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A SYSTEMATIC STUDY OF P_1-P_3 SPANNING SIDECHAINS FOR THE INHIBITION OF HIV-1 PROTEASE

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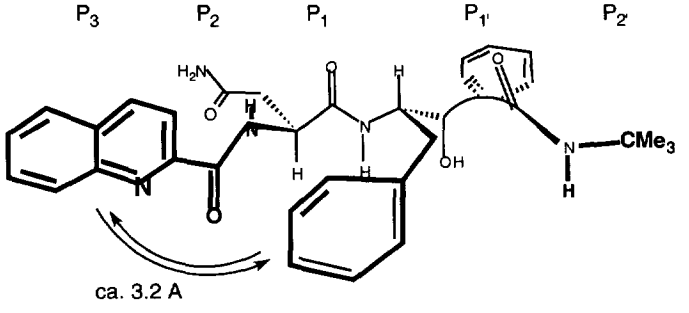
Abstract. Using information obtained from the co-crystal structure of an initial peptidomimetic lead complexed with HIV-1 protease, a series of inhibitors was constructed with substituents designed to span from the P_1 to the P_3 pockets of the enzyme. In accord with prediction, systematic extension of the P_1 substituent with large, lipophilic groups leads to enhancements in binding potencies for this class of inhibitors. Surprisingly, inhibitors with large substituents at both P_1 and P_3 are also well-tolerated by the enzyme, providing compounds with subnanomolar binding affinities for HIV-1 protease.

Human immunodeficiency virus type-1 (HIV-1) protease is currently of great interest as a potential therapeutic target for the treatment of acquired immunodeficiency syndrome (AIDS).[1] Efforts to identify inhibitors of this aspartyl protease have been aided by the wealth of structural information available on this enzyme: a number of high resolution co-crystal structures of inhibitors complexed to HIV-1 protease have been solved which have served as a springboard for the rational design of new classes of inhibitors.[1,2] We recently reported on the discovery of **1**, a potent inhibitor of HIV-1 protease containing a novel Phe-Pro dipeptide isostere.[3] In this communication, we wish to describe our research on the crystal structure-guided design of analogs of this lead inhibitor incorporating modified P_1 substituents.[4]

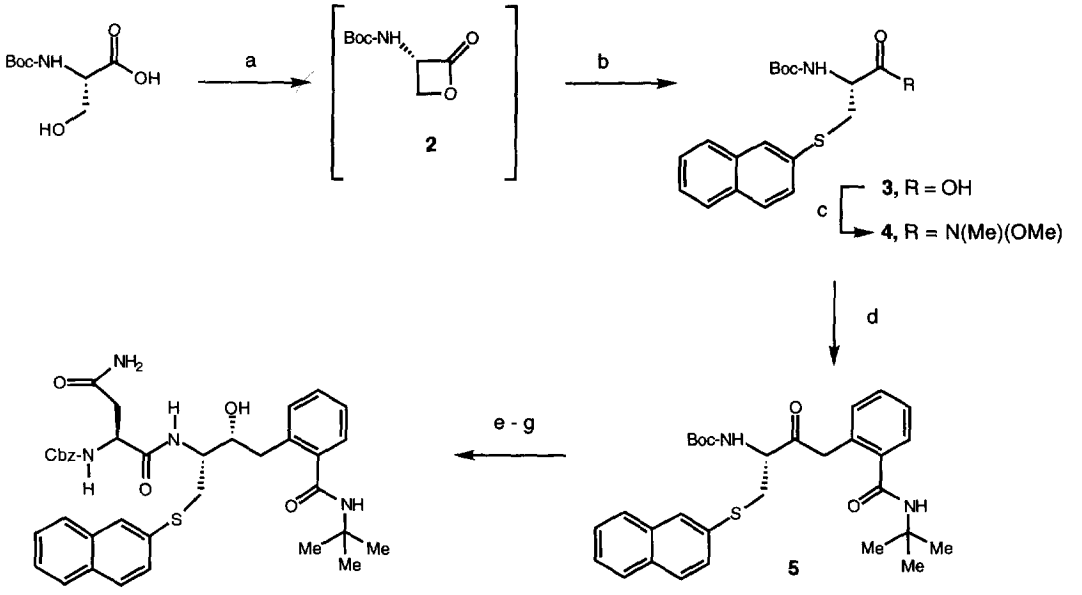
A cartoon rendition of the enzyme-bound conformation of **1** is illustrated in Figure 1. As is the case with other peptidomimetic inhibitors of HIV-1 protease, **1** binds in an extended beta sheet conformation, with its sidechains in a staggered array.[2,5] Notably, the P_1 phenyl group and P_3 quinoline ring of the inhibitor are proximate, with a distance of only 3.2 Å separating nearest atomic neighbors. Based on this observation, it was decided to explore the synthesis of compounds with expanded P_1 substituents capable of spanning from the P_1 pocket into the P_3 pocket of HIV-1 protease. It was simultaneously concluded that truncation of the P_3 substituent would be required to accommodate such P_1 modifications. Related work on the design and synthesis of HIV protease inhibitors containing O-alkylated tyrosine substituents which extend from the $P_{1'}$ to $P_{3'}$ subsites of the enzyme has recently been reported by Thompson and coworkers.[6]

Figure 1: Enzyme-bound Conformation of **1** Highlighting Proximity of P_1 and P_3 Substituents



Synthesis candidates were chosen through the combined use of computer modeling and assessment of synthetic feasibility. It was decided to construct P_1 analogs in which the phenyl group was replaced with either a S-phenyl or a 2-S-naphthyl substituent. P_3 was then varied systematically within each of these series (quinaldoyl, Cbz, Ac, H) to explore our truncation hypothesis. A representative synthesis is illustrated in Scheme 1. Vederas β-lactone chemistry provided an expedient route to the required non-natural amino acid intermediates (e.g. **3**).[7] It was found especially convenient to generate β-lactone **2** *in situ*: treatment with the desired thiolate anion followed by extractive work-up readily yielded the desired acid free of triphenylphosphine oxide and diacylhydrazide impurities. Conversion to the corresponding *N*-methoxy-*N*-methyl amide **4** provided a suitable acylating agent for use in our previously reported dianion acylation chemistry.[3] Reduction of ketone **5** with sodium borohydride then yielded the desired R alcohol stereoisomer (11:1 selectivity) as anticipated.[3] Protecting group removal followed by amine acylation afforded inhibitors for testing.

Scheme 1: Representative P_1-P_3 Spanning Inhibitor Synthesis

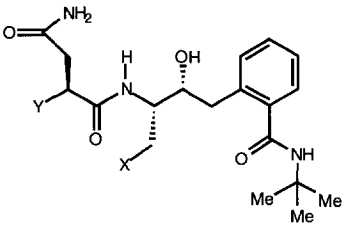


Reagents: a) PPh_3, DEAD, THF, -78°C; b) Na-2-naphthylthiolate, DMF; c) EDC, HOBt, MeNH(OMe), THF (45% from Boc-L-Serine); d) (N-*t*-butyl)-*o*-toluamide, 2 equiv. *sec*-BuLi, THF, -78°C; **4** (85%); e) $NaBH_4$, EtOH; f) TFA, CH_2Cl_2 (51% from **5**); g) Cbz-Asn, DCC, HOBt, DMF (70%).

HIV-1 protease (HIVP) inhibition data for the series revealed several trends (Table 1).[8] Compounds containing a 2-S-naphthyl substituent are consistently 10-20 fold more potent than their S-phenyl analogs (e.g. **8** versus **12**). In accord with prediction, it is possible to dramatically truncate the P_3 substituent if a bulky P_1 substituent is employed. For example, **12** (X=2-S-naphthyl, Y=Ac-NH) binds with equal affinity to the lead **1** (X=phenyl, Y=quinaldic-NH). However, complete excision of the P_3 substituent (e.g. **13**, Y=H) leads to a poor enzyme inhibitor. In addition, compounds possessing large substituents at both P_1 and P_3 are

exceptionally potent enzyme inhibitors, with **10** (X=2-S-naphthyl, Y= quinaldic-NH) binding with picomolar affinity. This final result is unanticipated: we had predicted that **10** would be a poor enzyme inhibitor because of unfavorable steric interactions between these two large substituents.

Table 1



LY #	X	Y	IC_{50} (nM)
6	S-Ph	Quinaldic-NH	1.1±0.3 (n=2)
7		Cbz-NH	14±3.5 (n=2)
8		Ac-NH	19 (n=1)
9		H	>150 (n=1)
10	2-S-Naphthyl	Quinaldic-NH	< 0.25 (n=3)
11		Cbz-NH	1.5±0.8 (n=2)
12		Ac-NH	1.1±0.5 (n=2)
13		H	>80 (n=1)
1	Ph	Quinaldic-NH	1.4±1.0 (n=10)

In order to assess the validity of our P_1-P_3 spanning hypothesis, compound **12** was co-crystallized with HIV-1 protease, and the co-crystal structure was solved at 1.9 Å resolution.[9] As expected, the inhibitor binds in an extended β-sheet conformation and occupies the P_3 through $P_{2'}$ pockets of the enzyme. The transition state mimetic hydroxyl group is favorably disposed to pick up key interactions with active site aspartic acid residues, and a highly localized water molecule is present in the flap region of the enzyme which serves to relay hydrogen bonds from enzyme to inhibitor. The acetamide group of **12** acts as both a hydrogen bond donor and acceptor, perhaps offering an explanation for the >80-fold loss in activity upon excision of this group.[10] Figure 2 shows a superposition of the enzyme-bound conformations of **1** (yellow) and **12** (blue). The two inhibitors bind in a highly analogous manner. If one focuses on the P_1 and P_3 residues of both inhibitors, one can see that the 2-S-naphthyl side chain of **12** does indeed span from the P_1 to P_3, neatly overlaying with both the P_1 phenyl and P_3 quinoline groups of **1** as predicted. Based on this structural data, the high binding affinity of **10** is not easily rationalized. Although it is possible that **10** adopts an alternate mode of binding to HIV-1 protease, modeling work leads us to speculate that **10** binds in a manner similar to **12**, with its P_3 quinaldoyl group displaced into another region of the relatively large P_3 pocket by the 2-S-naphthyl substituent of the inhibitor.

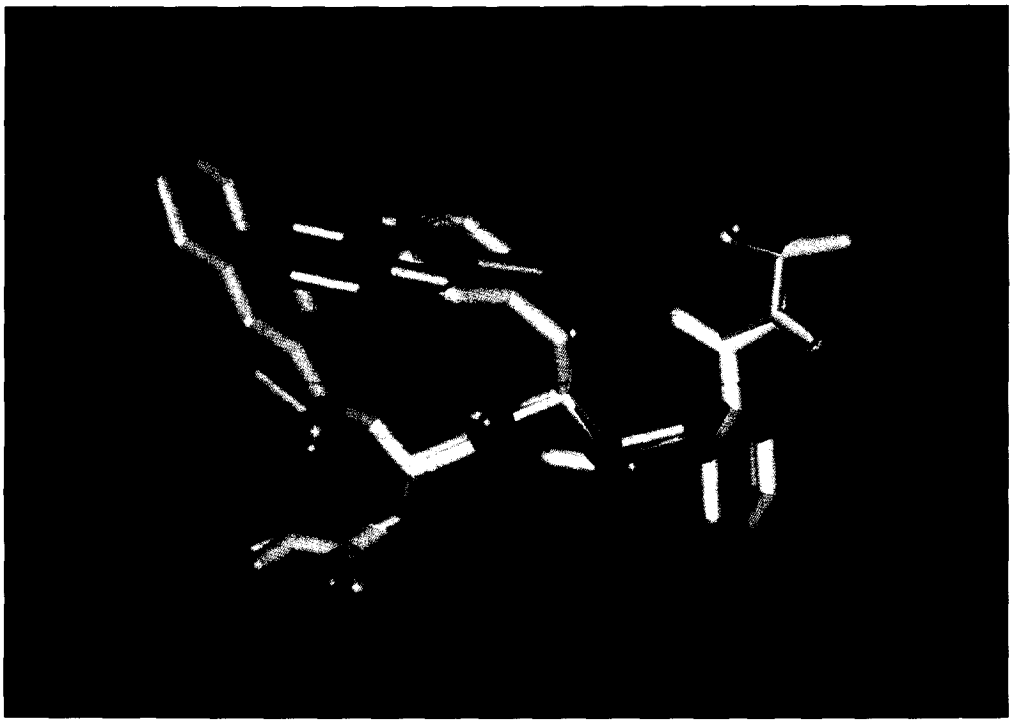
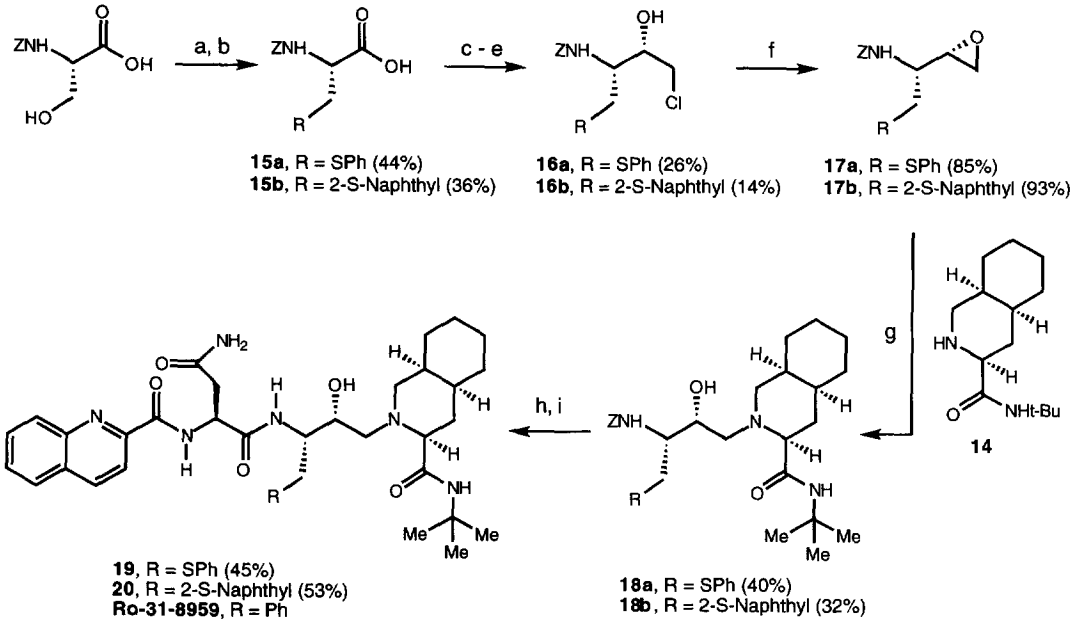


Figure 2: Superposition of the HIV-1 protease-bound conformations of **12** (blue) and **1** (yellow).

Although a number of the compounds listed in Table 1 are potent inhibitors of HIV-1 protease, this enzyme activity does not translate into desirable antiviral activity in whole cell assays: all of the illustrated inhibitors are at least 10-fold poorer antivirals than **1** (ED_{50} = 23 nM).[11]

In an effort to improve antiviral activity, the benzamide portion of these inhibitors was replaced by the saturated bicyclic amine **14** previously reported by Roberts et al. in the synthesis of the HIV protease inhibitor **Ro-31-8959**.[12] Scheme 2 illustrates the synthesis of two members of this series. Vederas β-lactone chemistry was again utilized to access the required amino acid intermediates, this time employing carbobenzyloxy (Cbz)-protected L-serine as the starting material. All subsequent steps were carried out in direct analogy to the sequence reported for the synthesis of **Ro-31-8959**. Diazoketone formation followed by treatment with hydrochloric acid yielded the desired chloroketones in moderate yields. Reduction with sodium borohydride occurred with modest stereoselection (3:1) in favor of the needed R-stereoisomers. Base treatment delivered the epoxides, which were then reacted with the known bicyclic amine **14**.[12] Treatment of the product amino alcohols with HBr/AcOH followed by coupling with quinaldoyl-L-asparagine provided the desired targets.

Scheme 2



Reagents: a) PPh_3, DMAD, THF, -55°C; b) Na-arylthiolate, THF; c) i-BuOCOCl, Et_3N, THF; CH_2N_2; d) HCl, Et_2O; e) $NaBH_4$, THF/H_2O; f) KOH, EtOH; g) **14**, EtOH (80°C); h) HBr, AcOH; i) quinaldoyl-L-Asn, DCC, HOBt, DMF.

Compounds **19** (HIVP IC_{50} = 1.5 ± 0.2 nM, n=2) and **20** (HIVP IC_{50} = <0.25 nM, n=2) are potent inhibitors of HIV-1 protease with IC_{50}s that closely parallel those seen for the corresponding benzamide analogs **6** (HIVP IC_{50} = 1.1± 0.3 nM, n=3) and **10** (HIVP IC_{50} < 0.25 nM, n=2). In addition, marked improvements in whole cell antiviral activity are seen upon incorporation of bicyclic amine **14** (**19** CEM ED_{50} = 1.3 nM, n=1; **20** CEM ED_{50} = 1.4 nM, n=1).[11,13]

In summary, crystal structure-based design has been employed as a tool to aid in the discovery of a novel series of HIV protease inhibitors which contain substituents that span from the P_1 to P_3 pockets of the enzyme, and further optimization work has been performed to successfully enhance the antiviral activity of these compounds. Further studies involving the chemistry, X-ray crystallography, and biology of these inhibitors and related derivatives will be reported in due course.[4,14]

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10. A reviewer has suggested that this >80-fold activity loss may instead be due to the loss of the chiral center which orientates the asparagine side chain.

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13. **Ro-31-8959** is also a potent enzyme inhibitor (HIVP IC50 = 1.8 nM, n=1) with good whole cell activity (CEM ED50 = 1.5 nM, n=1) in the assays described in References 8 and 11.

14. See subsequent two papers in this issue.

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