

3-Tetrahydrofuran and Pyran Urethanes as High-Affinity P₂-Ligands for HIV-1 Protease Inhibitors

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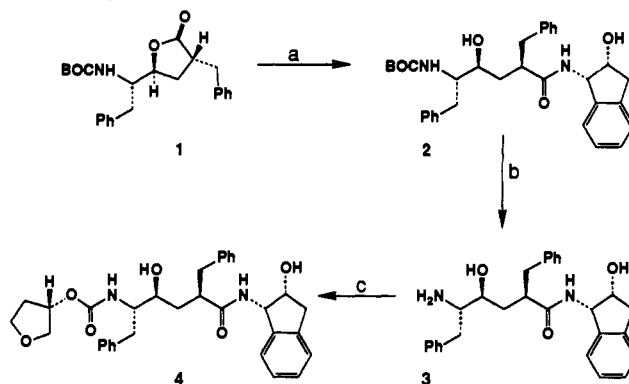
Acquired immunodeficiency syndrome (AIDS) resulting from infection with the human immunodeficiency virus (HIV),¹ continues to be one of the most challenging problems in medical history. Through substantial advances in molecular and cellular biology, much has been learned with unprecedented rapidity about the virus HIV-1. Since the virally encoded HIV-1 protease is responsible for proteolytic processing of the *gag* and *gag-pol* polyproteins to form infectious virions,² inhibition of this enzyme has become an attractive therapeutic target for AIDS chemotherapy.³

Consequently, several reports of novel cyclic and conformationally constrained ligands that mimic natural amino acid side chains have aided the design of HIV-1 protease inhibitors with improved pharmacokinetic properties. To date, perhaps the most notable are the *cis*-decahydroisoquinoline for the P₁' binding region,⁴ the 1(*S*)-amino-2(*R*)-hydroxyindan as P₂' ligand,⁵ and the 2-quinolinoyl moiety as the P₃ ligand.⁴ As part of our continuing efforts in the design of novel, cyclic ligands for the HIV-1 protease substrate binding site, we have incorporated various constrained urethanes in place of N-terminus acyclic *tert*-butylurethane. In this communication, we report that the urethanes of commercially available 3-tetrahydrofurans and easily accessible 3-tetrahydropyrans are high affinity ligands for the S₂ binding domains of the HIV-1 protease.

The known lactone 1 has been previously converted to compound 2 by our laboratories.^{5,6} As depicted in Scheme I, treatment of compound 2 with trifluoroacetic acid in methylene chloride at 0 °C for 30 min furnished the amine 3 in good yield. Urethanes 4-9, as shown in Table I, were then prepared by alkoxy-carbonylation⁷ of the corresponding alcohol with amine 3. For example, active carbonate, obtained by reaction of 3(*S*)-hydroxytetrahydrofuran, disuccinimidyl carbonate, and triethylamine in acetonitrile, readily reacted with amine 3 to provide the urethane 4 in good yield after silica gel chromatography.

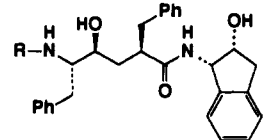
Various urethanes in the hydroxyethylamine series were prepared as shown in Scheme II. Previously reported⁸ azido epoxide 10 and decahydroisoquinoline⁹ derivative 11 were heated at reflux in 2-propanol for 12 h to furnish the azido alcohol 12 in 83% yield after silica gel chromatography. Catalytic hydrogenation of this resulting azide with 10% palladium on charcoal in a mixture of tetrahydrofuran and methanol (4:1) in the presence of

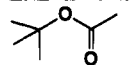
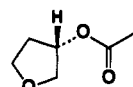
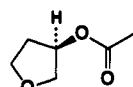
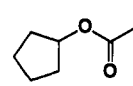
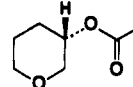
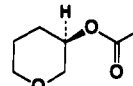
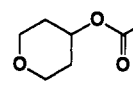
Scheme I*



^a (a) Reference 5. (b) Trifluoroacetic acid, CH₂Cl₂, 0 °C, 30 min. (c) Tetrahydrofuranylsuccinimidyl carbonate, CH₂Cl₂, Et₃N.

Table I. Structure and Inhibitory Potencies of Urethanes in Hydroxyethylene Series



compd	R	mp (°C)	IC ₅₀ ^a (nM)	CIC ₉₅ ^b (nM)
2			0.3	400
4		231-33	<0.03	3
5		217-19	0.03	100
6		237-38	0.33	400
7		228-30	<0.03	50
8		223-24	<0.03	25
9		215-17	1.22	

^a The HIV protease inhibitor Ro-31-8959⁴ displaced an IC₅₀ value of 0.23 nM (±0.1, *n* = 3) in this assay system.¹¹ ^b The CIC₉₅ value for Ro-31-8959 was 22 nM (±7, *n* = 10) in this assay.¹⁶ No overt cytotoxicity was observed for any compounds tested under the conditions used for the cell-based assay.¹⁷

acetic acid afforded the amine 13 in 98% yield. The amine 13 was then converted to the urethanes 14 and 16-20 using the alkoxy-carbonylation reaction as described above. The structures of all new compounds, thus obtained, were established by ¹H NMR spectroscopy, elemental analysis, and mass spectral analysis.¹⁰

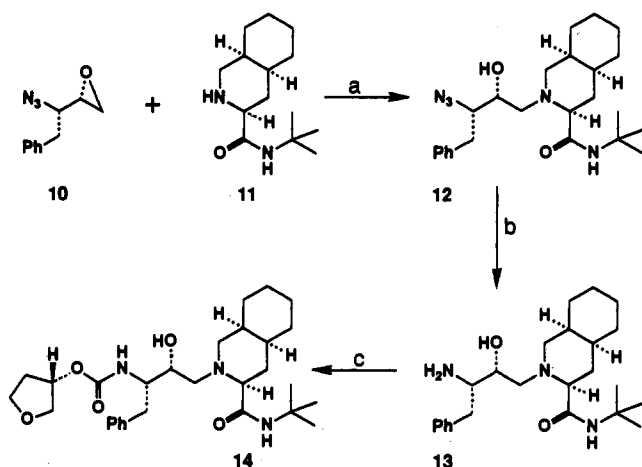
From Table I, it can be seen that the 3-tetrahydrofuranyl- or 3-tetrahydropyranylurethanes are very high affinity ligands into the S₂ binding region of the enzyme HIV-1 protease. Replacement of BOC urethane of compound 2 with 3(*S*)-tetrahydrofuranyluurethane (compound 4, IC₅₀ < 0.03 nM) resulted in over a 10-fold increase

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Scheme II*



^a (a) 2-Propanol, 80 °C, 12 h. (b) H₂, 10% Pd-C, acetic acid, MeOH-THF, 12 h. (c) Tetrahydrofuranylsuccinimidyl carbonate, CH₂Cl₂, Et₃N, 23 °C, 12 h.

Table II. Structure and Inhibitory Potencies of Urethanes in Hydroxyethylamine Series

compd	R	mp (°C)	IC ₅₀ (nM)
15			>3000
14		80–82	160
16		65–68	694
17		87–88	314
18		89–90	48.9
19		95–96	392
20		92–93	308

in its inhibitory potency.¹¹ Tetrahydrofuranylurethane with 3(*R*) configuration (compound 5) also exhibited similar potency-enhancing effect. The potency-enhancing effect of 3-tetrahydrofuranylurethanes was also successfully extended to sterically more demanding 3-tetrahydropyranylurethanes. As is evident, irrespective of ring stereochemistry, both 3(*S*) and 3(*R*) configurations¹² have shown a potency-enhancing effect by a factor of greater than 10-fold relative to BOC derivative 2. Examination of cyclopentylurethane 6 established that the presence of ring oxygen was essential for potency enhancement. As is evident from the lower activity (IC₅₀ = 1.22 nM) of the

4-tetrahydropyranylurethane 9, the position of pyran oxygen (3-position) is critical. The reason for this high affinity could be due to hydrophobic binding along with some specific interaction of ring oxygen as well as urethane oxygen in the S₂ binding domain of the HIV-1 protease. However, the proper understanding of this effect should await the solution of the X-ray crystal structure of a protein–ligand complex.¹³

Incorporation of various 3-tetrahydrofuranyl- or tetrahydropyranylurethanes as the P₂ ligand in the hydroxyethylamine series has resulted in similar potency enhancement. As shown in Table II, the tetrahydrofuranylurethane with 3(*S*) configuration (compound 14, IC₅₀ = 160 nM) has exhibited more than 18-fold potency increase relative to BOC derivative 15.¹⁴ The ring stereochemistry and conformational rigidity associated with various urethane moieties have a major effect on the *in vitro* potencies in this series. Tetrahydrofuranylurethane with 3(*R*) configuration (compound 16) showed a 4-fold loss in its inhibitory potency. Dihydropyranylurethane 17 has an IC₅₀ value of 314 nM, and saturation of the dihydropyran ring resulted in slight loss in potency (compound 19, IC₅₀ 392 nM). Incorporation of dihydropyranylurethane at P₂ subsite with 3(*R*) configuration (compound 18) afforded the most potent compound in this series with an IC₅₀ value of 49 nM, a greater than 61-fold potency enhancement over BOC urethane 15. The conformational rigidity as well as the ring stereochemistry of this urethane moiety was presumably making favorable interactions with the residues in the S₂ subsite.¹³ In accord with earlier observation, saturation of the dihydropyran ring of 19 resulted in loss of potency (compound 20, IC₅₀ = 308 nM).

Several compounds in the hydroxyethylene series have been tested for their ability to inhibit the spread of HIV-1 in MT4 human T-lymphoid cells infected with the IIIb isolate.^{5a} As evident in Table I, substitution of BOC urethane either with 3-tetrahydrofuranyl or 3-tetrahydropyranylurethane not only increases intrinsic potency against the enzyme but generally leads to significant enhancement of antiviral potency as well. Compound 4 has prevented the spread of HIV-1 at a concentration of 3 nM (CIC₉₅), a greater than 133-fold potency enhancement over BOC derivative 2. Furthermore, introduction of 3(*R*)-tetrahydropyranylurethane as P₂ ligand (compound 8) attenuated the CIC₉₅ by a factor of 15 compared to 2. In the hydroxyethylamine series, antiviral potencies of compound 14 and 18 have been determined and their CIC₉₅ values were 800 (*n* = 3)¹⁵ and 1600 nM, respectively.

In conclusion, urethanes of 3-tetrahydrofurans and pyrans are high-affinity ligands for the S₂ binding domains of the HIV-1 protease. Incorporation of these ligands provided potent inhibitors in the hydroxyethylene and hydroxyethylamine series with picomolar and nanomolar *in vitro* potencies. Of particular interest, substitution of the BOC urethane in compound 2 with urethane of commercially available 3-(*S*)-hydroxytetrahydrofuran led to a significant enhancement of antiviral potency (CIC₉₅, 3 nM) in compound 4. Further investigations of these ligands are in progress.

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Supplementary Material Available: Experimental procedures and spectral data for compounds 3, 4, and 12–14 and elemental analysis and mass spectral data for compounds 4–9 and 14–20 (6 pages). Ordering information is given on any current masthead page.

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- (17) Direct assay of cytotoxicity was found to be highly dependent on culture medium and cell type. Unless overt toxicity was noted during the spread assay, no further measure of cytotoxicity was performed.