.* **2007**, *50*, 4509–4515.

(30) Kear, J. L.; Blackburn, M. E.; Veloro, A. M.; Dunn, B. M.; Fanucci, G. E. Subtype polymorphisms among HIV-1 protease variants confer altered flap conformations and flexibility. *J. Am. Chem. Soc.* **2009**, *131*, 14650–14651.

(31) Coman, R. M.; Robbins, A. H.; Goodenow, M. M.; Dunn, B. M.; McKenna, R. High-resolution structure of unbound human immunodeficiency virus 1 subtype C protease: implications of flap dynamics and drug resistance. *Acta Crystallogr., D: Biol. Crystallogr.* **2008**, *D64*, 754–763.

(32) Liu, F.; Kovalevsky, A. Y.; Louis, J. M.; Boross, P. I.; Wang, Y. F.; Harrison, R. W.; Weber, I. T. Mechanism of drug resistance revealed by the crystal structure of the unliganded HIV-1 protease with F53L mutation. *J. Mol. Biol.* **2006**, *358*, 1191–1199.