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Epsilon substituted lysinol derivatives as HIV-1 protease inhibitors

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ABSTRACT

A series of HIV-1 protease inhibitors containing an epsilon substituted lysinol backbone was synthesized. Two novel synthetic routes using *N*-boc-L-glutamic acid alpha-benzyl ester and 2,6-diaminopimelic acid were developed. Incorporation of this epsilon substituent enabled access to the S2 pocket of the enzyme, affording high potency inhibitors. Modeling studies and synthetic efforts suggest the potency increase is due to both conformational bias and van der Waals interactions with the S2 pocket.

Highly active anti-retroviral therapy (HAART) includes a combination of protease inhibitors, reverse transcriptase inhibitors, and integrase inhibitors. Since HAART has become available, it has led to a significant reduction of HIV-associated morbidity and mortality.[1] However, more than a decade of long-term use in AIDS patients has led to the development of increasing amounts of drug resistance. Therefore, it is essential to develop improved or additional HAART components in order to gain a higher barrier to resistance.[2] HIV-1 protease is an excellent therapeutic target since it is responsible for the maturation of infectious virions and the correct and timely processing of viral precursor proteins.[3] Recently, compounds containing a novel HIV-1 protease inhibitor scaffold, the lysinol sulfonamide (Fig. 1), were reported as showing excellent enzyme inhibitory potencies and anti-viral activities.[4] One such inhibitor, compound **1**, is a protease inhibitor that may force HIV-1 into a unique mutational pathway that results in low levels of resistance and may be detrimental to the capacity of viral replication.[5] Compound **1** had an enzyme assay IC_{50} value of 0.50 nM.[4] Further SAR studies showed that incorporating an isoamyl group into the P1′ area of the scaffold afforded a compound with a greater than 10-fold increase in potency against purified enzyme (IC_{50} = 0.03 nM) as compared to **1**.

To further improve the potency, we examined the crystal structures of known HIV-1 protease inhibitors and substrates to identify additional binding opportunities. This analysis immediately suggested substitution along the lysinol backbone, particularly at the epsilon position, in order to enhance binding in the S2 site. This site is largely unfilled by **1**, despite the fact that it is known to accommodate a wide size and variety of substrate sidechains (i.e., Val, Ile, Asn, and Thr).[6] Note for example the *t*-butyl substituent in indinavir (Fig. 2), which occupies space not accessed by the alkyl chain of the lysinol scaffold in **1**. The concept of epsilon substitution was tested initially via synthesis of the racemic methyl substituted inhibitor.

This compound was shown to be active, and synthesis of the individual isomers indicated that the eutomer was ~50-fold more active than the distomer (Table 1). Molecular modeling studies were initiated to predict the absolute stereochemistry of the active isomer.

Docked poses for *S*- and *R*-methyl substituents **10b** and **10c** were derived from the X-ray structure of **1** bound in the HIV-1 protease active site (PDB code 2QMP). Inhibitor models were energy minimized in the active site using the MMFF94 force field[7] with a 2*r* distance dependent dielectric constant. All titratable enzyme

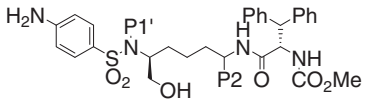

Figure 1. General structure of lysinol sulfonamide HIV-1 protease inhibitors, compound **1**: P1′ = *i*Bu, P2 = H.

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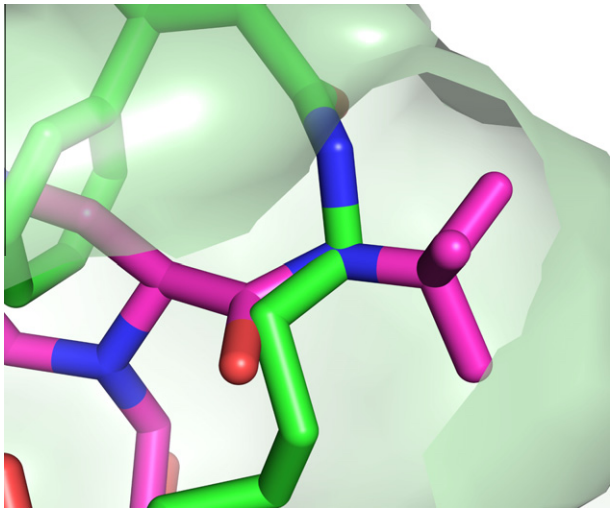


Figure 2. Comparison of substituents in the S2 pocket in the crystal structures of indinavir (magenta) and **1** (green).

Table 1
HIV-1 protease enzyme and cell inhibition for P2 analogs

Compound	P2	IC_{50} (nM)	SPREAD IC_{95} (nM) (10% FBS)
1	H	0.50[a,b]	
10a	H	0.03 (±0.015)[c]	12 (±8)[c]
10b	Me (*S*)	0.007 (±0.003)[c]	6.6[b]
10c	Me (*R*)	0.4 (±0.35)[c]	98 (±70)[c]
10d	Et (*S*)	0.016 (±0.003)[c]	10 (±7)[c]
10e	Pr (*S*)	0.10 (±0.02)[c]	56[b]
10f	iPr (*R*)	0.10 (±0.03)[c]	37 (±12)[c]
10g	CH_2OH (*R*)	0.013[b]	230[b]

[a] Data published in Ref. 4.
[b] Values are means of two experiments.
[c] Values are means of at least three experiments.

residues were charged with the exception of Asp_{25A} which was protonated on OD1. Modeled bound poses were subsequently re-scored with Xscore.[8]

Although either isomer could be accommodated geometrically in the S2 pocket (Fig. 3A), both scoring metrics favored the *S* isomer **10b** (MMFF E_{inter} = −56.3 kcal/mol; Xscore = 8.02) over the *R* isomer **10c** (MMFF E_{inter} = −54.5; Xscore = 8.00). A subsequent X-ray structure of the more active isomer (PDB code 3M9F) confirmed this stereochemical prediction (Fig. 3B).

In an attempt to further fill the S2 pocket of the enzyme, both hydrocarbon alkyl and hydroxymethyl substituents were incorporated into the epsilon position (P2) of the lysine backbone. This was accomplished via two novel synthetic schemes starting with *N*-boc-L-glutamic acid alpha-benzyl ester and 2,6-diaminopimelic acid, respectively.

The synthesis of **10** was completed in 12 steps starting from the commercially available *N*-boc-L-glutamic acid alpha-benzyl ester **2** (Scheme 1).[9] The esterification of **2** followed by Boc protection and subsequent reduction using diisobutylaluminum hydride gave the fully protected aldehyde **3** in good yield.

Compound **3** underwent a Henry reaction by treatment with an appropriately substituted nitroalkyl group.[10] the resulting Henry adduct **4** underwent elimination followed by hydrogenation to afford the amine **5**. Coupling of **5** with *S*-Moc-diPhe-Osu[11] gave **6**. Deprotection of **6** gave the amine hydrochloride **7** which was sulfonylated to afford a mixture diastereomers **8**. The nitro arylsulfonamide mixture was subjected to silica gel chromatography in order to isolate each of the diastereomers. Both diastereomers were carried through the remaining synthesis separately, but in all cases the more polar compound proved to be the desired entity.

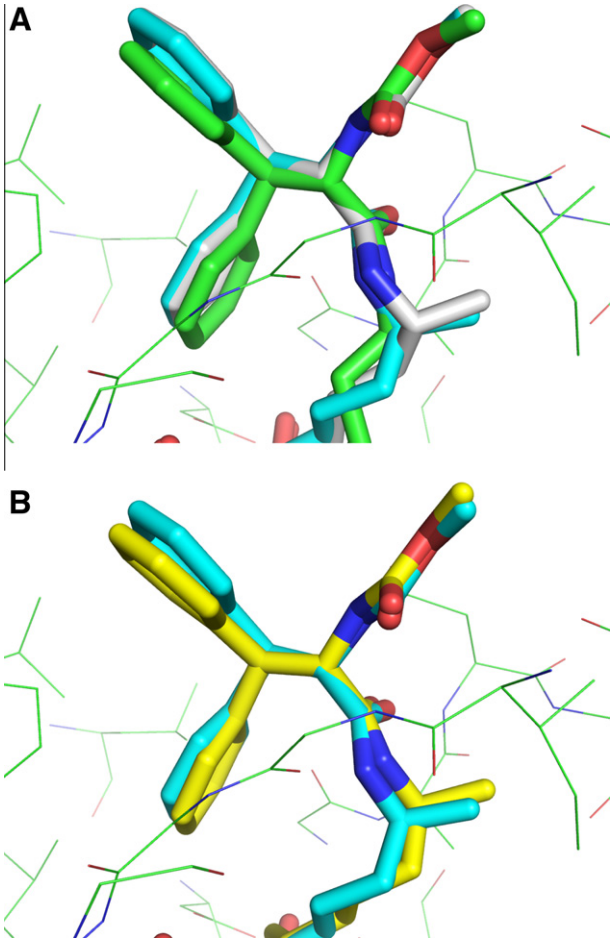
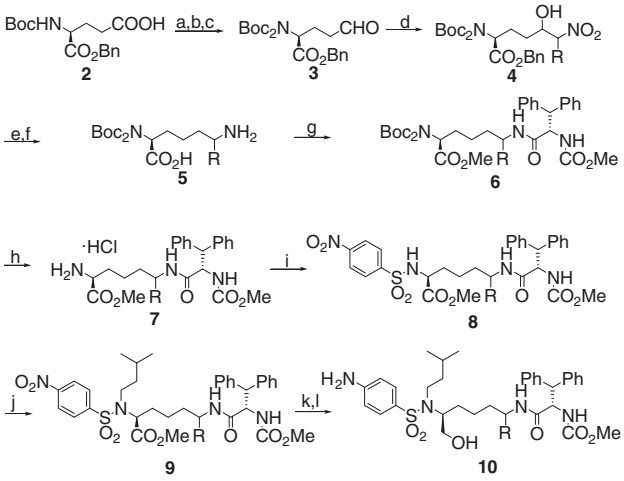


Figure 3. (A) Comparison of *S*- and *R*-methyl isomers **10b** (cyan) and **10c** (white) relative to the crystal structure of **1** (green). (B) Modeled pose (cyan) versus X-ray structure (yellow) of **10b**.



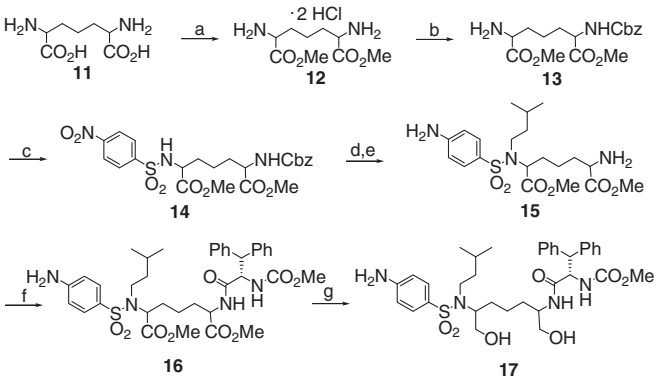
Scheme 1. Reagents and conditions: (a) $TMSCHN_2$, MeOH, DCM 0 °C; (b) Boc_2O, DMAP, CH_3CN, 90% for two steps; (c) DIBAL, ether −78 °C, 83%; (d) RCH_2NO_2, 1,1,3,3-tetramethylguanidine, toluene 0 °C to rt, R = Me, Et, Pr, *i*Pr; (e) MsCl, TEA, DMAP, toluene 80 °C, 20–95% for two steps; (f) H_2, $Pd(OH)_2$, EtOH 45 psi; (g) *S*-Moc-diPhe-OSu, $NaHCO_3$, THF/acetone; (h) HCl, MeOH 0 °C; (i) 4 NO_2-Ph-SO_2Cl, TEA, DCM, 7–11% for four steps, single diastereomer; (j) isoamylalcohol, TPP, DIAD, THF, 57–92%; (k) H_2, $Pd(OH)_2$, MeOH, 93–100%; (l) $LiBH_4$, MeOH, THF, 50–71%.

Based on the biological activity and crystal structure of **10b**, the stereochemistry of this desired compound was assumed to be *S,S,S* for the ethyl and propyl P2 substituents and *S,R,S* for the isopropyl P2 substituents. Mitsunobu alkylation with isoamyl alcohol resulted in **9**, which was then hydrogenated and reduced with lithium borohydride to afford the alcohol **10**.

The synthesis of **10**, where R = CH_2OH, was attempted using Scheme 1 but failed due to the occurrence of a retro-Henry reaction. Therefore, the preparation of this target was accomplished in seven steps starting from the commercially available 2,6-diaminopimelic acid **11** (Scheme 2).[9] Diesterification of **11** resulted in the dihydrochloride **12**, which was mono protected with Cbz chloride to afford **13**. Sulfonylation followed by Mitsunobu alkylation and subsequent hydrogenation resulted in the amine **15**. Coupling of **15** with *S*-Moc-diPhe-OSu followed by ester reduction with lithium borohydride resulted in **17** as a diastereomeric mixture of four compounds. This mixture was subjected to chiral chromatography to afford the individual diastereomers, which were then tested to identify the most active entity **10g**. While two of the four diastereomers showed some biological activity, once again the most polar compound proved to be the most potent entity. It was assumed, based on the crystal structure of **10b** and biological activity, that the stereochemistry of **10g** was *S,R,S*.

In addition to the enzyme assay, compounds were tested in an acute infection assay, or SPREAD assay, in the presence of 10% fetal bovine serum (FBS).[12] In order to optimize activity in both the enzyme and SPREAD assays, SAR was explored in the P2 region with several alkyl groups (Table 1). As previously described, incorporation of a methyl group resulted in an increase in enzyme potency compared to the unsubstituted compound **10a**. As predicted by molecular modeling, the *S* enantiomer **10b** was favored over the *R* enantiomer **10c** with a fourfold increase in enzymatic potency and a twofold increase in SPREAD potency as compared to **10a**.

However, increasing the size of the substituent to ethyl (**10d**) led to only a modest twofold increase in intrinsic potency over **10a**, despite the appearance of additional accessible space in the S2 pocket (Fig. 2). This observation suggests that an epsilon substituent may increase potency primarily by biasing the conformation toward the bio-active geometry. In order to assess this possibility, conformational searches of the crystal structure geometries of **1** and **10b** were performed within Maestro[13] using the MMFF94 force field,[7] an implicit water model, mixed torsional/low mode sampling with a 20 kJ/mol energy window, and subsequent Boltzmann population analysis.[14] The results suggest both **1** and **10b** can adopt low-energy conformations that are 'bent', that is, contain a gauche kink in the lysine scaffold as required in the bio-active



Scheme 2. Reagents and conditions: (a) HCl, MeOH 0 °C to rt; 100%; (b) CbzCl, TEA, DCM 0 °C to rt, 14%; (c) 4 NO_2-Ph-SO_2Cl, TEA, DCM, 87%; (d) isoamylalcohol, TPP, DIAD, THF, 90%; (e) H_2, $Pd(OH)_2$, MeOH; (f) *S*-Moc-diPhe-OSu, $NaHCO_3$, THF/acetone, 53% over two steps; (g) $LiBH_4$, MeOH, THF, 16% single diastereomer.

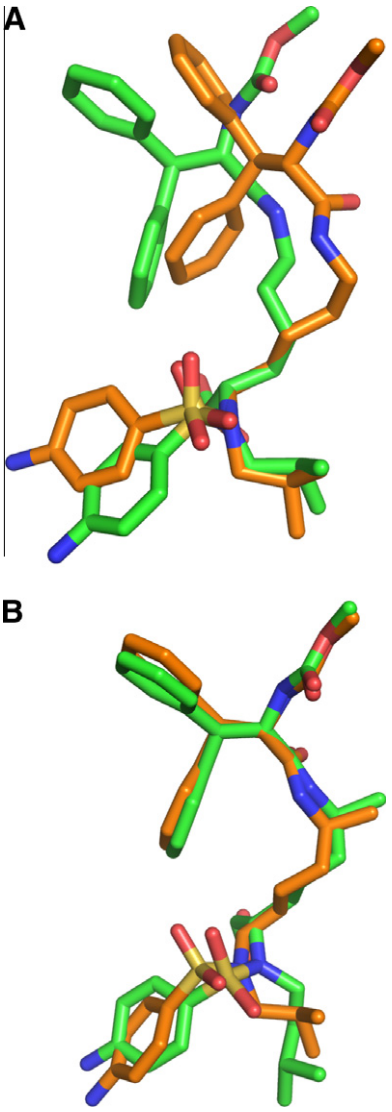


Figure 4. Comparison of the crystal structure geometry (in green) relative to the lowest energy similar conformer (in orange) for **1** and **10b**. (A) The lowest energy 'bent' conformer computed for **1** is 1.2 kJ/mol above the minimum and deviates significantly from the crystal structure geometry. (B) By contrast, the lowest energy conformer for **10b** is 'bent' and closely resembles the crystal structure geometry.

conformation. The relative populations based on Boltzmann analysis are 60% (**1**) and 67% (**10b**), respectively. However, the 'bent' geometry corresponds to the lowest energy conformer for **10b** (as compared to a difference of 1.2 kJ/mol for **1**). In addition, this **10b** conformer much more closely resembles the crystal structure (Fig. 4). These results are consistent with the hypothesis that conformational bias may play a significant role in binding.

Increasing the substituent size to propyl (**10e**), and isopropyl (**10f**) resulted in a dramatic decrease in intrinsic potency, perhaps because the substituent is now too large to fit optimally in the S2 pocket. As the size of the alkyl substituent increases, the SPREAD potency decreases. This may be attributable to increased hydrophobicity, as calculated log *P* values for these compounds show an upward trend.

Once the optimum size of the alkyl chain was determined, polarity was introduced in the P2 region with a hydroxymethyl group (**10g**). While we were delighted with the return of intrinsic potency with **10g**, we were disappointed to find that the SPREAD potency diminished greatly when compared to the methyl substituted compound **10b**. Interestingly both the methyl (**10b**) and

Table 2
Pharmacokinetics in dogs

Compound	Oral AUC[a] (μM h)	C_{Trough}[a] (nM)
10b	0.32	2.0
10d	1.05	5.1

[a] Values are means of at least two experiments.

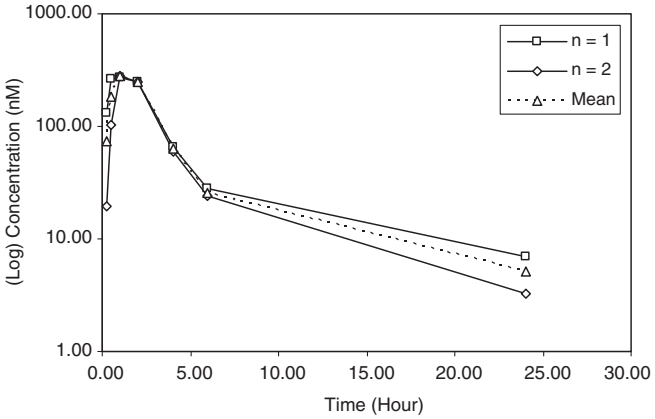


Figure 5. Plasma concentrations (nM) of **10d** in 4 mg/kg PO dosed beagle dogs.

ethyl (**10d**) compounds had potencies comparable to **10a** both in the 10% SPREAD and in the more stringent SPREAD assay in the presence of 50% normal human serum (NHS).[12] In the latter assay the unsubstituted compound **10a** showed a serum adjusted IC_{95} value of 130 nM, while **10b** and **10d** were 120 nM and 74 nM, respectively.

The pharmacokinetic properties of several of these new compounds were measured to further characterize this series of HIV-1 protease inhibitors (Table 2).

As a result, the methyl substituted compound **10b** and the ethyl substituted compound **10d** were each dosed at 4 mpk in PEG 400 to beagle dogs, and drug levels were measured. Both compounds showed good overall oral exposure over the course of the experiment and comparable 24 h trough drug levels. Ethyl substituted inhibitor **10d** was calculated to have a 6 h oral half-life (Fig. 5).

In addition to pharmacokinetic studies, the ethyl substituted compound was subjected to a panel of mutant viruses,[15] and this resulted in a comparable mutation profile to compound **1**.

In summary, replacement of the P1′ isobutyl substituent in **1** with an isoamyl substituent, coupled with epsilon substitution of the lysinol sulfonamide scaffold, yielded potent HIV-1 protease inhibitors. In particular, methyl and ethyl substitution on this position of the lysinol backbone enabled access to the S2 pocket of the enzyme, resulting in an increase in intrinsic potency as compared to the P2 unsubstituted compound. Modeling studies and synthetic efforts attribute the gain in potency as much to conformational bias of the inhibitor as to complementarity to the active site. Two novel synthetic routes using *N*-boc-L-glutamic acid alpha-benzyl ester and 2,6-diaminopimelic acid were developed. Further studies optimizing the other substituents of the lysinol scaffold that resulted in both improved functional potency and PK will be reported in due course.

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