ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett Letter

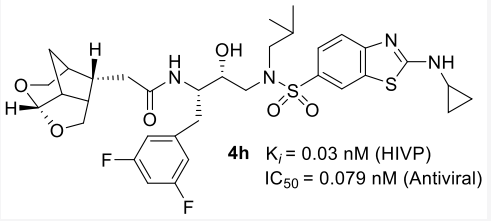
Design, Synthesis, and X-ray Studies of Potent HIV-1 Protease Inhibitors with P2-Carboxamide Functionalities

Arun K. Ghosh,* Alessandro Grillo, Jakka Raghavaiah, Satish Kovela, Megan E. Johnson, Daniel W. Kneller, Yuan-Fang Wang, Shin-ichiro Hattori, Nobuyo Higashi-Kuwata, Irene T. Weber, and Hiroaki Mitsuya

Cite This: https://dx.doi.org/10.1021/acsmedchemlett.9b00670 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The design, synthesis, biological evaluation, and X-ray structural studies are reported for a series of highly potent HIV-1 protease inhibitors. The inhibitors incorporated stereochemically defined amide-based bicyclic and tricyclic ether derivatives as the P2 ligands with (*R*)-hydroxyethylaminesulfonamide transition-state isosteres. A number of inhibitors showed excellent HIV-1 protease inhibitory and antiviral activity; however, ligand combination is critical for potency. Inhibitor **4h** with a difluorophenylmethyl as the P1 ligand, crown-THF-derived acetamide as the P2 ligand, and a cyclopropylaminobenzothiazole P2′-ligand displayed very potent antiviral activity and maintained excellent antiviral activity against selected multidrug-resistant HIV-1 variants. A high resolution X-ray structure of inhibitor **4h**-bound HIV-1 protease provided molecular insight into the binding properties of the new inhibitor.



KEYWORDS: *HIV-1 protease, cyclic ethers, carboxamide, multidrug-resistant, X-ray structure, backbone binding*

HIV-1 protease inhibitor drugs are important elements of current combined antiretroviral therapy (cART).[1,2] The use of cART has dramatically reduced both the mortality and morbidity rates among HIV-infected patients.[3,4] However, the emergence of multidrug-resistant HIV-1 variants has raised concerns as to the long-term viability of current treatment options.[5,6] In our continuing studies toward X-ray structure-based design of novel HIV-1 protease inhibitors to combat multidrug resistance, we recently reported a number of very potent inhibitors with intriguing structural features.[7,8] Our main inhibitor design strategies are based upon incorporation of nonpeptide cyclic-ether templates to effectively mimic peptide bond interactions in the active site.[9,10] Also, we strive to maximize active site ligand-binding site interactions, particularly to promote a robust network of hydrogen bonding interactions with the backbone atoms of HIV-1 protease. Over the years, these design principles have guided our development of HIV-1 protease inhibitors to combat drug resistance.[11,12] The latest FDA-approved protease inhibitor drug, darunavir (**1**, Figure 1) has been developed based upon these design concepts.[13,14] Darunavir emerged as a first-line therapy which is often preferred for cART-naïve HIV/AIDS patients due to its tolerance and a high genetic barrier to the development of drug-resistant viruses compared to other HIV-1 protease inhibitors.[15,16] This is possibly due to darunavir's unique dual mechanism of action as it inhibits the catalytic protease dimer and also protease monomer dimerization to form the active enzyme.[17,18] Darunavir (**1**) has been designed with a stereochemically defined bicyclic (3*R*,3a*S*,6a*R*)-*bis*-tetrahydrofuran heterocycle as the P2-ligand linked with a urethane functionality to a (*R*)-hydroxyethylamine sulfonamide dipeptide isosteric scaffold.[10] Detailed X-ray structural studies of darunavir-bound HIV-1 protease revealed critical darunavir-protease interactions responsible for its high affinity as well as its potent activity against multidrug-resistant HIV-1 variants.[19,20] Structurally, darunavir forms extensive van der Waals as well as hydrogen-bonding interactions throughout the protease active site.[19,20] In the S2 subsite, the *bis*-THF ligand oxygens are within hydrogen bonding distance to backbone Asp30 and Asp29 amide NHs. The urethane functionality also plays an important role in directing specific orientation of the *bis*-THF ligand oxygens for hydrogen bonding interactions. Both urethane NH and the carbonyl groups play an important role. The NH group forms a strong hydrogen bond with the Gly27 carbonyl of HIV-1 protease. The bicyclic ring of *bis*-THF, on the other hand, is engaged in extensive van der Waals interactions in the active site. The P2 urethane carbonyl functionality is very important. It forms a water-mediated tetracoordinated hydrogen bonding interaction with one of the sulfonamide oxygens of darunavir and amide

Special Issue: Medicinal Chemistry: From Targets to Therapies

Received: December 30, 2019
Accepted: February 27, 2020

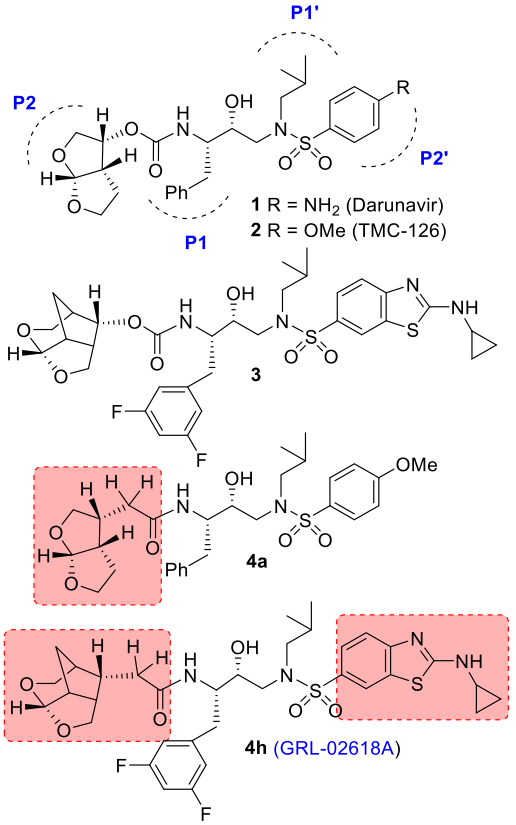


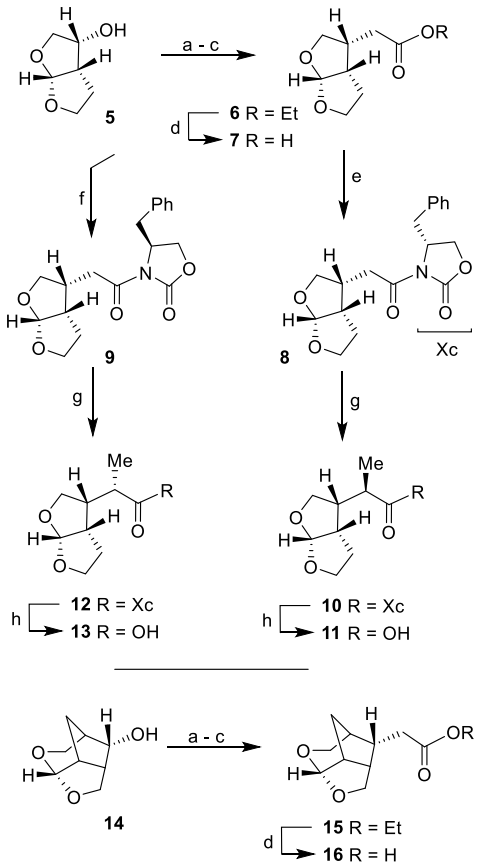
Figure 1. Structures of HIV-1 protease inhibitors **1**−**3**, **4a**, and **4h**.

NHs of Ile50 and Ile50′ in the flaps of HIV-1 protease. Similar water-mediated interactions have been observed with other HIV-1 protease inhibitors.[21,22]

Based upon the critical ligand-binding site interactions, we recently designed a crown-like tetrahydropyranotetrahydrofuran with a bridged methylene group as a very effective P2 ligand as represented in inhibitor **3** (Figure 1) with exceptional antiviral activity against multidrug-resistant HIV-1 strains.[7,23] The crown-THF P2 ligand is linked to an optimized nonpeptide transition-state mimetic via a urethane functionality. The X-ray structural studies of inhibitor **3**-bound HIV-1 protease show that compound **3** makes more extensive molecular interactions compared to darunavir.[7,8] The urethane-functionality, in general, shows good metabolic stability and drug properties.[24] However, one of the major metabolites of darunavir is the cleavage of the urethane functionality.[24,25] In general, carboxamides are metabolically more stable than their carbamate counterparts.[26] In a preliminary effort to improve drug properties, we sought to replace the urethane group. The carbamate group of darunavir has a direct role in drug−target interactions. In particular, the carbonyl oxygen forms a water-mediated hydrogen bond with the backbone Ile50′ amide NH in the S2 subsite.[21,22] We have explored replacement of the urethane with functionalities that can effectively link the P2 ligand with the transition-state mimetic amine and adopt favorable conformational and electronic bias to promote hydrogen bonding and van der Waals interactions in the active site. Herein we report our design of a new class of potent HIV-1 protease inhibitors incorporating stereochemically defined acetamide-based *bis*-tetrahydrofuran and hexahydro-methanofuropyran-derived P2-ligands. A number of inhibitors exhibited very potent enzyme inhibitory and antiviral activity. In particular, inhibitor **4h** maintained exceptional antiviral potency against selected multidrug-resistant HIV-1 variants. Our high resolution X-ray structural studies of inhibitor **4h**-bound HIV-1 protease provided important molecular insight into the ligand-binding site interactions.

The synthesis of various *bis*-THF-derived carboxamide P2 ligands started from known optically active alcohol **5**.[27−29] As shown in Scheme 1, alcohol **5** was converted to ethyl ester **6** in

Scheme 1. Synthesis of Carboxylic Acid P2 Ligands[a]



[a]Reaction conditions: (a) DMP, Na_2HPO_4, CH_2Cl_2, 23 °C, 3 h; (b) NaH, $(EtO)_2P(O)CH_2CO_2Et$, THF, 0 to 23 °C, 4 h (71% over 2-steps); (c) H_2, PtO_2 (cat), EtOAc, 23 °C, 2 h, (99%); (d) aq. LiOH, THF-H_2O (1:1) 23 °C, 3 h (96%); (e) pivaloyl chloride, LiCl, Et_3N, (*R*)-2-benzyl oxazolidinone, THF, 0 to 23 °C, 4 h (72%); (f) pivaloyl chloride, LiCl, Et_3N, 0 °C, then (*S*)-4-benzyl-2-oxazolidinone, THF, 0 to 23 °C (70%); (g) NaHMDS, THF, −78 °C, 45 min, then MeI, −78 °C, 4 h (64% for **10**; 61% for **12**); (h) aq. LiOH, H_2O_2, 0 to 23 °C, 3 h (87% for **11** and 86% for **13**). For ester **15** (71% yield over 3-steps) and for acid **16** (91%).

a three step sequence. Oxidation of alcohol **5** was carried out with Dess−Martin periodinane (DMP)[30] in CH_2Cl_2 in the presence of Na_2HPO_4 at 23 °C for 3 h to provide ketone. The resulting ketone was subjected to Horner−Wadsworth−Emmons reaction[31] with sodium hydride and triethyl phosphonoacetate in THF at 0 to 23 °C for 4 h to afford a mixture of α,β-unsaturated ester in 71% yield over two steps. The resulting unsaturated ester was hydrogenated with a catalytic amount of PtO_2 under a hydrogen-filled balloon at 23 °C for 2 h to provide near quantitative yield of the saturated ester **6** (91:9 mixture, 71% yield over 3-steps). The mixture

https://dx.doi.org/10.1021/acsmedchemlett.9b00670
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

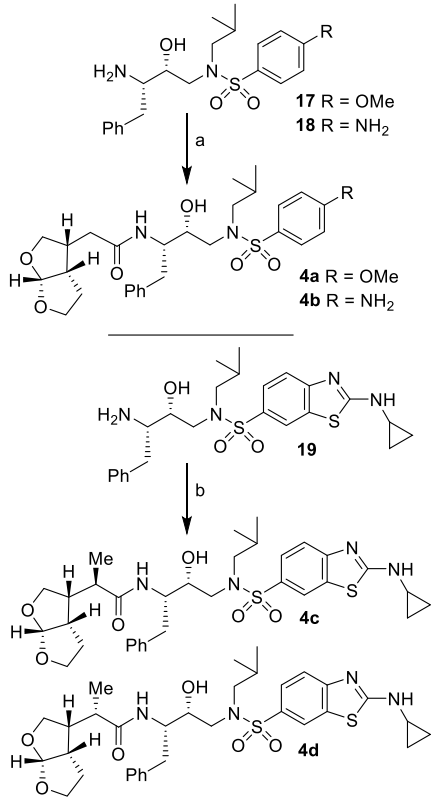
could not be separated by column chromatography. Saponification of the diastereomeric mixture of ester **6** with aqueous LiOH in THF at 0 to 23 °C for 3 h furnished carboxylic acid ligand **7** in 96% yield. Carboxylic acid **7** was utilized to incorporate methyl group to make van der Waals interactions in the active site of HIV-1 protease.

Thus, carboxylic acid **7** was converted to carboxamide **8** as follows.[32] Commercially available (*R*)-4-benzyl-2-oxazolidinone was mixed with acid **7** and LiCl in THF. The resulting lithio derivative was reacted with pivaloyl chloride and Et_3N at 0 to 23 °C for 4 h to provide oxazolidinone derivative **8** in 72% yield. Oxazolidinone derivative **9** with (*S*)-4-benzyl-2-oxazolidinone was prepared in a similar manner. For stereoselective incorporation of methyl group, oxazolidinone derivative **8** was treated with NaHMDS in THF at −78 °C for 45 min to provide the corresponding sodium enolate. Reaction of the enolate with methyl iodide at −78 °C for 4 h furnished methylated product **10** in 64% yield.[33] The corresponding diastereomeric oxazolidinone **12** afforded alkylation product **9** in 61% yield.[34] The removal of the oxazolidinone group was carried out by exposure to lithium hydroperoxide at 0 to 23 °C for 3 h to provide methylated ligand carboxylic acids **11** and **13** in 87% and 86% yields, respectively. The synthesis of carboxylic acid ligand containing crown-THF scaffold was carried out from the known optically active alcohol **14**.[7,8] This was converted to ethyl ester derivative in a three step process involving (1) DMP oxidation of alcohol **14** to the corresponding ketone; (2) Horner−Emmons reaction of the resulting ketone with triethyl phosphonoacetate to provide the corresponding α,β-unsaturated ester; and (3) hydrogenation of α,β-unsaturated ester using 10% Pd/C as the catalyst to provide **15** in 71% yield over 3-steps. Saponification of ethyl ester **15** using aqueous LiOH in THF furnished optically active ligand acid **16** in 91% yield.

The synthesis of various inhibitors containing *bis*-tetrahydrofuranyl acetamide as the P2 ligand is shown in Scheme 2. The known[7,8] 4-methoxybenzenesulfonamide **17** and 4-aminobenzenesulfonamide **18** isosteres were reacted with carboxylic acid **7** in the presence of HATU, DIPEA in DMF at 23 °C for 24 h to provide carboxamide inhibitors **4a** and **4b** in 77% and 79% yield, respectively. For the synthesis of inhibitors **4c** and **4d**, the known[7] amine **19** was coupled with acids **11** and **13** using HATU and DIPEA as described above to provide inhibitors **4c** and **4d** in 65% and 69% yield, respectively. The synthesis of various inhibitors containing *crown*-THF-derived acetamide as the P2 ligand is shown in Scheme 3. Amines **17**, **18**, **19**, and **20** were reacted with optically active carboxylic acid **16** in the presence of HATU, DIPEA in DMF at 23 °C as described above affording carboxamide inhibitors **4e**, **4f**, **4g**, and **4h** in very good yields.

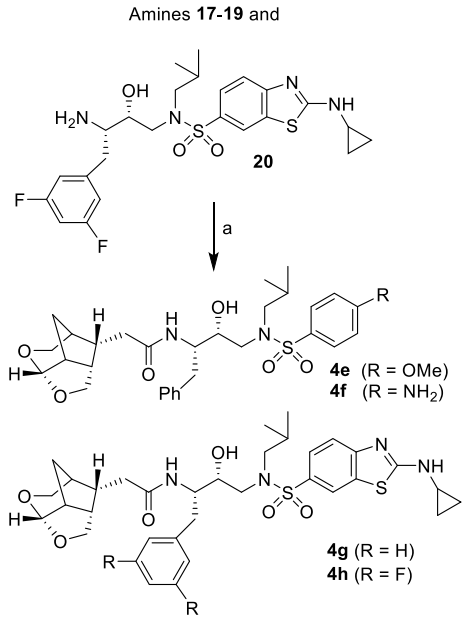
We have evaluated various carboxamide derivatives in Table 1 in both enzyme inhibitory and antiviral assays. HIV-1 protease inhibitory K_i was determined using the procedure described by Toth and Marshall.[35] Antiviral IC_{50} values were determined using MT-2 human T-lymphoid cells exposed to HIV-1_{LAI}.[36,37] Interestingly, both *bis*-tetrahydrofuranylacetamide derivatives **4a** and **4b** displayed significantly reduced HIV-1 protease inhibitory activity (K_i values of 0.28 nM and 17.4 nM, respectively) compared to darunavir (**1**) or its methoxy derivative **2** (entries 1 and 2) which showed K_i of 14 pM. Both carboxamide derivatives **4a** and **4b** did not show any appreciable antiviral IC_{50} value, while darunavir displayed antiviral IC_{50} value of 3.6 nM in the same assay. We then evaluated the effect of methyl group on the carboxamide ligand. Compound **4c** with a (*R*)-methyl substituent on the P2

Scheme 2. Synthesis of Carboxamide-Derived Inhibitor 4a−d[a]



[a]Reaction conditions: (a) acid **7**, HATU, DIPEA, DMF, 23 °C, 24 h, (77% for **4a** and 79% for **4b**); (b) acid **11** or acid **13**, HATU, DIPEA, DMF, 23 °C, 24 h (65% for **4c** and 69% for **4d**).

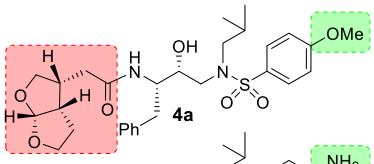
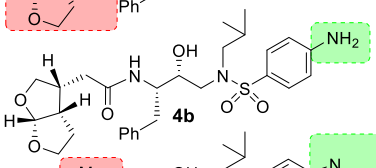
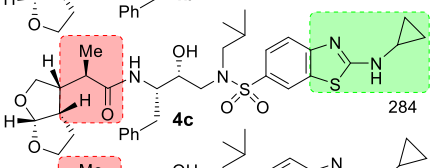
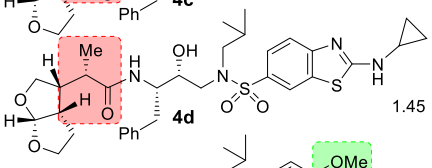
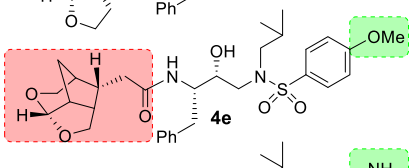
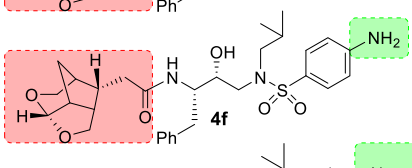
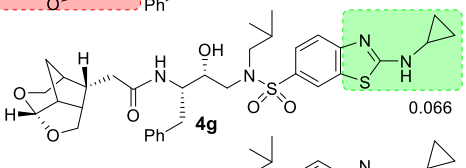
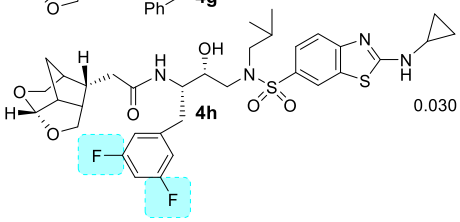
Scheme 3. Synthesis of Carboxamide-Derived Inhibitors 4e−g[a]



[a]Reaction conditions: (a) acid **16**, HATU, DIPEA, DMF, 23 °C, 24 h (73% for **4e**, 77% for **4f**, 70% for **4g**, and 68% for **4h**).

https://dx.doi.org/10.1021/acsmedchemlett.9b00670
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 1. HIV-1 Protease Inhibitory and Antiviral Activity of 4a−h

Entry	Inhibitor	K_i (nM)[a]	IC_{50}(nM)[b]
1.	 **4a**	0.28	>1000
2.	 **4b**	17.4	>1000
3.	 **4c**	284	>1000
4.	 **4d**	1.45	164
5.	 **4e**	0.032	2.9
6.	 **4f**	0.022	2.6
7.	 **4g**	0.066	2.6
8.	 **4h**	0.030	0.079

[a] K_i (HIV-1 protease) values represent at least 5 data points. Standard error in all cases was less than 7%. Darunavir exhibited K_i = 16 pM. [b] IC_{50} (HIV-1LAI) values are means of at least three experiments. Standard error in all cases was less than 5%. Darunavir exhibited antiviral IC_{50} = 3.2 nM, saquinavir IC_{50} = 21 nM; inhibitor **3**, K_i = 14 pM, IC_{50} = 17 pM.

ligand and aminobenzothiazole as the P2′-ligand showed reduction of K_i value compared to unsubstituted compound **4a**. Incorporation of methyl group did not improve antiviral activity of (*R*)-methyl derivative **4a**. Inhibitor **4d** with a (*S*)-methyl substituent on the P2 ligand however, showed improvement in both K_i and antiviral activity (entry 4) compared to its stereoisomeric derivative **4c** (entry 4). We have shown that the crown-THF ligand maintained similar backbone hydrogen bonding interactions as *bis*-THF ligand. In addition, crown-THF forms enhanced van der Waals interactions in the active site. We therefore, investigated inhibitors incorporating crown-THF-derived carboxamide derivatives as the P2 ligand. In inhibitor **4e**, we incorporated crown-THF containing acetamide derivative as the P2 ligand. The resulting inhibitor **4e** with a 4-methoxy sulfonamide as the P2′-ligand exhibited HIV-1 protease inhibitory K_i of 32 pM and antiviral IC_{50} value of 2.9 nM, which are comparable to the corresponding carbamate-derived inhibitors. Inhibitor **4f** with 4-aminosulfonamide as the P2′-ligand also showed very potent enzyme K_i as well as antiviral activity similar to darunavir and its methoxy derivatives (**1** and **2**). Combination of crown-THF containing acetamide derivative as the P2 ligand and cyclopropylamino benzothiazole as the P2′-ligand resulted in inhibitor **4g** which displayed with very potent K_i value as well as antiviral IC_{50} value (entry 7). We then examined the combined effect of the difluorophenylmethyl group as the P1-ligand in inhibitor, crown-THF-derived acetamide as the P2 ligand, and a cyclopropylaminobenzothiazole P2′-ligand. The resulting inhibitor **4h** displayed very potent activity with a K_i value of 30 pM and antiviral IC_{50} value of 79 pM (entry 8).

Based upon the antiviral data in preliminary studies, we then selected inhibitors **4e** and **4h** for further evaluation against a panel of highly multidrug-resistant HIV-1 variants that had been selected *in vitro* with widely used FDA-approved HIV-1 protease inhibitors, LPV, ATV, and DRV. Each of these HIV-1 variants were selected *in vitro* by propagating HIV-1_{NL4-3} or HIV_{8MIX}, a mixture of eight multidrug resistant clinical isolates, as starting viral populations in the presence of increasing concentrations of each drug (up to 5 μM) in MT-4 cells as reported by us previously.[36,38] The results are shown in Table 2. As can be seen, two current clinically used protease inhibitor drugs, LPV and ATV, lost significant activity against three highly PI-resistant HIV-1 variants examined. DRV displayed relatively better results; however, it too failed to effectively block replication of highly DRV-resistant HIV-1 variants. Inhibitor **3** containing a carbamate-linked crown-THF P2-ligand, an aminobenzotriazole P2′-ligand, and a difluorophenylmethyl as the P1-ligand showed variants tested except for highly DRV-resistant $HIV_{DRV}{}^{R}{}_{P51}$ variant selected in the presence of DRV over 51 viral passages. Inhibitor **4e** containing carboxamide-linked P2-crown-THF ligand and 4-methoxysulfonamide as the P2′-ligand showed comparable antiviral activity to DRV. Inhibitor **4h** containing a carboxamide-linked crown-THF P2-ligand, an aminobenzotriazole P2′-ligand and a difluorophenylmethyl as the P1-ligand also maintained superior activity against all HIV-1 variants comparable to one of our most potent carbamate-derived inhibitor **3**. Carboxamide derived inhibitor **4h** exerted exceptionally potent antiviral activity against HIV-$1_{LPV}{}^{R}{}_{5\ \mu M}$, HIV-$1_{ATV}{}^{R}{}_{5\ \mu M}$, and HIV-$1_{DRV}{}^{R}{}_{P20}$ with IC_{50} values ranging from 1.1 to 5.4 pM. Furthermore, against highly DRV-resistant variants, HIV-$1_{DRV}{}^{R}{}_{P30}$ and $HIV_{DRV}{}^{R}{}_{P51,}$ inhibitor **4h** maintained antiviral IC_{50} values of 39 pM and 14 nM, respectively. Our X-ray crystallographic studies of inhibitor **4h**-bound HIV-1 protease provided molecular insight into the carboxamide-derived inhibitor's potency.

We determined high resolution X-ray crystal structure of inhibitor **4h**-bound wild-type HIV-1 protease. The X-ray structure was determined at the near-atomic resolution of 1.18 Å to establish accurate protein−inhibitor interactions.[39] The complex crystallized in orthorhombic space group $P2_12_12$ with one protease homodimer per asymmetric unit. The inhibitor binds in the active site in two alternate conformations related by 180° rotation with relative occupancy of 0.55/0.45. The two alternate conformations are very similar so only the major one will be described here. Inhibitor **4h** contains a crown-THF-derived carboxamide P2 ligand. It is similar to inhibitor **3**

https://dx.doi.org/10.1021/acsmedchemlett.9b00670
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 2. Antiviral Activity of Compounds 4e and 4g against a Panel of Multidrug-Resistant HIV-1 Variants

	IC_{50} (nM) ± SD[a,b]					
Virus species	LPV	ATV	DRV	Compound **3**	Compound **4e**	Compound **4h**
HIV-1_{NL4-3}	27 ± 2	5.0 ± 1	4.6 ± 0.5	0.0042 ± 0.00006	1.0 ± 0.04	0.0045 ± 0.0004
HIV-$1_{LPV^{R}\ 5\ \mu M}$	>1,000	309 ± 25	50 ± 13	0.00018 ± 0.00006	88 ± 22	0.0011 ± 0.0004
HIV-$1_{ATV^{R}\ 5\ \mu M}$	340 ± 44	>1,000	11 ± 1.2	0.0025 ± 0.0003	9.0 ± 1.4	0.0054 ± 0.0006
HIV-$1_{DRV^{R}\ P20}$	>1,000	>1,000	316 ± 41	0.00055 ± 0.00007	299 ± 23	0.0040 ± 0.0001
HIV-$1_{DRV^{R}\ P30}$	>1,000	>1,000	424 ± 100	0.0047 ± 0.0004	425 ± 108	0.039 ± 0.012
HIV-$1_{DRV^{R}\ P51}$	>1,000	>1,000	>1,000	5.0 ± 0.4	>1,000	14 ± 1.2

[a]The amino acid substitutions identified in the protease-encoding region compared to the wild-type HIV-1_{NL4-3} were L23I, E34Q, K43I, M46I, I50L, G51A, L63P, A71V, V82A, and T91A in HIV-$1_{ATV\text{-}5\ \mu M}$; L10F, V32I, M46I, I47A, A71V, and I84V in HIV-$1_{LPV\text{-}5\ \mu M}$; L10I, I15V, K20R, L24I, V32I, M36I, M46L, L63P, V82A, and L89M in HIV-$1_{DRV^{R}\ P20}$; L10I, I15V, K20R, L24I, V32I, M36I, M46L, L63P, K70Q, V82A, I84V, and L89M in HIV-$1_{DRV^{R}\ P30}$; L10I, I15V, K20R, L24I, V32I, L33F, M36I, M46L, I54M, L63P, K70Q, V82I, I84V, and L89M in HIV-$1_{DRV^{R}\ P51}$. [b]The IC_{50} (50% inhibitory concentration) values were determined by using MT-4 cells as target cells. MT-4 cells (10^5/mL) were exposed to 100 $TCID_{50}$s of each HIV-1, and the inhibition of p24 Gag protein production by each drug was used as an end point. All assays were conducted in duplicate, and the data shown represent mean values (±S.D.) derived from the results of two independent experiments. LPV, lopinavir; ATV, atazanavir; DRV, darunavir.

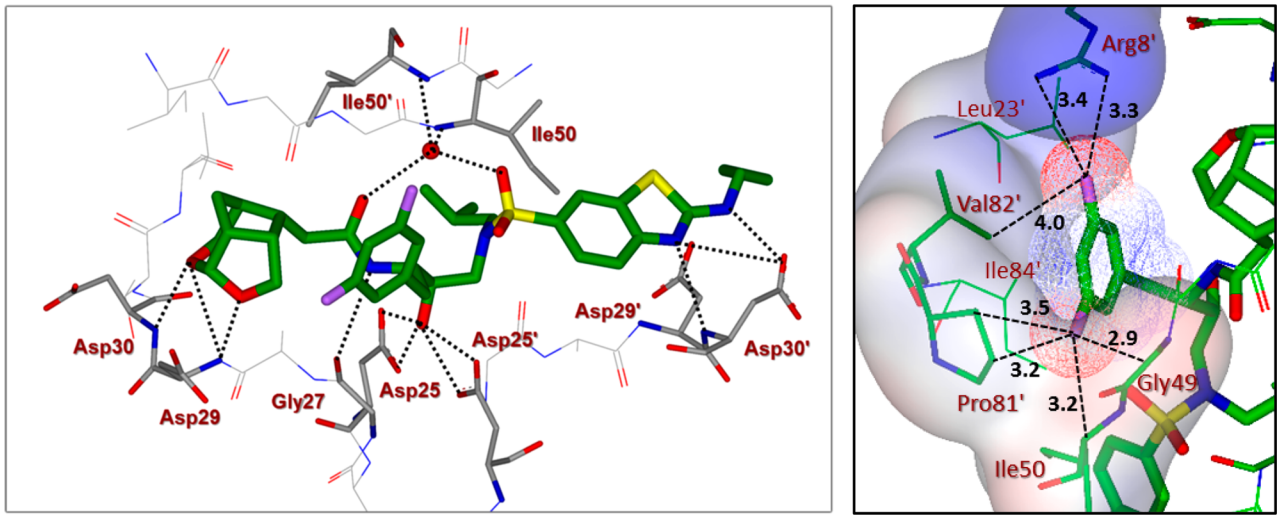


Figure 2. (A) Inhibitor **4h**-bound X-ray structure of HIV-1 protease. The major orientation of the inhibitor is shown. The inhibitor carbon atoms are shown in green, the water molecule is a red sphere, and the hydrogen bonds are indicated by dotted lines. (B) The van der Waals surface and interactions of the fluorinated P1 ligand are shown (PDB ID: 6VCE).

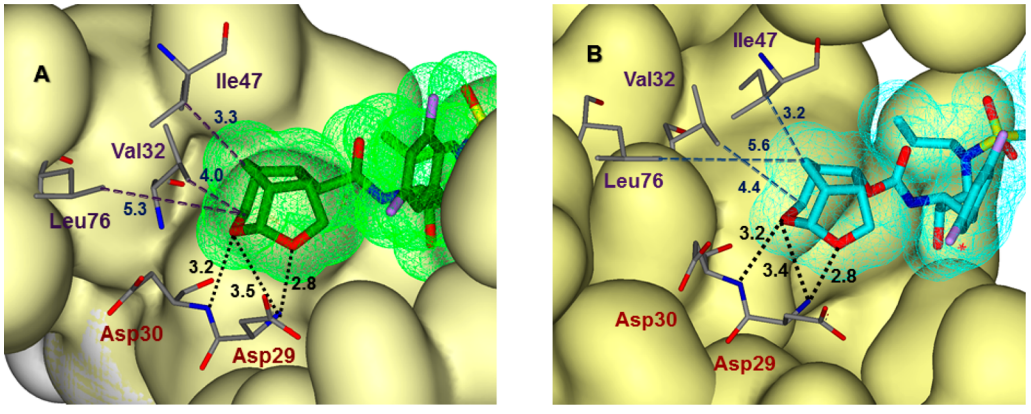


Figure 3. Side by side comparison of the new carboxamide P2-ligand containing *Crown*-THF moiety of inhibitor **4h** (A, green carbon chain) with the urethane *Crown*-THF moiety of inhibitor **3** (B, cyan carbon chain) inside the S2 subpocket. Both ligands form extensive van der Waals interactions (Val32 and Ile47) in the S2 subsite. Also, they are located close to the periphery of the protease active site and form three strong hydrogen bonds in a similar fashion (black dotted lines).

except that the P2 urethane linker has been replaced with a carboxamide linker. The overall dimer structure is similar to inhibitor **3**-HIV-1 protease complex[8] with a low RMSD of 0.13 Å for 198 equiv Cα atoms. The largest deviation of 0.6 Å between the two structures occurs at residue Gly49′ of the flap region. As highlighted in Figure 2, all major hydrogen bonds

https://dx.doi.org/10.1021/acsmedchemlett.9b00670
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

observed between inhibitor **3** and the backbone atoms of protease are retained in the structure of **4h**-bound HIV-1 protease.

The two fluorine atoms in the P1 group of inhibitor **4h** preserve the important halogen bond interactions with the protease seen for the structure of **3**-bound HIV-1 protease. One fluorine atom forms a halogen bond with the amide NH of Ile50. The second fluorine atom forms multipolar interactions with one conformation of the guanidium group of Arg8′, which is involved in a critical intersubunit ion pair with Asp29. Both alternate conformations of the guanidium group of Arg8′ preserve the ion pair with Asp29. Compared to the urethane linker of inhibitor **3**, the C atom in the carboxamide linker of inhibitor **4h** shifts toward Cδ1 of Ile84 by almost 0.9 Å and forms a new van der Waals interaction. The positions of nearby atoms from the carbonyl group in the linker to the whole crown-like P2 group also shift and consequently alter the van der Waals interactions of inhibitor with Gly49 and Ile50. The semirigid crown-like P2 group retains the shape and relative positions of P2 group of inhibitor **3**. However, inhibitor **4h** may show slight improvement in interactions with protease. We have compared hydrogen bonding and van der Waals interactions of the crown-THF urethane ligand of inhibitor **3** with crown-THF-derived acetamide ligand of inhibitor **4h** in the X-ray structures. As shown in Figure 3, the hydrogen bonding distances of acetal oxygens with Asp29 and Asp30 backbone amide NHs are nearly identical. All van der Waals interactions with Ile47 and Val32 are also very similar, although inhibitor **4h** forms a stronger van der Waals contact with Val32. Furthermore, aminobenzothiazole P2′ ligands form similar hydrogen bonding with Asp30′ backbone amide NH and van der Waals contacts in the S2′ subsite. These extensive active site interactions are responsible for the inhibitor's high affinity for HIV-1 protease as well as its potent antiviral activity against multidrug-resistant HIV-1 strains.

In conclusion, we investigated the replacement of urethane functionality of darunavir with designed carboxamide derivatives that can maintain key backbone binding interactions in the HIV-1 protease active site. Compounds with *bis*-tetrahydrofuran-derived carboxamide resulted in substantial reduction of potency compared to the corresponding carbamate derivatives. However, incorporation of crown-THF-derived carboxamide derivatives provided very potent inhibitors. In particular, compound **4h** is remarkably potent (K_i = 30 pM; IC_{50} = 79 pM), several orders of magnitude more potent than the latest approved PI drug, darunavir. Inhibitor **4h** also maintained very potent antiviral activity against a wide spectrum of multidrug-resistant HIV-1 variants. The observed antiviral activity of compound **4h** is superior to darunavir. An inhibitor **4h**-bound HIV-1 protease X-ray structure revealed extensive interactions of the inhibitor in active site. Particularly, the compound maintained a network of hydrogen bonding interactions with the backbone amide NHs in the active site. Further optimization of inhibitor's properties is in progress.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.9b00670.

Synthesis of inhibitors, full NMR spectroscopic data for all final compounds, X-ray structural data for inhibitor **4h**-bound HIV-1 protease, molecular formula strings and some data, and Virus and cell biology protocols (PDF)

Accession Codes

Inhibitor **4h**-bound HIV-1 protease X-ray structure is 6VCE. Authors will release the atomic coordinates and experimental data upon article publication.

■ AUTHOR INFORMATION

Corresponding Author

Arun K. Ghosh − *Department of Chemistry and Department of Medicinal Chemistry, Purdue University, West Lafayette, Indiana 47907, United States;* orcid.org/0000-0003-2472-1841; Email: akghosh@purdue.edu

Authors

Alessandro Grillo − *Department of Chemistry and Department of Medicinal Chemistry, Purdue University, West Lafayette, Indiana 47907, United States*

Jakka Raghavaiah − *Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States*

Satish Kovela − *Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States*

Megan E. Johnson − *Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States*

Daniel W. Kneller − *Department of Biology, Georgia State University, Atlanta, Georgia 30303, United States*

Yuan-Fang Wang − *Department of Biology, Georgia State University, Atlanta, Georgia 30303, United States*

Shin-ichiro Hattori − *Department of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute, Tokyo 162-8655, Japan*

Nobuyo Higashi-Kuwata − *Department of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute, Tokyo 162-8655, Japan*

Irene T. Weber − *Department of Biology, Georgia State University, Atlanta, Georgia 30303, United States;* orcid.org/0000-0003-4876-7393

Hiroaki Mitsuya − *Department of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute, Tokyo 162-8655, Japan; Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, United States; Department of Clinical Sciences, Kumamoto University Hospital, Kumamoto 860-8556, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.9b00670

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health (Grant AI150466, AKG and Grant AI150461, ITW) and a Georgia State University Molecular Basis of Disease fellowship (DWK). X-ray data were collected at the Southeast Regional Collaborative Access Team (SER-CAT) beamline 22ID at the Advanced Photon Source, Argonne National Laboratory. Use of the Advanced Photon Source was supported by the US Department of Energy, Basic Energy

Sciences, Office of Science, under Contract No. W-31-109-Eng-38. This work was also supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health (HM), and in part by grants for the promotion of AIDS research from the Ministry of Health; grants from Welfare and Labor of Japan (HM); grants for the Research Program on HIV/AIDS from the Japan Agency for Medical Research and Development (AMED) under grant numbers JP15fk0410001 and JP16fk0410101 (HM); a grant from the National Center for Global Health and Medicine (NCGM) Research Institute (HM); and a Grant-in-Aid for Scientific Research (Priority Areas) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Monbu Kagakusho)(HM). The authors would like to thank the Purdue University Center for Cancer Research, which supports the shared NMR and mass spectrometry facilities. The authors also thank Manabu Aoki for discussion.

■ ABBREVIATIONS

ATV, atazanavir; bis-THF, bis-tetrahydrofuran; cART, combined antiretroviral therapies; DIPEA, *N*,*N*-diisopropyletylamine; DRV, darunavir; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; HMDS, hexamethyldisilazane; LPV, lopinavir

■ REFERENCES

(1) Naggie, S.; Hicks, C. Protease inhibitor-based antiretroviral therapy in treatment-naive HIV-1-infected patients: the evidence behind the options. *J. Antimicrob. Chemother.* **2010**, *65*, 1094−1099.
(2) Broder, S. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. *Antiviral Res.* **2010**, *85*, 1−18.
(3) Lohse, N.; Hansen, A. B.; Gerstoft, J.; Obel, N. Improved survival in HIV-infected persons: consequences and perspectives. *J. Antimicrob. Chemother.* **2007**, *60*, 461−463.
(4) Montaner, J. S. G.; Lima, V. D.; Barrios, R.; Yip, B.; Wood, E.; Kerr, T.; Shannon, K.; Harrigan, P. R.; Hogg, R. S.; Daly, P.; Kendall, P. Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study. *Lancet* **2010**, *376*, 532−539.
(5) Esté, J. A.; Cihlar, T. Current status and challenges of antiretroviral research and therapy. *Antiviral Res.* **2010**, *85*, 25−33.
(6) Waters, L.; Nelson, M. Why do patients fail HIV therapy? *Int. J. Clin. Prac.* **2007**, *61*, 983−990.
(7) Ghosh, A. K.; Rao, K. V.; Nyalapatla, P. R.; Osswald, H. L.; Martyr, C. D.; Aoki, M.; Hayashi, H.; Agniswamy, J.; Wang, Y.-F.; Bulut, H.; Das, D.; Weber, I. T.; Mitsuya, H. Design and development of highly potent HIV-1 protease inhibitors with a crown-like oxotricyclic core as the P2-ligand to combat multidrug-resistant HIV variants. *J. Med. Chem.* **2017**, *60*, 4267−4278.
(8) Ghosh, A. K.; Rao, K. V.; Nyalapatla, P. R.; Kovela, S.; Brindisi, M.; Osswald, H. L.; Reddy, B. S.; Agniswamy, J.; Wang, Y.-F.; Aoki, M.; Hattori, S.-i.; Weber, I. T.; Mitsuya, H. Design of highly potent, dual acting and central nervous system penetrating HIV-1 protease inhibitors with excellent potency against multidrug-resistant HIV-1 variants. *ChemMedChem* **2018**, *13*, 803−815.
(9) Ghosh, A. K.; Chapsal, B. D. Design of the anti-HIV-1 protease inhibitor darunavir in *Introduction to biological and small molecule drug research and development: theory and case studies*; Ganellin, C. R., Jefferis, R., Roberts, S., Eds.; Elsevier: London, 2013; pp 355−384.
(10) Ghosh, A. K.; Sridhar, P. R.; Kumaragurubaran, N.; Koh, Y.; Weber, I. T.; Mitsuya, H. Bis-tetrahydrofuran: a privileged ligand for darunavir and a new generation of HIV protease inhibitors that combat drug resistance. *ChemMedChem* **2006**, *1*, 939−950.
(11) Ghosh, A. K.; Anderson, D. D.; Weber, I. T.; Mitsuya, H. Enhancing protein backbone binding—A fruitful concept for combating drug-resistant HIV. *Angew. Chem., Int. Ed.* **2012**, *51*, 1778−1802.
(12) 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.
(13) Ghosh, A. K.; Dawson, Z. L.; Mitsuya, H. Darunavir, a conceptually new HIV-1 protease inhibitor for the treatment of drug-resistant HIV. *Bioorg. Med. Chem.* **2007**, *15*, 7576−7580.
(14) de Béthune, M. P.; Sekar, V.; Spinosa-Guzman, S.; Vanstockem, M.; De Meyer, S.; Wigerinck, P.; Lefebvre, E. Darunavir (Prezista, TMC114): From bench to clinic, improving treatment options for HIV-infected patients. In *Antiviral Drugs: From Basic Discovery Through Clinical Trials*; John Wiley & Sons, Inc.: NJ, 2011; pp 31−45.
(15) Koh, Y.; Nakata, H.; Maeda, K.; Ogata, H.; Bilcer, G.; Devasamundram, 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. Novel *bis*-tetrahydrofuranylurethane-containing nonpeptidic protease inhibitor (PI) UIC-94017 (TMC114) with potent activity against multi-PI-resistant human immunodeficiency virus in vitro. *Antimicrob. Agents Chemother.* **2003**, *47*, 3123−3129.
(16) De Meyer, S.; Azijn, H.; Surleraux, D.; Jochmans, D.; Tahri, A.; Pauwels, R.; Wigerinck, P.; de Béthune, M. P. TMC114, a novel human immunodeficiency virus type 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range of clinical isolates. *Antimicrob. Agents Chemother.* **2005**, *49*, 2314−2321.
(17) Koh, Y.; Matsumi, S.; Das, D.; Amano, M.; Davis, D. A.; Li, J.; Leschenko, S.; Baldridge, A.; Shioda, T.; Yarchoan, R.; Ghosh, A. K.; Mitsuya, H. Potent inhibition of HIV-1 replication by novel nonpeptidyl small molecule inhibitors of protease dimerization. *J. Biol. Chem.* **2007**, *282*, 28709−28720.
(18) Hayashi, H.; Takamune, N.; Nirasawa, T.; Aoki, M.; Morishita, Y.; Das, D.; Koh, Y.; Ghosh, A. K.; Misumi, S.; Mitsuya, H. Dimerization of HIV-1 protease occurs through two steps relating to the mechanism of protease dimerization inhibition by darunavir. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 12234−12239.
(19) 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.
(20) Kovalevsky, A. Y.; Liu, F.; Leshchenko, S.; Ghosh, A. K.; Louis, J. M.; Harrison, R. W.; Weber, I. T. Ultra-high resolution crystal structure of HIV-1 protease mutant reveals two binding sites for clinical inhibitor TMC114. *J. Mol. Biol.* **2006**, *363*, 161−173.
(21) Wlodawer, A.; Vondrasek, J. Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249−284.
(22) Ogden, R. C.; Flexner, C. W. *Protease inhibitors in AIDS therapy*; Marcel Dekker, Inc.: New York, NY, 2001.
(23) Aoki, M.; Hayashi, H.; Rao, K. V.; Das, D.; Higashi-Kuwata, N.; Bulut, H.; Aoki-Ogata, H.; Takamatsu, Y.; Yedidi, R. S.; Davis, D. A.; Hattori, S.-i.; Nishida, N.; Hasegawa, K.; Takamune, N.; Nyalapatla, P. R.; Osswald, H. L.; Jono, H.; Saito, H.; Yarchoan, R.; Misumi, S.; Ghosh, A. K.; Mitsuya, H. A Novel central nervous system-penetrating protease inhibitor overcomes human immunodeficiency virus 1 resistance with unprecedented aM to pM potency. *eLife* **2017**, *6*, No. e28020.
(24) Ghosh, A. K.; Anderson, D. D.; Mitsuya, H. The FDA approved HIV-1 protease inhibitors for treatment of HIV/AIDS. In *Burger's Medicinal Chemistry, Drug Discovery and Development*, 7th ed.; Abraham, D. J., Rotella, D. P., Eds.; 2010; Vol. 7, pp 1−72.
(25) de Meyer, S.; Vangeneugden, T.; van Baelen, B.; de Paepe, E.; van Marck, H.; Picchio, G.; Lefebvre, E.; de Béthune, M.-P. Resistance profile of darunavir: Combined 24-week results from the POWER trials. *AIDS Res. Hum. Retroviruses* **2008**, *24*, 379−388.

https://dx.doi.org/10.1021/acsmedchemlett.9b00670
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

(26) Karaman, R. Prodrugs design based on inter- and intramolecular chemical processes. *Chem. Biol. Drug Des.* **2013**, *82*, 643−668.
(27) 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.
(28) 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.
(29) 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 (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-ol. *Org. Lett.* **2005**, *7*, 5917−5920.
(30) Dess, D. B.; Martin, J. C. Readily accessible 12-I-5 oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones. *J. Org. Chem.* **1983**, *48*, 4155−4156.
(31) Wadsworth, W. Synthetic applications of phosphoryl-stabilized anions. In *Organic Reactions*; Wiley: New York, 1977; Vol. *25*; pp 73−253.
(32) Alimardanov, A.; Nikitenko, A.; Connolly, T. J.; Feigelson, G.; Chan, A. W.; Ding, Z.; Ghosh, M.; Shi, X.; Ren, J.; Hansen, E.; Farr, R.; MacEwan, M.; Tadayon, S.; Springer, D. M.; Kreft, A. F.; Ho, D. M.; Potoski, J. R. Practical enantioselective synthesis of a 3-aryl-3-trifluoromethyl-2-aminopropanol derivative. *Org. Process Res. Dev.* **2009**, *13*, 1161−1168.
(33) Evans, D. A.; Ennis, M. D.; Mathre, D. J. Asymmetric alkylation reactions of chiral imide enolates. A practical approach to the enantioselective synthesis of α-substituted carboxylic acid derivatives. *J. Am. Chem. Soc.* **1982**, *104*, 1737−1739.
(34) The ^{1}H NMR analysis (400 MHz) after chromatography showed the presence of a 94:6 mixture of diastereomers, presumably resulting from the *cis/trans* mixture of acid **7**.
(35) Toth, M. V.; Marshall, G. R. A Simple, continuous fluorometric assay for HIV protease. *Int. J. Pept. Protein Res.* **1990**, *36*, 544−550.
(36) Koh, Y.; Amano, M.; Towata, T.; Danish, M.; Leshchenko-Yashchuk, S.; Das, D.; Nakayama, M.; Tojo, Y.; Ghosh, A. K.; Mitsuya, H. In vitro selection of highly darunavir-resistant and replication-competent HIV-1 variants by using a mixture of clinical HIV-1 isolates resistant to multiple conventional protease inhibitors. *J. Virol.* **2010**, *84*, 11961−11969.
(37) Aoki, M.; Danish, M. L.; Aoki-Ogata, H.; Amano, M.; Ide, K.; Das, D.; Koh, Y.; Mitsuya, H. Loss of the protease dimerization inhibition activity of tipranavir (TPV) and its association with the acquisition of resistance to TPV by HIV-1. *J. Virol.* **2012**, *86*, 13384−13396.
(38) Aoki, M.; Hayashi, H.; Yedidi, R. S.; Martyr, C. D.; Takamatsu, Y.; Aoki-Ogata, H.; Nakamura, T.; Nakata, H.; Das, D.; Yamagata, Y.; Ghosh, A. K.; Mitsuya, H. C-5-Modified tetrahydropyrano-tetrahydofuran-derived protease inhibitors (HIV-1 protease inhibitors) exert potent inhibition of the replication of HIV-1 variants highly resistant to various HIV-1 protease inhibitors, including darunavir. *J. Virol.* **2016**, *90*, 2180−2194.
(39) For details of X-ray studies, please see the Supporting Information.

https://dx.doi.org/10.1021/acsmedchemlett.9b00670
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX