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An enantioselective enzymatic desymmetrization route to hexahydro-4*H*-furopyranol, a high-affinity ligand for HIV-1 protease inhibitors

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ABSTRACT

An enantioselective synthesis of (3a*S*,4*S*,7a*R*)-hexahydro-4*H*-furo[2,3-*b*]pyran-4-ol, a high-affinity non-peptide ligand for a variety of potent HIV-1 protease inhibitors is described. The key steps involved a highly enantioselective enzymatic desymmetrization of *meso*-diacetate, an efficient transacetalization, and a highly diastereoselective reduction of a ketone. This route is amenable to large-scale synthesis using readily available starting materials.

The development of combined antiretroviral therapy (cART) with HIV-1 protease inhibitors dramatically transformed HIV/AIDS from a terminal disease to a chronic ailment.[1,2] This treatment regimen improved life expectancies of those patients with access to cART.[3,4] Despite this remarkable progress, there are numerous drug-related complications. Perhaps, the most concerning is the rapid emergence of multidrug-resistant HIV-1 variants which severely limit the current and future management of HIV/AIDS.[5–7] In our continuing efforts in developing novel protease inhibitors (PIs) with broad-spectrum activity against multidrug-resistant HIV-1 variants, we reported inhibitors containing fused *bis*-tetrahydrofuran and tetrahydropyranyl tetrahydrofuran represented in PIs **1**–**4** (Fig. 1).[8–10] Among these PIs, darunavir (**1**) is an FDA approved drug and it is used as a front-line therapy for HIV/AIDS patients.[11,12] Darunavir and its methoxy derivative (**1** and **2**) contain (3*R*,3a*S*,6a*R*)-*bis*-tetrahydrofuranyl (*bis*-THF) urethane as the P2 ligand.[8,9] Darunavir's exceptional antiretroviral activity against multidrug-resistant clinical isolates and high genetic barrier to resistance is attributed to the presence of the *bis*-THF ligand.[13–15] Darunavir exhibited dual mechanism of action by blocking HIV-1 protease enzymatic activity as well as by inhibiting dimerization of protease monomers.[16] Structurally, darunavir was designed to make extensive interactions in the active site of HIV-1 protease, particularly with the backbone atoms from S2 and S2′ subsites.[9,17]

In an effort to further optimize the *bis*-THF structural template of darunavir, we explored modifications that would enhance the 'backbone binding' as well as improve hydrophobic interactions in the protease active site.[10,17] Interestingly, the ring-fusion, stereochemistry, and position of urethane are critical to inhibitor potency. Inhibitor **3**, with a (3*R*,3a*S*,7a*R*)-hexahydro-4*H*-furo[2,3-*b*]pyran-3-ol, is significantly less potent than inhibitors **1** and

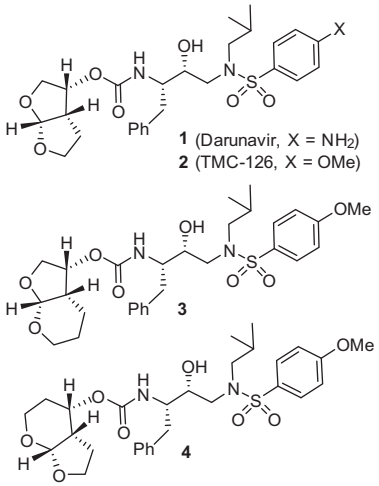

Fig. 1. Structures of protease inhibitors **1**–**4**.

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2.[8,11] However, incorporation of isomeric (3a*S*,4*S*,7a*R*)hexahydro-4*H*-furo[2,3-*b*]pyran-4-ol (*Tp*-THF) resulted in inhibitor **4** which showed excellent protease inhibitory activity (K_i = 2.7 pM) and improved antiviral activity in MT cells (IC_{50} = 0.5 nM) compared to inhibitor **2** (K_i = 14 pM, IC_{50} = 2.3 nM).[18] Furthermore, inhibitor **4** showed retention of antiviral activity against a panel of multidrug-resistant clinical HIV-1 strains with IC_{50} values in the nanomolar range and is superior to other approved PIs and comparable to darunavir.[15,18] The X-ray structural analysis revealed that the hydrogen bonding interactions of both P2 ligand oxygens are significantly stronger than the *bis*-THF ligand in darunavir or inhibitor **2** (Fig. 2).[19,20] This may be due to the fact that the *Tp*-THF ligand of inhibitor **4** has higher affinity for the active site of HIV-1 protease. For the synthesis of *Tp*-THF ligand, we previously converted optically active bicyclic lactone **5** to (3a*S*,4*S*,7a*R*)-hexahydro-4*H*-furo[2,3-*b*]pyran-4-ol **6**.[18] Lactone **5** was obtained from cyclopentene diacetate as the key starting material.[21] To expand the use of the *Tp*-THF ligand, we have now explored an alternative synthetic route using readily available and inexpensive starting materials. Herein, we report a convenient enantioselective synthesis of (3a*S*,4*S*,7a*R*,)-hexahydro-4*H*-furo[2,3-*b*]pyran-4-ol using an efficient enzymatic desymmetrization of *meso*-diacetate as the key reaction.

The synthetic strategy for optically active hexahydro-4*H*-furopyranol **6** is shown in Scheme 1. The 6,5-fused ring structure would be obtained through a transacetalization reaction on diol **7**. Optically active diol **7** can be derived from the oxidative cleavage of cyclohexene derivative **8**, which could be obtained from optically active alcohol **9**. Alcohol **9** can be prepared from commercially available *meso*-diacetate **10** by an enzymatic desymmetrization process.

The synthesis and enzymatic desymmetrization are shown in Scheme 2. *Cis-meso*-diacetate **10** was prepared in multigram quantity from commercially available, inexpensive, 1,2,3,6-tetrahydrophthalic anhydride **11** by LAH reduction followed by acetylation as reported in the literature.[22] Initially, we carried out enzymatic desymmetrization of *meso*-diacetate **10** by treatment with Porcine Pancreatic Lipase (PPL, 5% w/w, Sigma, type II, crude)[23] in 0.1 M phosphate buffer (pH 7) over 24 h to afford optically active monoacetate **9** in >95% *ee*. During this enzymatic reaction, aqueous 1 N NaOH was added dropwise to neutralize the acetic acid formed during the reaction.[22,24] However, when the reaction was performed on a gram-scale, inconsistent yields and varying degree of optical purity were observed. This is presumably due to non-enzymatic hydrolysis of the monoacetate **9** and diacetate **10** promoted by aqueous 1 N NaOH in the reaction. In a modified protocol, we subsequently used aqueous 1 N $NaHCO_3$ instead

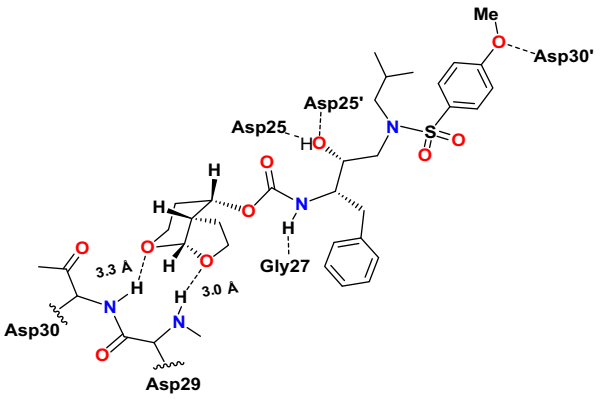
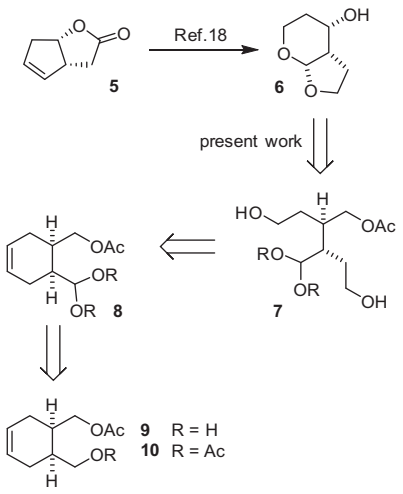
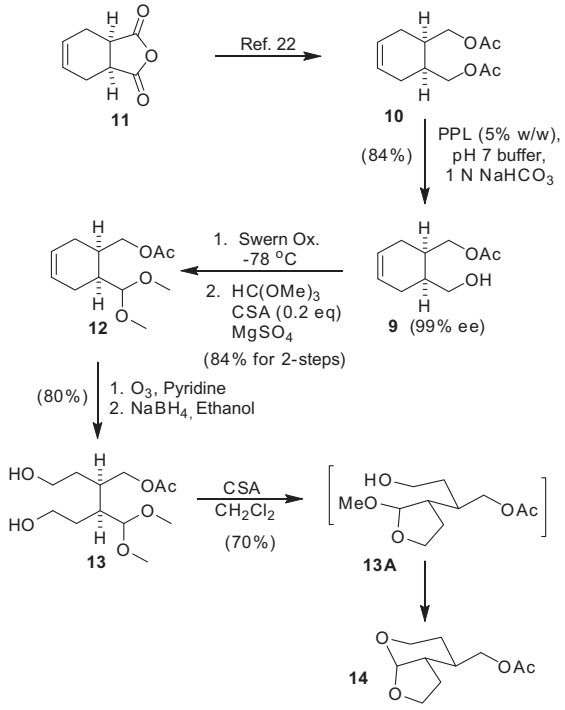


Fig. 2. The key hydrogen bonding interactions of hexahydro-4*H*-furopyranol ligand of inhibitor **4** with Asp29 and Asp30.



Scheme 1. An enzymatic desymmetrization strategy for hexahydro-4*H*-furopyranol.



Scheme 2. Synthesis of bicyclic acetal **14**.

of 1 N aqueous NaOH to neutralize the liberated acetic acid. This condition afforded monoacetate **9** in 84% yield and high enantiomeric purity (99% *ee*). The reaction yield and optical purity were reproducible even on a 60 g scale reaction.[25]

For the synthesis of the ligand alcohol **6**, monoacetate **9** was oxidized using Swern oxidation to provide the corresponding aldehyde in 94% yield. Protection of the aldehyde with ethylene glycol in presence of a catalytic amount of camphorsulfonic acid (CSA) gave the desired acetal in poor yield (11%). We screened a number of other protecting groups, acid catalysts and solvents and the results are shown in Table 1. The use of *bis*(trimethylsilyloxy) ethane (1.3 equiv) in the presence of TMSOTf afforded 30% yield of the corresponding 1,3-dioxolane derivative (entry 2). Reaction of aldehyde with trimethyl orthoformate (30 equiv) in methanol at 23 °C in the presence of a catalytic amount of PPTS afforded

Table 1
Optimization of acetal protection for compound **12**.

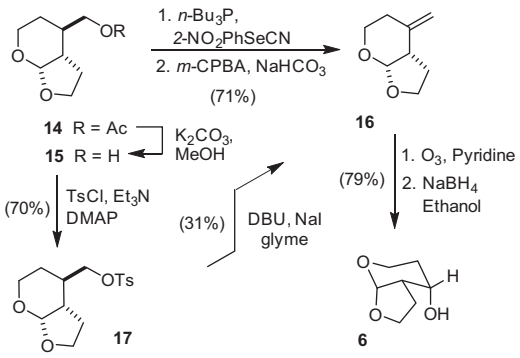
Entry	Reagent	Acid (equiv)	Temp (°C)	Time (h)	Yield (%)
1[a]	Ethylene glycol	CSA (0.2)	80	0.5	11
2[b]	$TMSO(CH_2)_2OTMS$	TMSOTf (13)	−78	2	30
3[c]	$HC(OMe)_3$	PPTS (0.3)	23	24	27
4[b]	$HC(OMe)_3$	PPTS (0.2)	23	72	76[d]
5[b]	$HC(OMe)_3$	CSA (0.2)	23	12	89[d]

[a] Solvent: benzene.
[b] Solvent: CH_2Cl_2.
[c] Solvent MeOH.
[d] Anhydrous $MgSO_4$ was used.

dimethyl acetal **12** in 27% yield (entry 3). When the reaction was carried out in CH_2Cl_2 with 0.2 equivalent of PPTS, acetal yield improved to 76% (entry 4). The use of a catalytic amount of CSA (0.2 equivalent) and in the presence of anhydrous $MgSO_4$ in CH_2Cl_2 at 23 °C for 12 h provided acetal **12** in 89% yield (entry 5).

Oxidative cleavage of the olefin by ozonolysis in the presence of pyridine as the organocatalyst[26] in CH_2Cl_2 at −78 °C, followed by reduction of the resulting crude dialdehyde with sodium borohydride at 0 °C to 23 °C afforded diol **13**. Treatment of diol **13** with a catalytic amount (30 mol%) of CSA in CH_2Cl_2 at 23 °C for 2 h furnished bicyclic acetal **14** via intermediate **13A** in 56% yield over 3 steps.

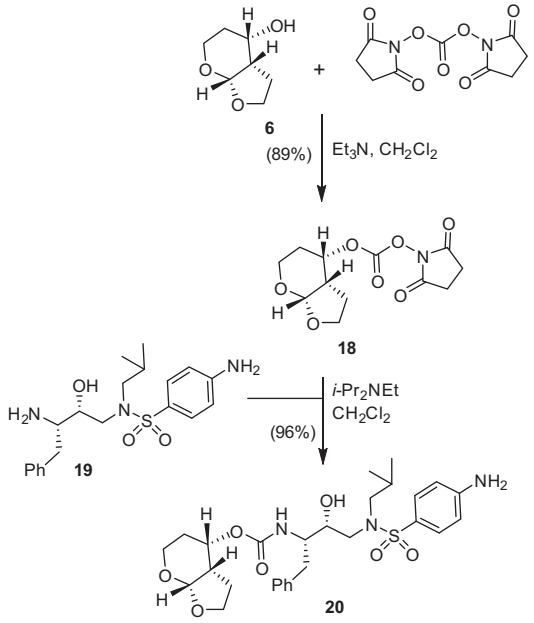
Bicyclic acetal **14** was converted to hexahydro-4*H*-furopyranol **6** as shown in Scheme 3. Removal of the acetate was carried out by treatment of **14** with potassium carbonate in methanol at 23 °C for 30 min to afford alcohol **15** in 98% yield. Alcohol **15** was subjected to an elimination reaction using a protocol developed by Grieco and co-workers.[27] Alcohol **15** was treated with *o*-nitrophenylselenonitrile and *n*-tributylphosphine in THF at 23 °C for 45 min to provide the corresponding selenide derivative. Oxidation of the resulting selenide with *m*-CPBA in CH_2Cl_2 at 23 °C for 10 min resulted in elimination of the corresponding selenoxide to afford olefin **16** in 71% yield over 2-steps. We also explored alternative pathways to convert alcohol **15** to olefin **16**. Tosylation of alcohol **15** with tosyl chloride in the presence of triethylamine and a catalytic amount of DMAP at 23 °C for 2 h provided tosylate derivative **17** in 70% yield. However, elimination of the tosylate with sodium iodide and DBU in 1,2-dimethoxyethane at 90 °C for 3 h furnished olefin **16** in low yield (31%).[28] Further attempted elimination of the tosylate with potassium *tert*-butoxide in DMSO provided only trace amount of elimination product and mostly resulted in the decomposition of the tosylate. We carried out oxidative cleavage of exocyclic olefin **16** by ozonolysis in CH_2Cl_2 at −78 °C to furnish the corresponding ketone in 88% yield. Reduction of the resulting ketone with sodium borohydride in ethanol at 23 °C for 15 min provided alcohol **6** ($[\alpha]_D^{23}$ −31.1 (*c* 1, $CHCl_3$)) in 90% yield.



Scheme 3. Synthesis of ligand alcohol **6**.

Optically active ligand alcohol **6** was converted to HIV-1 protease inhibitor **20** as shown in Scheme 4. Alcohol **6** was converted to the activated mixed carbonate **18** by treatment with disuccinimidyl carbonate in the presence of Et_3N in CH_2Cl_2 at 23 °C for 18 h.[29] Reaction of carbonate **18** with the known (hydroxyethyl)-sulfonamide isostere[30] **19** in the presence of diisopropylethylamine at 23 °C for 1 h afforded inhibitor **20** in 85% yield over 2 steps. Inhibitor **20** was evaluated in an enzyme inhibitory assay following a protocol described by Toth and Marshall.[31] As reported previously, inhibitor **20** showed a K_i value of 10 pM, comparable to that of Darunavir (16 pM).[18]

In conclusion, we have carried out an optically active synthesis of the P2 ligand, (3a*S*,4*S*,7a*R*)-hexahydro-4*H*-furo[2,3-*b*]pyran-4-ol. This nonpeptide ligand is a high affinity P2 ligand for a variety of exceptionally potent HIV-1 protease inhibitors. The key step involved the synthesis of optically active ((1*R*,6*S*)-6-(hydroxymethyl)cyclohex-3-en-1-yl)methyl acetate **9** by a very efficient enzymatic desymmetrization of *meso*-diacetate **10** using commercially available and inexpensive porcine pancreatic lipase. The enzymatic desymmetrization was carried out in large scale providing acetate **9** in high optical purity (up to 99% ee). Optically active alcohol **9** was efficiently converted to (3a*S*,4*S*,7a*R*)-hexahydro-4*H*-furo[2,3-*b*]pyran-4-ol. This ligand alcohol was then converted to HIV-1 protease inhibitor **20**. Further studies, particularly scopes and application are in progress in our laboratory.



Scheme 4. Synthesis of HIV-1 protease inhibitor **20**.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.07.010.

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