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LY316340: A POTENT HIV-1 PROTEASE INHIBITOR CONTAINING A HIGH AFFINITY OCTAHYDROTHIENOPYRIDINE HYDROXYETHYLAMINE ISOSTERE

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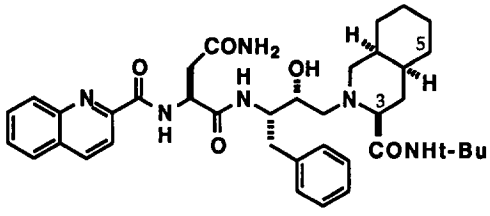
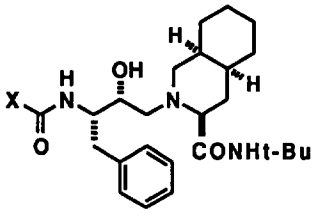
Abstract. Replacement of the decahydroisoquinoline group contained in Ro 31-8959 by a *cis*-octahydrothienopyridine moiety has provided a high affinity hydroxyethylamine isostere for use in HIV-1 protease inhibitors. Further gains in potency have been realized by incorporation of a sulfur atom into the P_1 benzyl group. Modification by a key P_2 ligand provided LY316340, a potent, orally absorbed inhibitor of HIV-1 protease.

Of the many HIV-1 protease inhibitors,[1] Ro 31-8959[2] still stands as one of the most potent antivirals. Attempts to prepare inhibitors that possess improved oral bioavailability in animals[2b] have succeeded, but often with concomitant loss of antiviral potency.[3] One approach toward achieving both of these goals has been to prepare inhibitors that only span part of the P_3-P_3' active site of the enzyme.[4] The Merck group has studied truncated analogs **1**[5] and **2**[6] of Ro 31-8959, in part based on the successful replacement of the asparagine of Ro 31-8959 by tetrahydrofuranylglycine.[7] Recently, a collaborative effort[8] has found substituted aryl amides as viable P_2 ligands, exemplified by inhibitors such as **3**. While these efforts have produced small, potent inhibitors, they fall short of the antiviral activity of Ro 31-8959. Increasing the intrinsic affinity of the hydroxyethylamine isostere should raise the overall potency of these truncated inhibitors. Heteroatom substitution for carbon atoms of the decahydroisoquinoline in combination with addition of a sulfur atom to the P_1 benzyl group has achieved this goal[9] and is the subject of this Letter.



Ro 31-8959

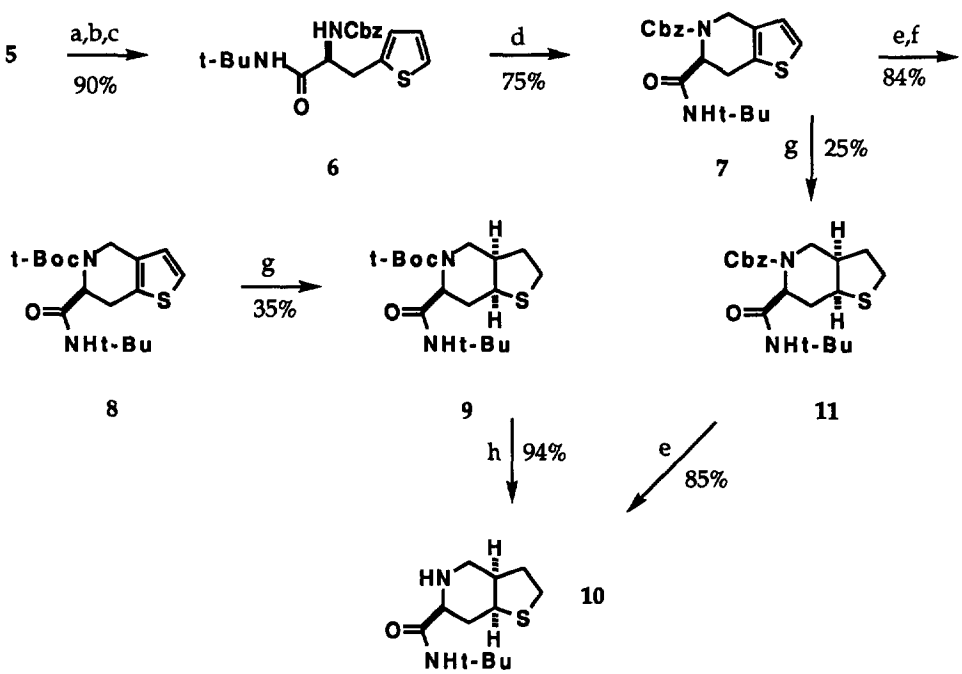


1: X = O-heterocycle
2: X = heterocycle
3: X = aryl
4: X = $OCH_2C_6H_5$

Examination of the X-ray co-crystal structure of Ro 31-8959 with HIV-1 protease[10] revealed the C-5 carbon atom of the decahydroisoquinoline ring to be accessible to solvent. It was anticipated that replacement of one or more carbon atoms by a heteroatom could have an impact on the affinity for this ligand for the enzyme. One such replacement, ethylene by sulfur, is precedented in medicinal chemistry and has the advantage of ready accessibility starting from the commercially available β-thienyl-L-alanine (**5**).[11]

The synthesis of the target ligand **10** is outlined below (Scheme). Treatment of **5** with benzyloxychloroformate and potassium carbonate in aqueous dioxane provided the corresponding Cbz protected amino acid. Subsequent treatment with isobutylchloroformate and N-methylmorpholine in THF followed by tert-butyl amine afforded amide **6** in 90% overall yield from **5**. Formation of the piperidine ring of **7** was accomplished in good yield by exposure of **6** to dimethoxymethane (DMM) and trifluoroacetic acid in refluxing trichloroethane. The results from these relatively mild conditions stand in contrast to those obtained from more classical Pictet-Spengler conditions ($HCHO_{(aq)}$/HCl; DMM/HOAc/H_2SO_4) which afforded only intractable mixtures. Concern about the instability of the Cbz group towards hydrogenation conditions led to its exchange for the Boc group by treatment of **7** with TMSI followed by $(Boc)_2O$, affording **8** in 84% yield. The key reduction of the thiophene ring was carried out by exposure of a solution of **8** in THF/EtOH at 80°C to 5% palladium on carbon under 3000 psi of hydrogen in a bomb to provide octahydrothienopyridine **9** in 35% yield (unoptimized) after chromatography and crystallization from ether/hexane. The relative configuration of the newly formed ring fusion stereocenters of the major isomer **9** is predicted by *cis* delivery of hydrogen *anti* to the C-9 carboxamide group[12] and was unambiguously determined by X-ray crystallography[13] on the subsequently obtained **11**. Recoverable from the mother liquors was an isomer of **9**, in 15% yield, consistent with the *cis* delivery of hydrogen *syn* to the C-9 carboxamide group. Deprotection of **9** by treatment with TFA / CH_2Cl_2 afforded **10** in 94% yield.

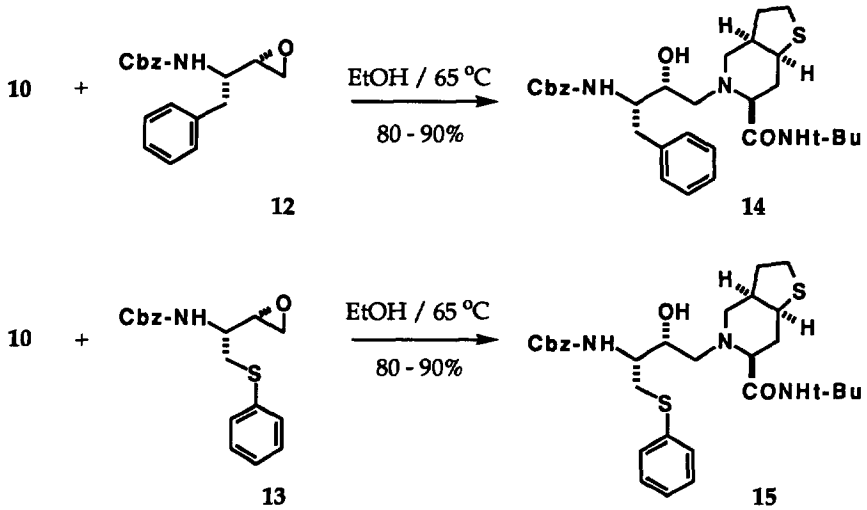
Scheme



REAGENTS: a. Cbz-Cl; b. i-BuOCOCl; c. tert-$BuNH_2$; d. $(CH_3O)_2CH_2$/TFA/$CH_2ClCHCl_2$; e. 4 equiv TMS-I / CH_2Cl_2 - CH_3CN; f. $(t\text{-Boc})_2O$ / CH_2Cl_2; g. 5% Pd/C; H_2 /3000 psi / 70°C/ THF - EtOH; h. TFA / CH_2Cl_2.

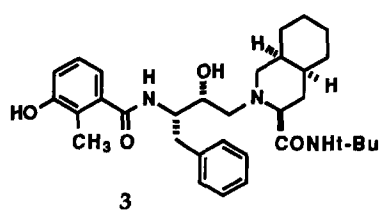
Upon scale-up, it was found that hydrogenation of 7 was more convenient than exchanging protecting groups, since the Cbz group was not removed to any significant extent.[14] Treatment of 7 under the above conditions provided a 25% yield of 11, after chromatography and crystallization from ether/hexane. The highly crystalline nature of 11 both facilitated its purification and provided X-ray quality crystals for structure determination. Deprotection with TMSI completed the synthesis of ligand 10.

Ligand 10 was incorporated into two hydroxyethylamine isosteres, one derived from epoxide 12[15] and the other from epoxide 13.[16] Heating a mixture of the two components in ethanol provided Cbz protected isosteres 14 and 15 in good yield.



These protected isosteres were evaluated as inhibitors of HIV-1 protease.[17] Protected isostere 4 exhibited an IC_{50} = 127 nM whereas 14 and 15 showed IC_{50}'s of 6.6 and 3.3 nM, respectively. Introduction of a sulfur atom into the ligand that occupies the P_1' pocket of the enzyme resulted in a remarkable gain in HIV-1 protease activity. These isosteres therefore became candidates for modification by the aforementioned low molecular weight P_2 ligands.

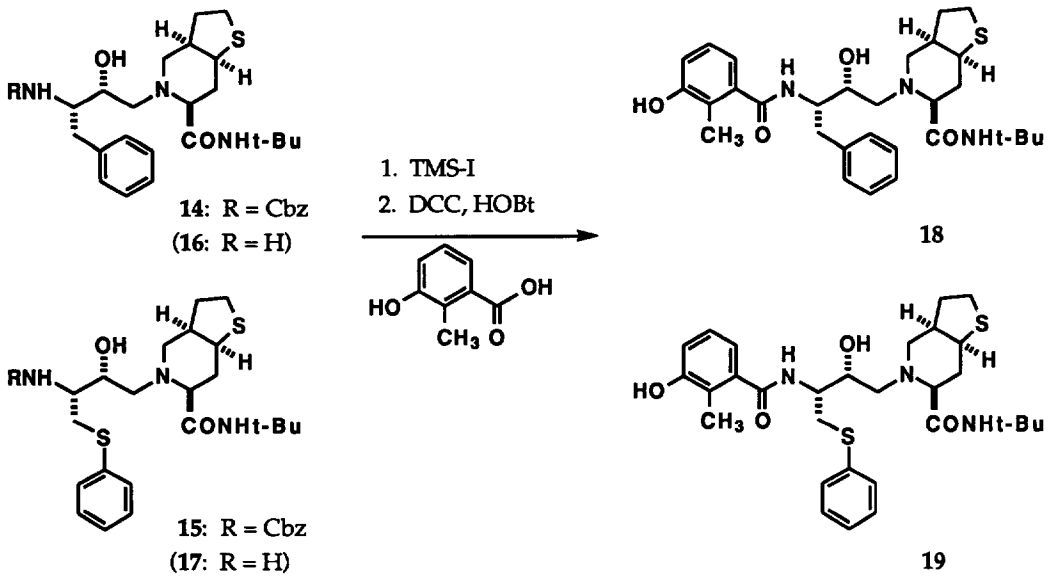
An ideal P_2 ligand for this purpose is the recently described[8] 3-hydroxy-2-methylbenzoyl function. Attachment of this group to the deprotected amine of decahydroisoquinoline 4 provided 3,[8] a moderately potent HIV-1 antiviral[18] (HXB-2 infected CEM-SS cells: IC_{50} = 33 nM, IC_{95} = 135 nM; Ro 31-5989: IC_{50} = 6.6 nM, IC_{95} = 21 nM) with good oral bioavailability in rats. It was envisaged that the same gains in potency observed above could be realized by the attachment of these new isosteres to this P_2 ligand, while maintaining the excellent pharmacokinetic properties exhibited by 3.



3

The targeted compounds were prepared as illustrated. Deprotection of 14 and 15 by treatment with TMSI afforded animes 16 and 17, in yields of 96% and 85%, respectively Acylation of 16 and 17 by the

HOBT active ester, generated *in situ*, of 3-hydroxy-2-methylbenzoic acid provided amides **18** and **19** (LY316340), in yields of 85% and 84%, respectively.



Compounds **18** and **19** proved to be potent HIV-1 protease enzyme inhibitors as well as excellent antivirals. The activity of these compounds relative to **3** and Ro 31-5989 is summarized below (Table).

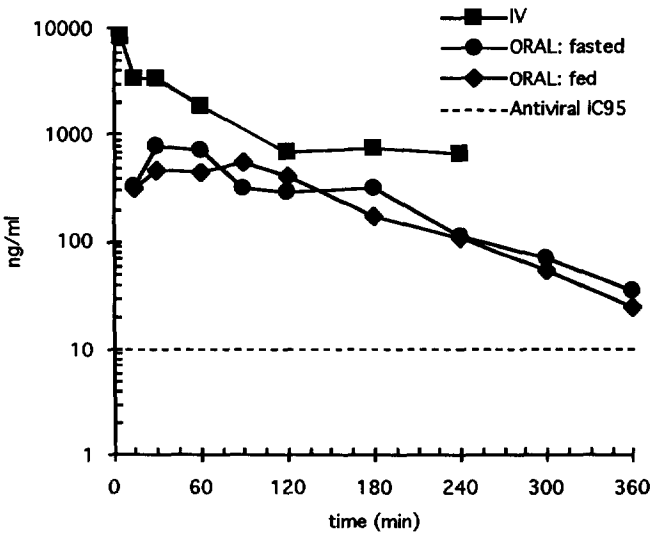
Table. HIV-1 protease activity (HIV-Pr) and HIV-1 antiviral activity (HXB2/CEM-SS) of phenols **18** & **19**.

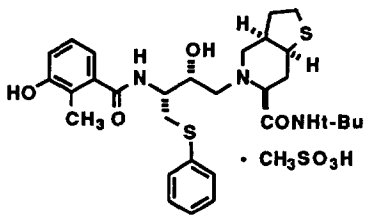
Assay	**3**	**18**	**19**	**Ro 31-5989**
HIV-Pr IC_{50} (nM)	13	0.5	0.3	1
HXB2/CEM-SS IC_{50} (nM)	33	24	5.1	6.6
HXB2/CEM-SS IC_{95} (nM)	135	1360	18	21

Although **18** showed about a 25-fold increase in enzyme inhibitory activity relative to **3**, the change in antiviral IC_{50} was not significant. The reason for the dramatically poorer IC_{95} is not clear.[19] The improved enzyme inhibitory activity of **19**, however, did translate into improvements in antiviral activity. Both the IC_{50} and IC_{95} showed about a 6-fold increase over **3**, yielding potency equivalent to that exhibited by Ro 31-5989. This level of potency ranks among the best for those inhibitors that spans only the P_2-P_2' pockets of the HIV-1 protease enzyme.

Pharmacokinetic studies in rats dosed the mesylate salt of **19** (LY316957/AG1350[20]) in water revealed 22% and 19% oral bioavailability of a 20 mg/kg dose, under the fasting and fed states, respectively (Figure).

Figure. Plasma concentration of LY316957/AG1350 in Fisher rats (n = 2) after a single 20 mg/kg dose.





LY316957 / AG1350

At this dose, the corresponding Cmax were 1.4 and 1.0 μM, and the plasma concentration covered the IC_{95} of HIV-1 infected cells for greater than 6 hours. Thus, **19** exhibits antiviral potency equivalent to that of Ro 31-5989 but with oral bioavailability in the rat many fold greater than the 3% previously reported.[2b]

In conclusion, the combination of high affinity isostere **17** with an appropriate P_2 ligand resulted in a potent, orally bioavailable HIV-1 protease inhibitor. Other combinations of these isosteres with selected P_2 and P_3-P_2 ligands follow.

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19. Other derivatives of **16** show more characteristic IC_{50}-IC_{95} relationships, see accompanying articles.
20. Development of the hydroxybenzamide based P_2 derivatives of **16** and **17** is being pursued by Agouron Pharmaceutical, San Diego, CA.

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