Communications to the Editor

Structure-Based Design of HIV-1 Protease Inhibitors: Replacement of Two Amides and a 10π-Aromatic System by a Fused Bis-tetrahydrofuran

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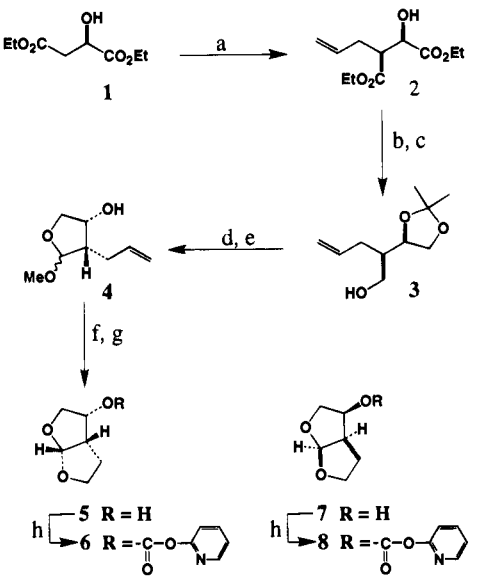
The replacement of the peptide bond with a functionality that mimics the biological mode of action continues to be a major strategy for drug design. As part of our continuing efforts in search of therapeutic agents for AIDS, we recently described a stereochemically defined tetrahydrofuran ring as a surrogate for the asparagine side chain in the design of HIV protease inhibitors.[1] We were intrigued by the possibility of further exploiting this concept with the ultimate goal of producing a polyether template to mimic the peptide region which binds to the viral enzyme.[2]

After visual inspection of the X-ray crystal structure of the HIV-1 protease-inhibitor (Ro 31-8959) complex,[3] it was tempting to speculate that a fused bicyclic tetrahydrofuran could effectively hydrogen bond to the NH of the Asp 29 and 30 residues and thereby replace the quinaldic amide–asparagine amide fragment of the Ro 31-8959 inhibitor. In the hydroxyethylamine isostere derived inhibitors of which Ro 31-8959 is prototypical, inclusion of a P_3 ligand is essential for low nanomolar activity against the HIV protease. Since there was considerable rotational freedom about the four bonds connecting the two carbonyls involved, a rigid cyclically constrained system might provide additional gains in binding energy, to offset loss of the P_3 hydrophobic binding of the quinoline ring. In this paper, we report the structure-based design of a fused bis-tetrahydrofuran that effectively replaces two amide bonds and a 10π-aromatic system of the present clinical candidate **12** (Ro 31-8959).[4]

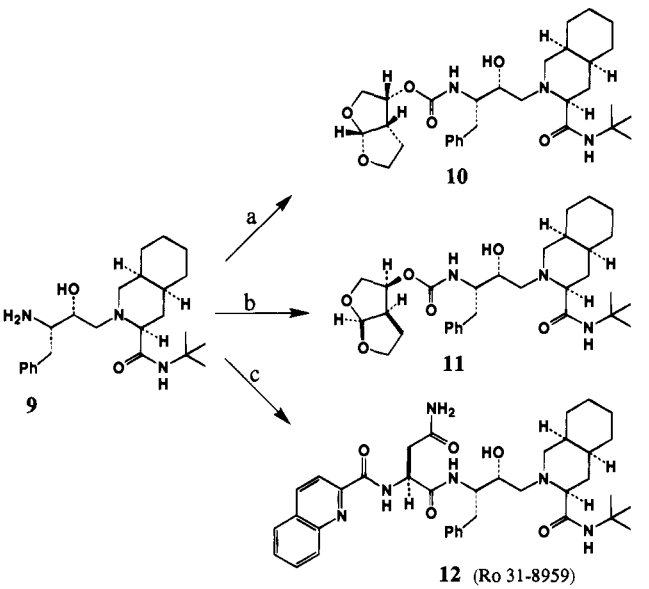
The synthetic route leading to the bis-tetrahydrofuran (bis-Thf) **4** is outlined in Scheme 1. As shown, optically pure (3*R*)-diethyl malate **1** was alkylated utilizing the procedure reported by Seebach.[5] The diastereomer **2** was obtained as the major (selectivity 12:1) product in 85% yield after distillation. The diastereomeric mixture was converted to the isopropylidene derivative **3** by LAH reduction in diethyl ether followed by treatment with a catalytic amount of *p*-TsOH in acetone at 23 °C for 12 h (59% isolated yield). Swern oxidation and subsequent reaction with camphorsulfonic acid (CSA) in methanol afforded the methyl acetal **4** as a mixture (ratio 4:1) in 73% yield. Methyl acetal **4** was converted to bis-Thf **5**

Scheme 1[a]



[a] Key: (a) LDA, CH_2=$CHCH_2Br$; (b) LAH, Et_2O; (c) acetone, *p*-TsOH; (d) Swern oxidation; (e) CSA, MeOH; (f) ozonolysis then $NaBH_4$; (g) CSA, CH_2Cl_2; (h) DPC, Et_3N, CH_2Cl_2.

Scheme 2[a]



[a] Key: (a) mixed carbonate **6**, CH_2Cl_2; (b) mixed carbonate **8**, CH_2Cl_2; (c) ref 1.

by the following reaction sequence: (1) ozonolytic cleavage of the terminal olefin, (2) $NaBH_4$ reduction of the resulting aldehyde in ethanol at 0 °C, and (3) exposure of the corresponding alcohol with CSA in methylene chloride at 23 °C for 12 h. The desired bis-Thf ligand **5** (α^{23}_D −4.3°, *c* 0.215, $CHCl_3$) was obtained in 81% yield (from **4**) after silica gel chromatography.[6] Similarly, bis-Thf ligand **7** (α^{24}_D +3.9°, *c* 0.32, $CHCl_3$) with a 3*S*,3a*R*,-6a*S*-configuration was synthesized, starting from optically pure (3*S*)-diethyl malate following the sequence of reactions described above. The ligands **5** and **7** readily reacted with dipyridyl carbonate and triethylamine in methylene chloride to furnish the correspond-

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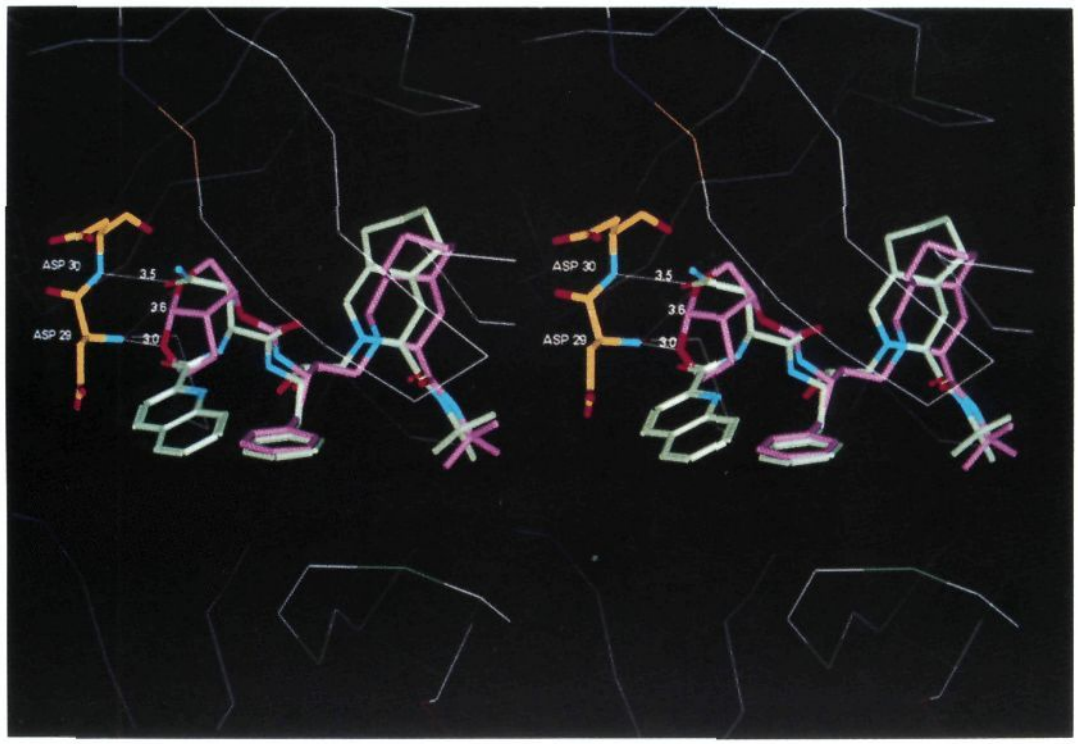

Figure 1. X-ray structure of the inhibitors **10** (magenta) and **12** (green) bound to the HIV-1 protease.

ing active carbonates **6** and **8** in good yields (75–80%).[7] *Reaction of the active carbonate* **6** *with the hydroxy*ethylamine[1b] isostere **9** in methylene chloride at 23 °C provided only the inhibitor **10** (white solid, mp 98–101 °C) by ^{1}H NMR and HPLC analyses (96% yield). Similarly, treatment of active carbonate **8** with amine **9** afforded the inhibitor **11** (white solid, mp 85–89 °C) in 94% isolated yield.

The binding properties of the inhibitors **10** and **11** were determined by enzyme inhibition assays.[8] As is evident, the ring stereochemistry and conformational rigidity associated with bis-Thf ligands have a significant effect on their in vitro potencies. The inhibitor **10** derived from (3*R*,3a*S*,6a*R*-bis-Thf has shown an enzyme inhibitory activity (IC_{50}) of 1.8 ± 0.2 nM (n = 6). In comparison, inhibitor **12** (Ro 31-8959), containing asparagine in the P_2-position and quinaldic amide in the P_3-position, exhibited an IC_{50} value of 0.23 ± 0.10 nM (n = 3). The inhibitor with (3*S*,3a*R*,6a*S*)-bis-Thf as the P_2-ligand **11**, IC_{50} = 6.4 nM (n = 2) was less potent than **10**. More strikingly, the enhanced inhibitory potency of **10** relative to the (3*S*-tetrahydrofuranylurethane (IC_{50} 132 nM; CIC_{95} >800 nM) or the BOC derivative (IC_{50} >3 μM)[9] was also reflected in its antiviral potency. Inhibitor **10** has prevented the spread of HIV-1 in MT4 human T-lymphoid cells infected with IIIb isolate[10] at an average concentration (n = 4) of 46 ± 4 nM (CIC_{95}). In head to head comparison, inhibitor **10** was equipotent to present clinical candidate **12** (Ro 31-8959), CIC_{95} = 23 ± 7 nM.[11] In contrast, inhibitor **11** has shown an antiviral potency of 200 nM.

In an effort to gain insight into the ligand binding site interactions, a single crystal of the inhibitor **10** complexed with HIV-1 protease was generated, and the three-dimensional structure was determined by X-ray diffraction to 2.10-Å resolution.[12] A stereoview of the bound conformation of inhibitors **10** (magenta) and **12** (green) as determined by X-ray crystallographic analysis *of the enzyme–inhibitor complex is shown in* Figure 1.[13] The (*R*)-hydroxyl group of inhibitor **10** is positioned symmetrically between the two aspartates of the enzyme. Both the asparagine of **12** and the bis-Thf of **10** are located in the S_2 subsite.[14] As shown, bis-Thf oxygen-1 of **10** and the P_2 asparagine carbonyl of **12** are within hydrogen bonding distance (3.5 and 3.2 Å, respectively) to the Asp 30 NH present in the S_2 binding domain of the HIV-1 protease. Also, the bis-Thf oxygen-6 and the P_3 quinoline amide carbonyl of **12** interact with the Asp 29 NH (bonding distance 3.0 and 3.3 Å, respectively) positioned in the region. Like other reported protein–ligand complex structures, the P_2 bis-Thf urethane carbonyl and the *tert*-butyl amide carbonyl of **10** hydrogen bond to the critical water molecule that interacts with the flap Ile 50 NH residues.

Thus, incorporation of a conformationally constrained bis-Thf as the P_2 ligand provided an inhibitor **10**, with comparable *in vitro* antiviral activities to inhibitors in the hydroxyethylamine class with both P_2 and P_3 ligands. Design of such a high-affinity ligand led to improved aqueous solubility,[15] decreased log P value,[16] and reduction in molecular weight due to exclusion of the P_3 ligand. The molecular weight of the bis-Thf is essentially half the combined molecular weight of the P_2 asparagine and P_3 quinoline ligands of inhibitor **12** (Ro 31-8959). The present studies offer many important aspects of the molecular design that could facilitate the design of other novel protease inhibitors with improved biological actions.

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Supplementary Material Available: Experimental procedures and spectral data for compounds **2**–**12** and log *P* determination protocol for **10** and **12** (9 pages). Ordering information is given on any current masthead page.

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(3) A complex between **12** (Ro 31-8959, L-697, 803) and HIV-1 protease was crystallized in the space group $P6_1$, $a = b = 63.33$ and $c = 83.31$ Å. A model for the complex was partially refined against data extending from 8.0 to 2.2 Å in resolution; the *R* value for the current model is 0.196, and deviations from ideal bond distances are 0.018 Å. Full details of the crystallographic analysis will be published elsewhere.

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(12) A complex between **10** (L-739, 594) and HIV-1 protease was crystallized in the space group $P6_1$, $a = b = 63.40$ and $c = 83.48$ Å. A model for the complex was refined to completion against data extending from 8.0 to 2.1 Å in resolution; the *R* value for the final model is 0.166 and deviations from ideal bond distances are 0.018 Å. Full details of the crystallographic analysis will be published elsewhere.

(13) Figure 1 was generated by superposition of the X-ray crystal structure of **12** on the protein–inhibitor complex of **10**, in the same frame of references.

(14) For X-ray analysis of the compound **12** (Ro 31-8959) and HIV protease complex, see: (a) Martin, J. A. Recent Advances in the Design of HIV Protease Inhibitors. *Antiviral Res.* **1992**, *17*, 265–278. (b) Krohn, A.; Redshaw, S.; Ritchie, J.; Graves, B. J.; Hatada, M. H. Novel Binding Mode of Highly Potent HIV-Proteinase Inhibitors Incorporating the (R)-Hydroxyethylene Isostere. *J. Med. Chem.* **1991**, *34*, 3340–42.

(15) Aqueous solubility of inhibitor **10** was determined to be 0.235 mg/mL in phosphate buffer (pH = 7.4). Under these same conditions, compound **12** was less than 0.01 mg/mL. The (3*S*)-tetrahydrofuranylurethane (ref 8) was also found to be less soluble (0.15 mg/mL) in this buffer.

(16) The log *P* value of inhibitors **10** and **12** were determined to be 3.5 and 5.7 respectively. The protocol for log *P* determination is provided in the supplementary material.