Synthesis and Structure–Activity Relationships of a Series of Penicillin-Derived HIV Proteinase Inhibitors: Heterocyclic Ring Systems Containing P_1' and P_2' Substituents

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As an extension of our earlier work based upon a single penicillin-derived thiazolidine moiety we have found that the decahydroisoquinoline grouping, also present in Ro 31-8959, is an effective replacement for one of the thiazolidine units in C_2 symmetric penicillin-derived dimers. Reaction of racemic epoxide **6** with [3*S*-[3α,4aα,8aα]]-decahydro-*N*-(1,1-dimethylethyl)-3-isoquinolinecarboxamide gave diasteroisomers **34a** and **34b**. The stereochemistry of the hydroxyl grouping of **34a** was determined to be (*S*). Reaction of the amines derived from **34a** and **34b** with thiazolidine **8a** gave **50** and **51**, respectively. Compound **50** was a potent inhibitor of HIV proteinase (IC_{50} = 23 nM) with antiviral activity against HIV-1 *in vitro* (EC_{50} C8166 cells = 50 nM). However, a poor pharmacokinetic profile in the dog for compound **50** and its analogues, in keeping with earlier studies on penicillin-derived dimers in three species, precluded their development as potential antivirals.

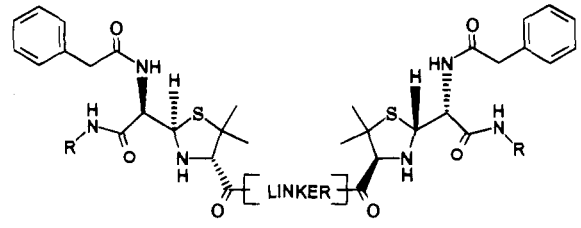
Introduction

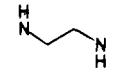
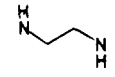
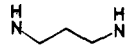
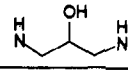
Human immunodeficiency virus (HIV)[1] is the causative agent of acquired immune deficiency syndrome (AIDS). A late stage in the replication of HIV involves the key proteolytic processing of polyproteins by a viral enzyme, HIV proteinase.[2] Thus the inhibition of HIV proteinase has become an important therapeutic target in the search for an effective antiviral agent.[3] A number of groups have developed peptide-based inhibitors of HIV-1 proteinase using the natural substrate sequences as starting points with the scissile bond being replaced with a transition-state mimetic. Many of these inhibitors have been shown to block the replication of HIV-1 *in vitro*. However, the combination of high molecular weight and peptidic character of these molecules has made it difficult to achieve the balance of physicochemical properties necessary to provide an acceptable *in vivo* profile. Nevertheless, a recent report from workers at Abbott[4] demonstrated that reduction in molecular weight may be achieved while retaining potency. Compound A-80987 has emerged from their studies as a potent anti-HIV agent *in vitro* with favorable pharmacodynamic properties *in vivo*.

In an earlier communication[5] we described our screening approach as a means of identifying novel inhibitors of HIV-1 proteinase. This led to a series of penicillin-derived C_2 symmetric dimers as potent enzyme inhibitors which also showed activity against the virus *in vitro*, *e.g.*, **1a** and **1b** (Table 1). An X-ray structure[6] of the ethylamide analogue **1a** complexed to the recombinant enzyme has shown a C_2 symmetric mode of binding with no significant interaction with the catalytic aspartates Asp25 and Asp25′. Subsequent comparisons[7] with **2** and **3** demonstrated that introduction of a hydroxyl group into the linker region of the dimers resulted in a favorable hydrogen bonding interaction to the aspartates. With the aid of computer-assisted molecular modeling we then developed a series of potent inhibitors, *e.g.* **4** (IC_{50} = 4.6 nM), having significantly reduced molecular weight and containing a stereochemically unique statine dipeptide isostere.[8]

In view of the success of this strategy based upon inhibitors containing a single penicillin-derived thiazolidine unit, we sought alternative means of interacting with the S_1' and S_2' subsites. The recently published work from Martin and co-workers[9] on the biological

Table 1. Anti-HIV Activity of Penicillin Dimers



No.	R	Linker	IC_{50}(μM)[a]	EC_{50}(μM)[b] (MT-4)
1a	Et		0.005	5.4
1b	$PhCH_2$		0.0009	0.29
2	$PhCH_2$		0.54	-
3	$PhCH_2$		0.005	2.6

[a] IC_{50} determinations were performed in duplicate at each concentration with mean values used for data analysis. Results were standardized, with a control used in all assays. [b] ED_{50} (MT-4) ≥ 100 μM.

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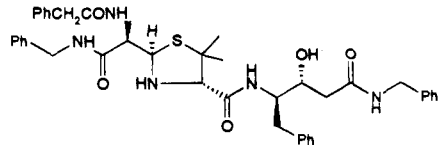
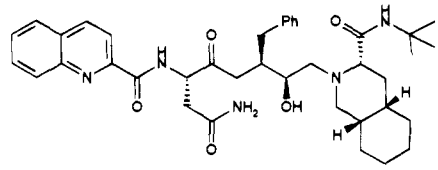


4



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properties of Ro 31-8959 (**5**) indicated that the decahydroisoquinoline moiety could fulfill this role and may well significantly affect the antiviral and pharmacokinetic properties of any "hybrid" molecules.

This paper describes the synthesis and structure–activity relationships of a series of "monomer" penicillin-derived analogues containing substituted heterocyclic ring systems designed to interact with the S_1' and S_2' subsites of HIV-1 proteinase.

Chemistry

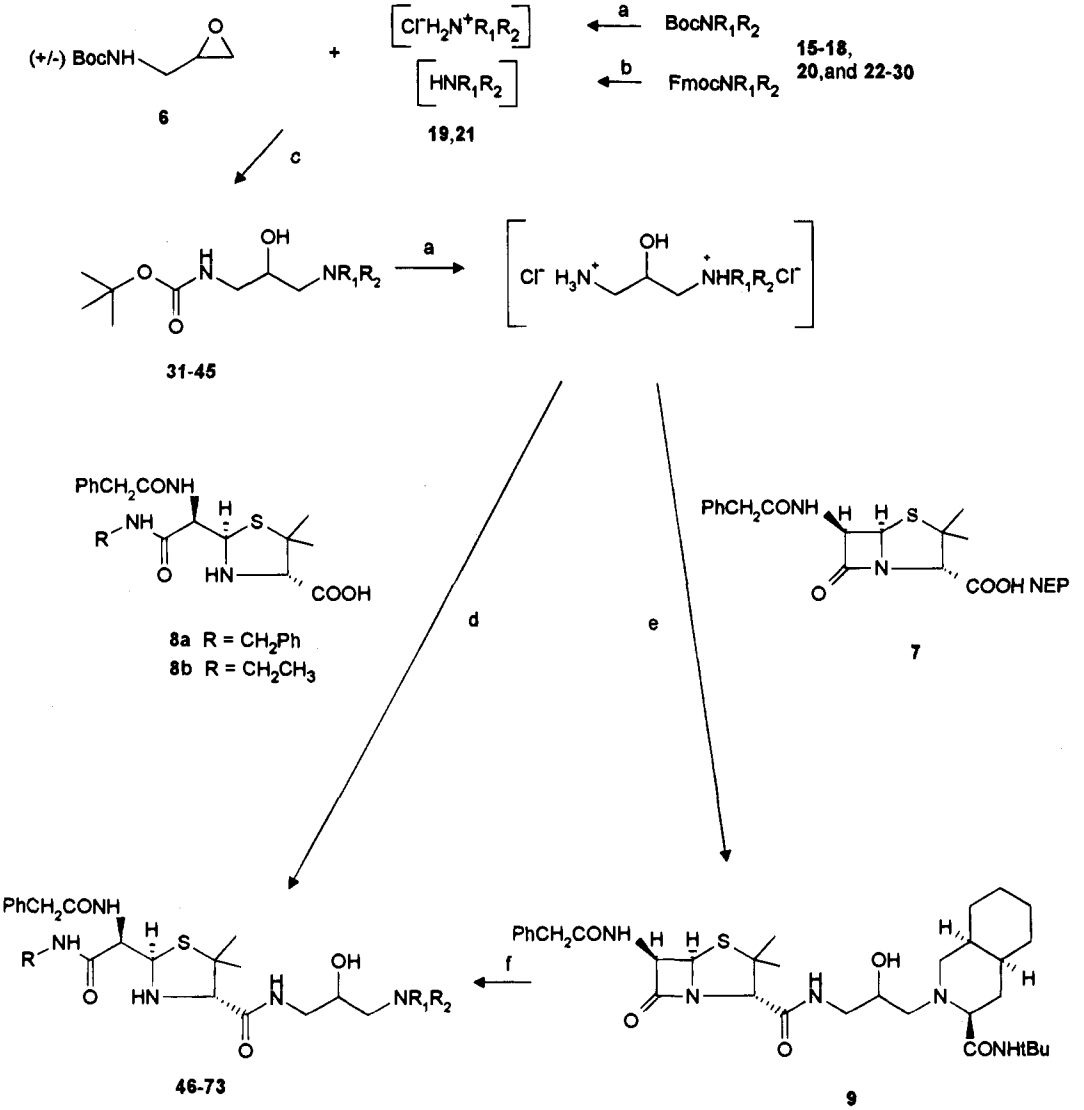
All the compounds **46**–**73** (Tables 5–7) were prepared by the routes outlined in Scheme 1. The amides **15**–**30** (Table 3) were prepared in good yield by reacting the appropriate amino acid with either isobutyl- or *tert*-butylamine using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as the coupling agent in DMF according to general procedure B. Acid **11**, the precursor to **17**, was derived from pipecolic acid using di-*tert*-butyl dicarbonate to introduce the protecting group. The thiazolidine **18** was synthesized from the known N-protected thiazolidine acid[10] and then converted to the hydrochloride salt **19** using HCl(g) in dioxan. The fully protected proline derivative **23** was prepared from acid **10** (Table 2), which itself was obtained from the commercially available *tert*-butyl ether of 4(*R*)-hydroxyproline. The Boc-protected 4(*S*)-ethoxyproline **13** was synthesized in 94% yield from the hydroxyl-containing precursor by alkylation with ethyl iodide and sodium hydride in tetrahydrofuran (THF). The *tert*-butylamide **25** was then synthesized in the conventional manner. A similar alkylation of **24** using benzyl bromide and sodium hydride in DMF gave the benzyl ether **26** in 55% yield. Piperazine **12** (Table 2) was derived from the known 4-benzyloxycarbonyl-protected amino acid.[11] The *N*-methylamino acid **14** was synthesized following the procedure of Freidinger and co-workers[12] and then converted to the amide **29**. Similarly prepared was the known[12] Fmoc-protected *N*-(cyclohexylmethyl)alanine required for the synthesis of compound **30**. The Fmoc group was removed using piperidine in DMF following general procedure D. Racemic epoxide **6**[13] was then ring opened in ethanol containing diisopropylethylamine (DIPEA) with the chiral amines derived from the protected intermediates of Table 3 (except **21** and **27**) to afford the hydroxyamines of Table 4 as mixtures of isomers at the hydroxyl center. Racemic thiazolidine **21** on the other hand was reacted in refluxing ethanol with the chiral epoxides (***S***)-**77a** and (***R***)-**77b** to limit the number of isomers and gave **37a** and **37b**, respectively. Similarly, the benzyloxycarbonyl-protected piperazine **42** was obtained using (*S*)-epoxide **77a**. The chiral (*S*)-epoxide **77a** was prepared *via* the route shown in Scheme 2. (*R*)-(+)-Glycidol **74a** was reacted with ammonia in 2-propanol for 2 days and the intermediate amine converted directly to the *tert*-butoxycarbonyl diol derivative **75a** in 73% yield. Reaction of the diol **75a** with *p*-toluenesulfonyl chloride in pyridine gave the primary tosylate **76a** in 62% yield which was converted to (*S*)-(−)-epoxide **77a** in 75% yield using sodium methoxide in methanol. Utilisation of (*S*)-(−)-glycidol **74b** using the same methodology gave (*R*)-epoxide **77b**. Reaction of racemic epoxide **6** with [3*S*-[3α,4aα,8aα]]-decahydro-*N*-(1,1-dimethylethyl)-3-isoquinolinecarboxamide[14] gave two diasteroisomers **34a** and **34b** that were separated by silica gel flash chromatography. The stereochemistry of the hydroxyl groups was determined by reaction of (*R*)-epoxide **77b** with the decahydroisoquinoline ring system to give a product **34a** that was co-retentive by TLC with the less polar component of the diastereomeric mixture obtained earlier. The more polar isomer, therefore, has the (*S*) chirality for the hydroxyl group. The intermediates **31**–**42** (Table 4) were deprotected with HCl(g) in dioxan and the unpurified salts then reacted with thiazolidine **8a**[15] (Scheme 1) using general procedure B to give analogues **46**–**62** (Table 5). Thiazolidine **8a**[15] itself was obtained from penicillin G *N*-ethylpiperidine (NEP) salt **7** using benzylamine to ring open the β-lactam. Compounds **43**–**45** were similarly converted to derivative **64**–**66** (Table 6) except that thiazolidine **8b**[16] obtained from penicillin G NEP salt **7** using ethylamine as the nucleophile afforded **64** and **65**. An alternative method was developed for the preparation of some of the analogues **46**–**73** (Scheme 1) whereby the β-lactam ring system was left intact until the final stage when intermediate **9**, which was used without purification, could be reacted with a variety of nucleophiles. Compounds **63** and **67**–**73** were synthesized using this methodology. The single diastereoisomers **63**, **67**, **68**, and **69** (Table 7) were prepared from the separated isomers **34a** and **34b** (Table 4) as appropriate. Derivative **72** was synthesized from **9** by ring opening the β-lactam with monoprotected ethylenediamine.[17] Removal of the *tert*-butoxycarbonyl group was achieved using trifluoroacetic acid (TFA) in dichloromethane to give an intermediate amine which was subsequently reacted with the 4-nitrophenyl ester of biotin to afford **73**.

Results and Discussion

Our initial targets were compounds **46** and **47** (Table 5) which were obtained from the isobutyl- and *tert*-butylamides of (*S*)-proline. Our expectation was that the proline amide would fit S_1' and S_2' in a similar manner to substrate sequences and inhibitors containing proline as the P_1' group, *e.g.*, JG365[18].

The modeling of compound **47** was based upon the X-ray co-crystal structure of **1a** with HIV proteinase.[6] The thiazolidine unit of **47** was placed in a similar position to half of the dimeric inhibitor and torsion angles adjusted to place the proline ring in S_1' and the *tert*-butyl group in S_2'. Particular attention was paid to the positioning and direction of the pendant hydroxyl and *tert*-butyloxycarbonyl groups such that interactions to the catalytic aspartates and the "flap" water (301) were maintained. A stereorepresentation is shown in Figure 1 of the overlay of JG365 with **47**. The coordi-

Scheme 1[a]



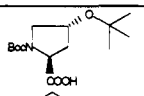
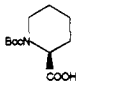
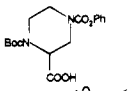
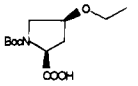
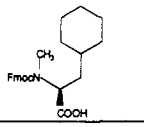
[a] (a) HCl in dioxane (b) 20% piperidine in DMF; (c) EtOH, 1.1 equiv of DIPEA used with HCl salt, room temperature 17–120 h; (d) 3.3 equiv of DIPEA, 1,1 equiv of TBTU DMF, room temperature 2–48 h; (e) as in d with decahydroisoquinoline moiety; (f) RNH_2 CH_2Cl_2, 1–5 d.

nates of JG365 were those determined from the X-ray crystallographically determined structure of synthetic HIV-1 proteinase bound to JG365.[18b]

However, the results we obtained for **46** and **47** (IC_{50} = 3.1 and 1.5 μM, respectively) (Table 5) were disappointing when compared to data in the literature[18,19] on JG365 and indicated that interactions were far from optimal. We then proceeded to increase the ring size and bulk of the P_1' group to give the pipecolic acid and decahydroisoquinoline analogues **48**, **49**, **50**, and **51**. We were surprised to find that we did not observe the increase in binding for the pipecolic acid analogues **48** and **49** that might have been expected based upon the work of Roberts and co-workers.[9a] However, modeling of **48** and **49** along similar lines to the proline analogue **47** and overlaying with JG365 indicated that there was an increase in space filling, but no major hydrophobic interactions with the protein were observed. The backbone of the proline analogue **47** does follow a different pathway to JG365, and as a consequence the proline ring is positioned in S_1' in a different plane and is rotated relative to JG365 (Figure 1). Despite the lack of improvement in inhibitory activity of **48** and **49**, the decahydroisoquinoline moiety provided a substantial increase in activity to 23 and 160 nM for the two diastereoisomers **50** and **51**, respectively, in keeping with our expectations. If **47**, **48**, and **49** are modeled as described above and overlayed with Ro 31-8959, the backbone again follows a slightly different course through the active site when compared to Ro 31-8959 and as a consequence the *tert*-butylamide group position in S_2' is different to Ro 31-8959 with the methyl groups not as deeply buried. The modeling of Ro 31-8959 was based upon the information available in the communication by Krohn and co-workers[19] and reflected the novel binding mode determined for Ro 31-8959. A similar protocol to that described above for **47**, **48**, and **49** was used to generate a model of compound **50**. This indicated (Figure 2) improved hydrophobic interaction in the S_1' pocket as the main reason for the observed increase in binding of **50** relative to **47**. Comparison with the model generated for Ro 31-8959 shows again that there is good correspondence in the positions of important binding functionality.

Our conclusions are that as a result of the modeled backbone paths of our inhibitors being slightly different to both JG365 and Ro 31-8959, we feel that the result observed is not unreasonable. Unfortunately, co-

Table 2. Structure and Physical Data for Intermediates 10–14

No.	Structure[a]	Formula[b]	$[\alpha]_D^{22}$	m.p.
10		$C_{14}H_{25}NO_5$	-32.6°(c 1.07,MeOH)	80°
11		$C_{11}H_{19}NO_4$	-48.0°(c 1.0,MeOH)	115-118°
12	(+/-) 	$C_{18}H_{24}N_2O_6$		foam
13		$C_{13}H_{23}NO_5 \cdot 0.5H_2O$	-28.5°(c 0.93,MeOH)	oil
14		$C_{25}H_{29}NO_4 \cdot 0.3H_2O$	-16.0°(c 0.25,$CHCl_3$)	foam

[a] 10 and 11 prepared from the commercially available amine using standard procedure A. [b] Satisfactory analyses (C, H, N; 0.4% of theoretical values) were obtained for all compounds.

crystallization studies with this type of inhibitor have proved unsuccessful.

Isomer **50** (Table 7) displayed similar antiviral activity to the thiazolidine dimer **1** (Table 1) (ED_{50} = 0.35 and 0.29 μM, respectively) in HIV-1-infected MT-4 cells yet was of substantially reduced molecular weight. Compound **50** also inhibited syncytia formation of HIV-1-infected C8166 cells (EC_{50} = 50 nM). The thiaproline analogue **52** showed a 7-fold increase in activity against HIV proteinase compared to **47** probably as a result of the increased hydrophobic contact in S_1'. A further 20-fold increase in binding was obtained by using the substituted thiazolidine derived from L-penicillamine to give **53** which has an IC_{50} = 21 nM against HIV-proteinase and shows antiviral activity in a whole cell assay EC_{50} = 1.7 μM.

Figure 3 shows the van der Waals contacts between the inhibitor thiazolidine residue bearing the *tert*-butylamide and the residues forming the S_1' pocket. It can be seen that with the additional lipophilic *gem*-dimethyl groups the ligand now fills the pocket more effectively.

We then proceeded to synthesize the tetramethylthiazolidine derivatives **54** and **55** in order to increase the bulk and lipophilicity still further. Using chiral epoxides (*S*)-**77a** and racemic thiazolidine **21** we obtained **37a** as an inseparable 1:1 mixture of diastereoisomers, as determined by NMR spectroscopy. Removal of the *tert*-butoxycarbonyl group from **37a** and coupling to the thiazolidine **8a** gave the final compound **54** as a 1:1 mixture of diastereoisomers. A similar series of reactions beginning with **21** and epoxide (*R*)-**77b** resulted in intermediate **37b** and final compound **55** as 1:1 mixtures, respectively. Compounds **54** and **55** are both 1:1 mixtures of diastereoisomers with enzyme inhibitory levels of IC_{50} = 93 and 190 nM, respectively. Therefore, this level of inhibitory activity obtained confirmed that compound **53** was the most potent identified in the series and that the increased lipophilicity had no beneficial effect. A further series of analogues **56–60** based upon *trans*- and *cis*-4-hydroxyproline were synthesized in an attempt to improve hydrophobic interactions in the S_1' pockets. However, the similar level of activity relative to the parent **46** and the insensitivity to stereochemistry demonstrated that there was little gain from hydrophobic interactions.

As compound **50** was the most potent in the series, a set of monocyclic derivatives **64–66** (Table 6) was made. The substantial loss in activity relative to **63** confirmed that the conformational constraint afforded by the bicyclic system was a requirement for high binding affinity.

Previous structure–activity studies[16] had shown that the P_3 group could be varied considerably without making marked differences to the level of enzyme inhibitory activity. We therefore embarked upon a program to vary the nucleophile used to open the β-lactam, in order to investigate the antiviral properties of analogues in cellular assays. As can be seen from Table 7 compound **50** with the (*S*)-hydroxyl showed a pronounced antiviral effect whereas the corresponding (*R*)-isomer was less active at the enzyme level and had a very poor anti-HIV effect. Subsequent analogues either maintained the (*S*)-stereochemistry **63** and **67–69** or were a mixture of isomers. Interestingly compounds **63**, **68**, and **69** had similar levels of enzyme and cellular activities and were all slightly less potent than **50** as antivirals. The P_3 group could therefore be varied substantially while retaining antiviral activity except for heptylamine analogue **71**, which proved inactive. Compound **73** which contains a biotin grouping was synthesized, utilizing **72** as an intermediate in an attempt to improve cellular penetration by utilization of an active transport system.[20] The poor activity in the cellular assay indicated that it was unlikely the compound was being actively transported into the cell.

No compound exhibited cytotoxicity in any of the cellular assays, nor did any show activity against other aspartyl proteinases (renin, pepsin, or cathepsin D) at concentrations up to 100 μg/mL.

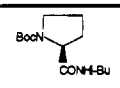
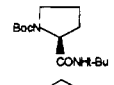
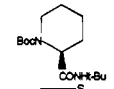
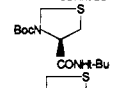
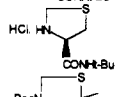
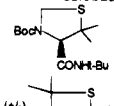
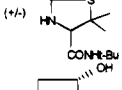
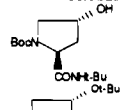
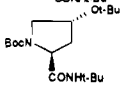
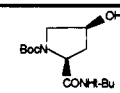
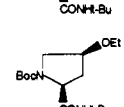
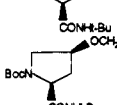
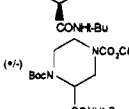
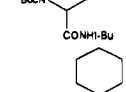
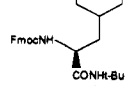
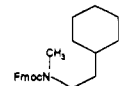
The pharmacokinetic profiles of compounds **50** and **68** were examined in the dog at a dose of 1 mg/kg iv and compared to the penicillin dimer **1a**. The plasma samples were analyzed by thermospray mass spectrometry, and the plasma level–time data used to calculate the pharmacokinetic parameters are shown in Table 8. The compounds were very rapidly eliminated from the plasma (Figure 4) presumably by biliary excretion which was the mode of elimination previously determined for the thiazolidine dimers.[16] The plasma levels at 3 h for **50** and **68** were 0.03 and 0.02 μg/mL, respectively, which were below the *in vitro* determined IC_{50} values.

Conclusion

We have identified a series of penicillin-derived inhibitors of HIV-1 proteinase that have a decahydroisoquinoline moiety as the P_1' group. Compound **50**, which has the (*S*) configuration for the secondary hydroxyl, was the most potent of the set in enzyme and cellular assays (EC_{50} = 50 nM, C8166 cells).

In keeping with results obtained from the earlier penicillin dimer series of HIV proteinase inhibitors we observed a poor pharmacokinetic profile for **50** and its lower molecular weight analogue **68** in the dog at a dose of 1 mg/kg iv. The disappointing pharmacokinetics, therefore, prevented the development of these compounds as potential antivirals in the treatment of HIV infections and concluded our interest in penicillin-derived HIV proteinase inhibitors.

Table 3. Structure and Physical Data for Intermediates **15–30**

No.	Structure[a]	Formula[b]	$[\alpha]_D^{22}$	m.p.
15		$C_{14}H_{26}N_2O_3$	-59.1°(c 1.0,MeOH)	91-93°
16		$C_{14}H_{26}N_2O_3$	-47.8°(c 0.98,MeOH)	119-121°
17		$C_{15}H_{28}N_2O_3$	-48.0°(c 1.0,MeOH)	127-129°
18		$C_{13}H_{24}N_2O_3S$	-142.0°(c 1.0,MeOH)	128-129°
19		$C_8H_{16}N_2O_3S.HCl$	-99.8°(c 1.0,MeOH)	244-5°
20		$C_{15}H_{28}N_2O_3S.0.1H_2O$	-5.9°(c 1.0,MeOH)	98-101°
21		$C_{12}H_{24}N_2OS$		140°
22		$C_{14}H_{26}N_2O_4$	+46.7°(c 1.0,MeOH)	188-190°
23		$C_{18}H_{34}N_2O_4$	-12.1°(c 1.2,MeOH)	188-190°
24		$C_{14}H_{26}N_2O_4.0.1H_2O$	-5.8°(c 0.95,MeOH)	126-128°
25		$C_{16}H_{30}N_2O_4.0.1H_2O$	-24.1°(c 1.2,MeOH)	61-64°
26		$C_{21}H_{32}N_2O_4$	-15.1°(c 0.63,MeOH)	oil
27		$C_{22}H_{33}N_3O_5$		110-111°
28		$C_{28}H_{36}N_2O_3$	+7.1°(c 0.2,$CHCl_3$)	96-98°
29		$C_{29}H_{38}N_2O_3.0.05CHCl_3$	-72.0°(c 1.0,$CHCl_3$)	146-148°
30		$C_{29}H_{38}N_2O_3.0.2H_2O$	-23.0°(c 1.00,MeOH)	foam

[a] Prepared using general procedure B. [b] Satisfactory analyses (C, H, N; 0.4% of theoretical values) were obtained for all compounds.

Experimental Section

Chemistry. Melting points were determined with a Gallenhamp melting point apparatus and are uncorrected. Optical rotations were determined on an Optical Activity AA100 polarimeter. Proton nuclear magnetic resource (NMR) spectra were recorded on a Varian XL200, a Varian VXR400, or a Bruker AM250 spectrometer. Chemical shifts were reported as δ units (ppm) relative to tetramethylsilane as internal standard. Infrared (IR) spectra were recorded on a Nicolet 5SXC FT-IR spectrometer. High-resolution mass spectra were determined using a Kratos-Concept. Merck Kieselgel 60 (230–400 mesh) was used for flash column chromatography. Reagents were from commercial sources and used without further purification.

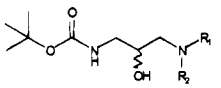
HIV-1 Proteinase Inhibition Assay. The IC_{50} values for the compounds of Tables 5–7 were determined using purified recombinant HIV-1 proteinase. IC_{50} values were obtained by assaying the enzyme against the synthetic substrate peptide KQGTVSFNFPQIT tritiated at the proline residue (Cambridge Research Biochemicals). The peptide was coupled *via* its N-terminal lysine to an Affi-gel 10/15 bead mixture (Bio-rad), and the immoblized peptide (1 part) was stored in the assay buffer: 20 nM MES (pH 6.0), 2 M NaCl, 5 mM DTT, and 2 mM EDTA (2 parts) at −20 °C until required. The assay was performed in 96-well microtiter filtration plates (Pall, Silent Monitor) using 10 μL of a solution of the test compound dissolved at a range of concentrations in 25% Me_2SO in water, 50 μL of HIV-1 proteinase (0.2 μg/mL), and 50 μL of radiolabeled bead suspension (diluted with assay buffer (40 parts)) per well. The plates were incubated at 37 °C for 60 min on a flat-bed shaker (80 rev/min) and then filtered into the wells of a collection plate on a vacuum manifold (Pall). The 10 μL of each filtrate was transferred to a 96-well scintillation plate (Wallac) and mixed with 50 μL of Hi-Load Scintillant (Wallac). Radioactivity was measured using an LKB 1205 Microbeta liquid scintillation counter (Wallac). IC_{50} determinations were performed in duplicate at each concentration with mean values used for data analysis. Results were standardized relative to the initial IC_{50} value obtained for compound **1a** which was thereafter used as a control.

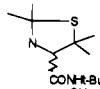
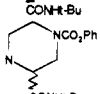
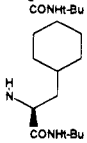
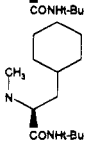
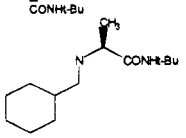
Inhibition of HIV-1 in Cell Culture. A. Inhibition of Formazan Conversion Assay.[21] MT-4 cells at 10^6 cells/mL in RPMI 1640 growth medium with HIV-1 (strain RF) at a multiplicity of infection of 2×10^{-3} infectious units/cell. Test compounds were dissolved in Me_2SO and serially diluted with RPMI 1640 growth medium in 10-fold steps from 100 to 0.01 μg/mL in 96-well microtiter plates. Then, 20 μL of infected or mock-infected cell suspension was added to each well (5×10^4 cells/well), and the plates were incubated at 37 °C in a 5% CO_2 atmosphere for 7 days in humidified containers. After incubation, 10 μL of MTT (7.5 mg/mL) was added, and the plates were incubated at 37 °C for a further 60 min. Acidified 2-propanol (150 μL) was added to each well, and the absorbance was measured at 540 nm using a Multiskan MC plate reader. Conversion of yellow MTT to its blue-black formazan derivative was maximal in uninfected cells and absent in untreated infected cells. The effective concentration (EC_{50}) required to inhibit the conversion of MTT by 50% was determined from the average of duplicate assays. Compounds were assayed for toxicity at identical concentrations.

B. Inhibition of Syncytium Formation Assay. C8166 cells were infected with HIV-1 (strain RF) at a multiplicity of infection of 1×10^{-3} infectious units/cell. Aliquots of 10^5 cells were added to each well of 24-well plates containing the test compounds dissolved in Me_2SO and serially diluted with RPMI 1640 growth medium from 50 to 0.05 μg/mL. Untreated infected cells and untreated uninfected cells were included as controls. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 3–4 days in humidified containers. The cells were examined daily for evidence of HIV-1-induced syncytium formation. The syncytia were quantified by reference to the untreated infected controls and the dose of compound required to reduce the cytopathic effect by 50% (EC_{50}) was calculated.

General Method A: Boc Protection of 10–12. The appropriate amino acid (1.0 equiv) was dissolved in aqueous dioxane (50% v/v) (0.5 M) containing either sodium carbonate or sodium hydroxide (1.0–40 equiv). Di-*tert*-butyldicarbonate (1–10 equiv) was added and the mixture stirred at room temperature for 2–18 h. The solution was extracted with ether (2×), ethyl acetate was then added, and the pH was adjusted to 2.0–3.0 with 2 N hydrochloric acid. The aqueous layer was further extracted with ethyl acetate, and combined organic phases were washed with brine and dried ($MgSO_4$), and the solvent was evaporated *in vacuo* to give the required

Table 4. Structure and Physical Data for Intermediates **31–45**



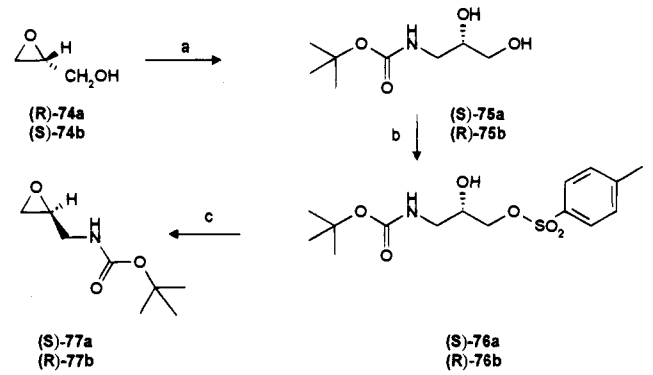
No.[a]	NR_1R_2	(OH)Stereochemistry	Formula[c]	m.p.
31			$C_{17}H_{33}N_3O_4.0.6H_2O$	
32			$C_{17}H_{33}N_3O_4$	
33			$C_{18}H_{35}N_3O_4$[d]	
34a		(R)	$C_{22}H_{41}N_3O_4$	161-163°
34b		(S)	$C_{22}H_{41}N_3O_4.0.25H_2O$	56-58°
35a		isomer 1	$C_{18}H_{35}N_3O_4S$	98-102°
35b		isomer 2	$C_{18}H_{35}N_3O_4S.0.25H_2O$	102-105°
36			$C_{16}H_{31}N_3O_4S$[b]	
37a		(S)	$C_{20}H_{39}N_3O_4S$[b]	
37b		(R)	$C_{20}H_{39}N_3O_4S$[b]	
38			$C_{17}H_{33}N_3O_5.0.5H_2O$	
39			$C_{21}H_{41}N_3O_5$	
40			$C_{19}H_{37}N_3O_5.0.6CHCl_3$	
41			$C_{24}H_{39}N_3O_5.0.5H_2O$	
42		(S)	$C_{25}H_{40}N_4O_6$	
43			$C_{21}H_{40}N_3O_4.1.0H_2O$	
44			$C_{22}H_{43}N_3O_4.0.5H_2O$	
45			$C_{22}H_{44}N_3O_4$[b]	

[a] Prepared using general procedures C and E. [b] Satisfactory accurate mass of MH^+, and HPLC analysis. [c] Satisfactory analyses (C, H, N; 0.4% of theoretical values) were obtained for all compounds. [d] A single diastereoisomer of undetermined hydroxyl stereochemistry was obtained pure and gave a satisfactory accurate mass of MH^+, and HPLC analysis.

product (85–90% yield), which was recrystallized from cyclohexane where appropriate.

Example of General Method A. (*R*)-3-(*tert*-Butoxycarbonyl)thiazolidine-4-carboxylic Acid.[10] Sodium bicarbonate (5.6 g, 66.7 mmol) was added portionwise to a stirred suspension containing L-thiazolidine-4-carboxylic acid (5.19 g, 39.0 mmol) in water (90 mL) and 1,4-dioxane (60 mL). The resulting solution was cooled in an ice bath, and a solution of di-*tert*-butyldicarbonate (10.8 g, 49.5 mmol) in dioxane (30 mL) was added. The mixture was stirred at room temperature for 3 h and then concentrated to *ca.* half volume *in vacuo*. The aqueous residue was washed with ethyl acetate (2 × 50 mL) and then acidified with solid citric acid until saturated. It was then extracted with ethyl acetate (4 × 200 mL), and the combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* to a white solid. This was crystallized from hot cyclohexane/ethyl acetate to give the title compound as colorless needles (7.91 g, 87%): mp 132–133 °C [lit.[10] mp 132–135 °C; $[\alpha]^{20}_D$ −114° (*c* 1, MeOH)]; $[\alpha]^{21}_D$ −106° (*c* 1.0, MeOH); ^1H NMR (DMSO-d_6) δ 1.40 (s, 9H), 3.5–3.0 (broad m, 2H), 4.50 and 4.32 (AB q, J = 12 Hz, 2H), 4.7–4.5 (br m, 1H), 12.9 (br s, 1H). Anal. ($C_9H_{15}NO_4S$) C, H, N.

Scheme 2[a]



[a] (a) Saturated ammoniacal 2-propanol, room temperature 48 h/Boc_2O dioxane, 0.5 M NaOH, room temperature 20 h; (b) *p*-toluenesulfonyl chloride/pyridine, room temperature 3 h; (c) 1.0 equiv of $NaOCH_3$ in methanol, room temperature 3 h.

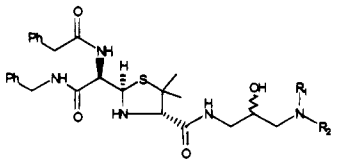
General Procedure B: Amide Formation Using TBTU/DIPEA 15–30, 46–51, 53–66. The appropriate acid (1.0 equiv) and amine or amine hydrochloride (1.0 equiv) were dissolved in DMF (0.1–0.7 M), and DIPEA (1.1 equiv, 1 extra equiv for RNH_2·HCl) and TBTU (1.1–1.2 equiv) were added. The mixture was stirred at room temperature for 2.0–48 h, until the reaction was judged complete by TLC analysis. The solution was partitioned between EtOAc and water, and the aqueous phase was further extracted with EtOAc (2×). The combined organic phases were washed with 2 N HCl, saturated aqueous $NaHCO_3$, and brine before drying ($MgSO_4$). The solvent was removed *in vacuo* and the resulting residue purified by silica gel chromatography to afford the desired amide in good in yield (40–95%).

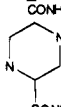
Example of General Method B. *N*-*tert*-Butyl-3-(*tert*-butoxycarbonyl)thiazolidine-4-carboxamide (18). A solution containing 3-(*tert*-butoxycarbonyl)thiazolidine-4-carboxylic acid (2.09 g, 8.96 mmol), *tert*-butylamine (1.04 mL, 9.90 mmol), DIPEA (1.72 mL, 9.87 mmol), and TBTU (3.47 g, 10.8 mmol) in DMF (20 mL) was stirred at room temperature under N_2 for 2 h and was then diluted with ethyl acetate (200 mL). The solution was washed with dilute HCl (2 × 50 mL), saturated aqueous sodium bicarbonate (2 × 50 mL), and brine (50 mL), dried ($MgSO_4$), and evaporated *in vacuo* to a crystalline solid. This was recrystallized from hot cyclohexane to give the title amide as colorless needles (2.36 g, 91%): mp 128–129 °C; $[\alpha]_D^{21}$ −142° (*c* 1.05, MeOH); ^1H NMR (DMSO-d_6) δ 1.25 (s, 9H), 1.38 (s, 9H), 2.95 (dd, J = 12.0, 6.0 Hz, 1H), 3.32 (br m, 1H), 4.60 and 4.32 (AB q, J = 12.0 Hz, 2H), 4.35 (br m, 1H), 7.50 (broad s, 1H). Anal. ($C_{13}H_{24}N_2O_3S$) C, H, N.

General Procedure C: Boc Deprotection of 15–18, 20, 22–27, and 31–45. The appropriate Boc-protected amine (1.0 equiv) was dissolved in dioxane (0.1–0.4 M), and 3-8 M HCl-(g) in dioxane (5–20 equiv) was added. The solution was stirred at room temperature for 4–20 h and the solvent then evaporated *in vacuo*. The unpurified hydrochloride salts were used in subsequent amide bond forming reactions.

Compounds **27** and **42** were deprotected using trifluoroacetic acid (7–20% v/v) in dichloromethane for 2–6 h at room temperature. Evaporation of the solvent *in vacuo* gave un-

Table 5. Activity against HIV Proteinase



No.[a]	NR_1R_2	(OH)Stereo-chemistry	Formula[c]	IC_{50}(μM)
46			$C_{35}H_{50}N_6O_5S.0.5H_2O$	3.1
47			$C_{35}H_{50}N_6O_5S$[b]	1.5
48		isomer 1	$C_{36}H_{52}N_6O_5S$[b]	>2.2
49		isomer 2	$C_{36}H_{52}N_6O_5S.0.5H_2O$	6.5
50		(S)	$C_{40}H_{58}N_6O_5S.0.5H_2O$	0.023
51		(R)	$C_{40}H_{58}N_6O_5S.0.5H_2O$	0.16
52[d]			$C_{34}H_{48}N_6O_5S_2.1.0H_2O$	0.45
53			$C_{36}H_{52}N_6O_5S_2.1.5H_2O$	0.021
54		(S)	$C_{38}H_{56}N_6O_5S_2.0.5H_2O$	0.19
55		(R)	$C_{38}H_{56}N_6O_5S_2.0.5H_2O$	0.093
56			$C_{35}H_{50}N_6O_6S.0.5CHCl_3$	5.1
57			$C_{39}H_{50}N_6O_6S.1.0H_2O$	1.6
58		isomer 1	$C_{37}H_{54}N_6O_6S.0.7H_2O$	>35
59		isomer 2	$C_{37}H_{54}N_6O_6S.0.15CHCl_3$	>35
60			$C_{42}H_{56}N_6O_6.0.5H_2O$	0.65
61		isomer 1 (S)	$C_{43}H_{57}N_7O_7S.0.6CHCl_3$	0.058
62		isomer 2 (S)	$C_{43}H_{57}N_7O_7S$	0.082

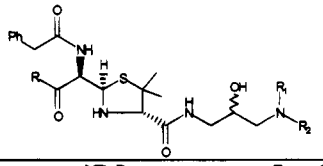
[a] Prepared using general procedure B. [b] Satisfactory accurate mass of MH^+, and HPLC analysis. [c] Satisfactory analyses (C, H, N; 0.4% of theoretical values) were obtained for all compounds. [d] Prepared using general procedure F.

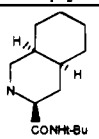
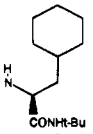
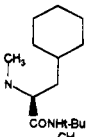
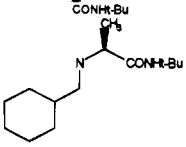
purified trifluoroacetate salts, which were used in subsequent amide bond forming reactions.

Example of General Method C. (*S*)-*N*-*tert*-Butylthiazolidine-4-carboxamide, Hydrochloride (19). A solution of HCl(g) in 1,4-dioxane (6 M, 10 mL) was added to a suspension containing the Boc-protected intermediate **18** (2.16 g, 7.49 mmol) in 1,4-dioxane (20 mL). The mixture was stirred at room temperature under nitrogen for 1.5 h, a further portion of HCl(g) dioxane (5 mL) was added, and the mixture was stirred for a further 3 h. The precipitate was then collected by filtration, washed with diethyl ether, and dried *in vacuo* to give the title salt as fine colorless needles (1.45 g, 86%): mp 244–5 °C; $[\alpha]^{20}_D$ −99.8° (*c* 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 9H), 3.12 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.48 (dd, *J* = 12.0, 8.0 Hz, 1H), 4.26 and 4.35 (AB q, *J* = 12.0 Hz, 2H), 4.30 (t, *J* = 8.0 Hz, 1H), 8.39 (s, 1H), 10.0 (br, 2H). Anal. ($C_8H_{16}N_2OS$·HCl) C, H, N.

General Method D: Fmoc Deprotection of 28–30. The appropriate Fmoc-protected amine (1.0 equiv) was dissolved in DMF (0.12 M) and piperidine added (20–25% v/v). The solution was stirred at room temperature for 1–2 h and then partitioned between water and ethyl acetate. The organic phase was washed with brine and dried ($MgSO_4$) and the solvent evaporated *in vacuo* to leave an oil. Purification by silica gel chromatography afforded the amine, which was used in subsequent amide bond forming reactions.

Table 6. Activity against HIV Proteinase



No.[a]	R	NR_1R_2	Formula[c]	IC_{50}(μM)
63[d]	EtNH		$C_{35}H_{56}N_6O_5S$[b]	0.031
64	EtNH		$C_{34}H_{56}N_6O_5S.2.0H_2O$	1.0
65	EtNH		$C_{35}H_{58}N_6O_5S.0.9EtOAc$	0.71
66	$PhCH_2NH$		$C_{40}H_{60}N_6O_5S.2.5H_2O$[e]	6.4

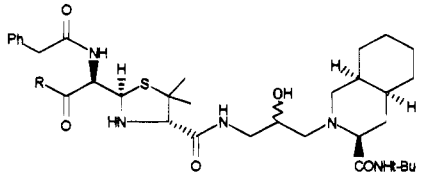
[a] Prepared using general procedure B. [b] Satisfactory accurate mass of MH^+, and HPLC analysis. [c] Satisfactory analyses (C, H, N; 0.4% of theoretical values) were obtained for all compounds. [d] Prepared using general procedure F. [e] N: calcd, 10.75; found, 11.36.

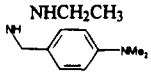
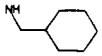
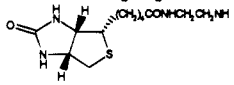
Example of General Method D. (2*S*)-α-(Methylamino)-*N*-(1,1-dimethylethyl)cyclohexanepropionamide. A solution containing Fmoc derivative **29** (100 mg, 0.216 mmol) in DMF (2 mL) was stirred at room temperature with piperidine (0.5 mL) for 1 h and then partitioned between ethyl acetate (25 mL) and water (25 mL). The aqueous phase was extracted with more ethyl acetate (2 × 25 mL), and the combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residual solid was purified by chromatography on silica gel. Elution with chloroform/methanol (30:1) gave the title amine as a white solid (38 mg, 77%): mp 59–61 °C; $[\alpha]^{23}_D$ +14.5° (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.1–0.8 (m, 2H), 1.5–1.1 (m, 6H), 1.40 (s, 9H), 1.9–1.5 (m, 5H), 2.25 (s, 3H), 2.9 (t, *J* = 5.0 Hz, 1H), 7.48 (s, 1H). Anal. ($C_{14}H_{28}N_2O$) C, H, N.

General Method E: Epoxide Ring Opening of 31–45. The appropriate amine or amine salt (1.0 equiv) was dissolved in ethanol (0.1–0.8 M), and diisopropylethylamine (1.1 equiv for the amine salt as necessary) was added. Epoxide **6** (1.0 equiv) in the case of compounds **31–36**, **38–41**, and **43–45** and epoxides (***R***)-**79b** and (***S***)-**79a** (1.0 equiv) in the case of compounds (**37b**, **37a**, and **42**, respectively) was added and the mixture stirred at room temperature for 17–120 h. The reaction mixture was heated at a temperature of 78, 65, and 50 °C for 2.5–3 d for the amines derived from **21**, **23**, and **30**, respectively. The reactions were monitored by TLC and on completion were concentrated *in vacuo* to oils. Purification by flash silica column chromatography afforded the required products in yields of 36–84%.

Example of General Method E. (2′*RS*,3″*S*,4a″*R*,8a″*S*)-*N*-[2-Hydroxy-3-[3-[[(1,1-dimethylethyl)amino]carbonyl]-decahydro-2-isoquinolinyl]propyl]carbamic Acid, 1,1-Dimethylethyl ester (34a,b). A solution containing *N*-*tert*-butyldecahydroisoquinoline-3-carboxamide[14] (1.0 g, 4.19 mmol)

Table 7. Anti-HIV and Activity of Selected Compounds



No.[a]	R	(OH)Stereo chemistry	Formula[c]	IC_{50}(μM)	EC_{50}(μM) MT4	EC_{50}(μM) C8166
50[d]	$NHCH_2Ph$	(S)	$C_{40}H_{58}N_6O_5S.0.5H_2O$	0.023	0.35	0.05
51[d]	$NHCH_2Ph$	(R)	$C_{40}H_{58}N_6O_5S.0.5H_2O$	0.16	13.6	
67	OCH_3	(S)	$C_{34}H_{53}N_5O_6S.1.0H_2O$	0.054	3.9	
68	NH_2	(S)	$C_{33}H_{52}N_6O_5S.0.35CHCl_3$	0.023	0.59	0.12
63	$NHCH_2CH_3$	(S)	$C_{35}H_{56}N_6O_5S$[b]	0.031	0.43	0.74
69		(S)	$C_{42}H_{63}N_7O_5S.1.0H_2O$	0.026	0.52	0.27
70			$C_{40}H_{64}N_6O_5S.1.0H_2O$	0.3		
71	$C_7H_{15}NH$		$C_{40}H_{66}N_6O_5S.0.4H_2O$	0.086	>100	
72	$BocNHCH_2CH_2NH$		$C_{40}H_{65}N_7O_7S.1.0CH_2Cl_2$	0.16	0.052	0.1
73[e]			$C_{45}H_{71}N_9O_7S_2.2.0H_2O$	0.12	18.0	
	Ro 31-8959			0.011	0.002	0.002

[a] Prepared using general procedure F. [b] Satisfactory accurate mass of MH^+, and HPLC analysis. [c] Satisfactory analyses (C, H, N; 0.4% of theoretical values) were obtained for all compounds. [d] Prepared using general procedure B. [e] Prepared by the method described in text.

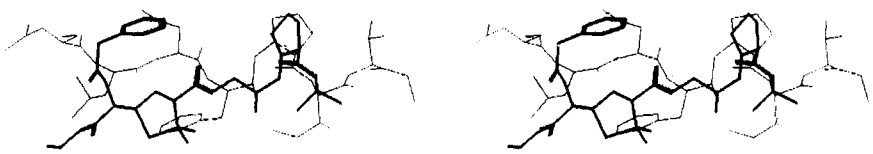


Figure 1. A stereorepresentation of JG365 (thin lines) overlayed with **47** (thick lines).

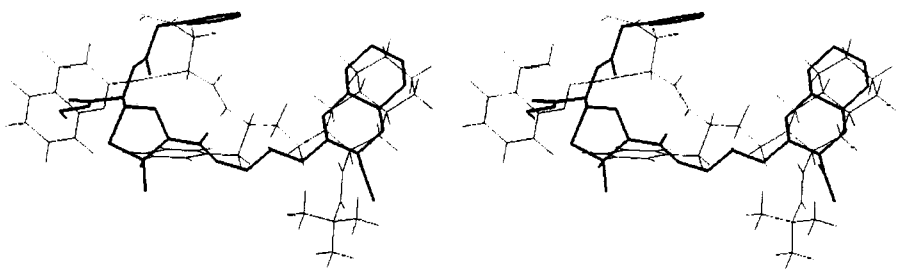


Figure 2. A stereorepresentation of Ro 31-8959 (thin lines) overlayed with **50** (thick lines).

and (±)-Boc epoxide **6** (0.73 g, 4.20 mmol) in absolute ethanol (50 mL) was stirred at room temperature under nitrogen for 20 h and then evaporated to dryness *in vacuo*. The residual foam was purified by chromatography on silica gel. Elution with chloroform/methanol (10:1) gave the title compound (isomer 1), **34a**, as a white solid (800 mg, 46%): mp 161–163 °C; $[\alpha]^{21}_D$ −114° (*c* 1.1, MeOH); ^1H NMR (DMSO-d_6) δ 1.26 (s, 9H), 1.35 (s, 9H), 2.1–1.0 (m, 14H), 2.52 (m, 1H), 2.24 (m, 1H), 2.90 (m, 3H), 3.58 (m, 1H), 4.78 (br s, 1H), 6.69 (t, J = 6.0 Hz, 1H), 7.38 (br s, 1H). Anal. ($C_{22}H_{41}N_3O_4$) C, H, N. Further elution of the column gave the title compound (isomer 2), **34b**, as a white solid (536 mg, 31%): mp 56–58 °C; $[\alpha]^{21}_D$ −100° (*c* 0.97, MeOH); ^1H NMR (DMSO-d_6) δ 1.26 (s, 9H), 1.37 (s, 9H), 2.1–1.0 (m, 14H), 2.28 (dd, J = 9.0, 11.0 Hz, 1H), 2.53 (m, obscured by solvent), 2.78 (d, J = 11.0 Hz, 1H), 3.01 (m, 2H), 3.55 (m, 1H), 4.63 (d, J = 5.0 Hz, 1H), 6.74 (t, J = 6.0 Hz, 1H), 7.37 (br s, H). Anal. ($C_{22}H_{41}N_3O_4{\cdot}H_2O$) C, H, N.

General Method F: β-Lactam Ring Opening 50–52, 63, 67–72. Penicillin G *N*-ethylpiperidine salt (1 equiv) was added to a solution of amine hydrochoride (1 equiv) derived from (**34a** or **34b** or a mixture thereof and **36** using procedure A) DIPEA (3 equiv) and TBTU (1.1 equiv) in DMF (0.1 M) at room temperature. After stirring for 2 h the reaction mixture was partitioned between ethyl acetate and water. The organic layer was separated, washed with saturated aqueous sodium hydrogen carbonate solution and brine, and dried ($MgSO_4$), and the solvent was evaporated *in vacuo* to afford an oil, which was stored at +4 °C. The oil (1.0 equiv) was dissolved in dichloromethane (0.04 M) and the appropriate amine (2 equiv) added to the stirred solution. After 1–5 days the reaction mixture was diluted with dichloromethane, washed with water and saturated sodium hydrogen carbonate solution, and dried ($MgSO_4$), and the solvent was evaporated *in vacuo* to afford an oil which was purified by flash silica column chromatography to furnish the required products in 16–51% yield. In the preparation of compound **69**, *p*-(dimethylamino)benzyl dihydrochloride (1.2 equiv) and DIPEA (4.8 equiv) were added to the solution of **9**. In the preparation of compound **68**, tetrahydrofuran (THF) was used as the solvent, and concentrated ammonia solution (excess) was added to the solution of **9**.

[2*R*-[2α(*R),4β[2′*R***S**,4″*R**]]]-4-[[[2-Hydroxy-3-[4-[[(1,1-dimethylethyl)amino]carbonyl]-3-thiazolidinyl]propyl]-amino]carbonyl]-5,5-dimethyl-α-[(phenylacetyl)amino]-*N*-(phenylmethyl)-2-thiazolidineacetamide (52).** Penicillin G *N*-ethylpiperidine salt (456 mg, 1.12 mmol) was added to a mixture containing amine hydrochloride (derived from **36**) (304 mg, 1.02 mmol), DIPEA (0.54 mL, 3.06 mmol), and TBTU (356 mg, 1.12 mmol) in DMF (16 mL). The resulting solution was stirred at room temperature under nitrogen for 21 h and then diluted with ethyl acetate. The mixture was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and

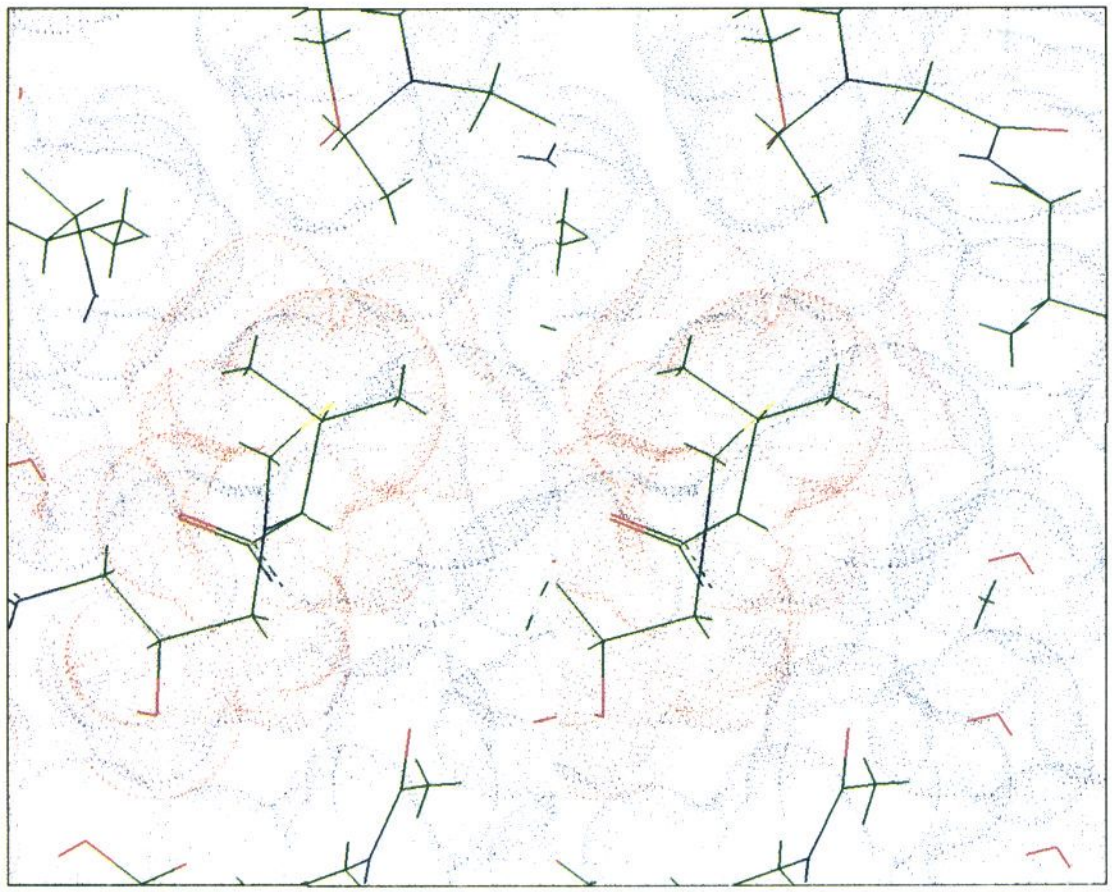


Figure 3. A stereorepresentation showing the S_1' pocket filled by the thiazolidine *gem*-dimethyl group.

Table 8

	1a	50	68
$t_{1/2}$ (h)	4	3	1.3, 1.2
clp (L/h)	14–24	27, 37	26, 23
Vdss (L)	20–80	70, 98	21, 19

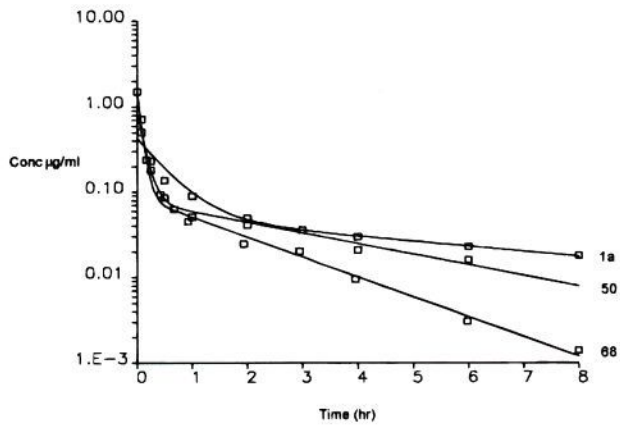


Figure 4. Plasma levels of unchanged drug **1a**, **50**, and **68** in dogs after an iv dose of 1 mg/kg.

evaporated *in vacuo* to dryness. The residual dark brown foam was purified by chromatography on silica gel. Elution with a gradient of ethyl acetate/methanol from 9:1 to 4:1 gave the crude intermediate amide as a brown foam: 244 mg (37%); ^{1}H NMR (DMSO-d_6) 1.27 (s, 9H), 1.42 (s, 3H), 1.61 (s, 3H), 2.25–2.42 (m, 2H), 2.8–3.1 (m, 4H), 3.5 (s, 1H), 3.55 and 3.56 (2s, 1H), 3.65 (m, 1H), 3.9 (m, 2H), 4.20 (m, 1H), 5.4–5.51 (m, 2H), 7.15–7.36 (m, 5H), 7.41 and 7.46 (2s 1H), 8.25 (m, 1H), 8.9 (m, 1H).

The above intermediate amide (206 mg) was dissolved in dichloromethane (10 mL), and benzylamine (46 mg, 0.43 mmol) was added. The mixture was stirred at room temperature for 5 days, diluted with ethyl acetate, and washed with dilute hydrochloric acid, saturated aqueous $NaHCO_3$, and brine. The organic extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo*, and the residual foam was purified by chromatography on silica gel. Elution with dichloromethane/methanol (9:1) gave a yellow foam which was purified further by chromatography on silica plates. Elution with the same solvents gave the title compound as a cream-colored foam (104 mg, 42%): $[\alpha]^{19}_D$ + 15.3° (*c* 0.50, DMSO); ^{1}H NMR (DMSO-d_6) δ 1.18 (s, 3H), 1.27 (s, 9H), 1.43 (s, 3H), 3.2–2.2 (complex multiplet, 2H), 3.7–3.2 (m, 8H), 3.90 (m, 3H), 4.05 (m, 1H), 4.30 (m, 2H), 4.45 (m, 1H), 4.70 (m, 1H), 5.10 (dd, J = 8.0, 4.0 Hz, 1H), 7.25 (m, 10H), 7.40 (d, J = 8.0 Hz, 1H), 7.50 (m, 1H), 8.32 (d, J = 7.0 Hz, 1H), 8.48 (m, 1H). Anal. ($C_{34}H_{48}N_6O_5S_2{\cdot}H_2O$) C, H, N.

***(R)*-1-[(*tert*-Butoxycarbonyl)amino]-2,3-dihydroxypropane (75b).** (*S*)-Glycidol **74b** (5.0 mL, 75.4 mmol) was dissolved in saturated ammoniacal 2-propanol (900 mL). The solution was allowed to stand at room temperature in a stoppered flask for 67 h and was then evaporated to dryness *in vacuo*. The residual oil was taken up in 1,4-dioxane (250 mL), aqueous sodium hydroxide (0.5 M, 250 mL) and di-*tert*-butyldicarbonate (18.5 g, 84.8 mmol) were added, and the mixture was stirred at room temperature for 18 h. The resulting suspension was concentrated *in vacuo* to *ca.* 200 mL, and the aqueous residue was saturated with sodium chloride, adjusted to pH 7 with 2 N HCl, and extracted with ethyl acetate (3 × 300 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to a viscous oil which was purified by chromatography on silica gel. Elution with ethyl acetate gave the title diol as a viscous colorless oil (7.68 g, 53%): $[\alpha]^{20}_D$ +13.6° (*c* 0.72, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H), 3.1 to 2.7 (complex m, 2H), 3.28 (t, J = 5.0 Hz, 2H), 3.43 (m, 1H), 4.48 (t, J = 5.0 Hz, 1H), 4.63 (d, J = 5.0 Hz, 1H), 6.61 (t, J = 6.0 Hz, 1H). Anal. ($C_8H_{17}NO_4{\cdot}0.2H_2O$) C, H, N.

(*R*)-1-[(*tert*-Butoxycarbonyl)amino]-2-hydroxy-3-[[(4-methylphenyl)sulfonyl]oxy]propane (76b). A solution containing the diol **75b** (4.49 g, 23.5 mmol) and tosyl chloride (5.01 g, 26.3 mmol) in pyridine (24 mL) was stirred at room temperature under N_2 for 3 h. The resulting mixture was diluted with ethyl acetate (600 mL) and washed with 2 N HCl (2 × 100 mL), water (100 mL), saturated aqueous sodium bicarbonate (2 × 100 mL), and brine (50 mL). The organic extract with dried ($MgSO_4$) and evaporated *in vacuo* to a viscous oil which was purified by chromatography on silica gel. Elution with a gradient of cyclohexane to cyclohexane/ethyl acetate (3:1) gave the title compound as a colorless oil (5.05 g, 62%): $[\alpha]^{21}_D$ +0.48° (*c* 1.25, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.43 (s, 3H), 3.7–3.0 (m, 3H), 4.1–3.8 (m, 3H), 4.95 (br, 1H). Anal. ($C_{15}H_{23}NO_6S \cdot 0.7H_2O$) C, H, N.

(*R*)-[[(*tert*-Butoxycarbonyl)amino]methyl]oxirane (77b). A solution containing the tosylate **76b** (2.98 g, 8.63 mmol) and sodium methoxide (0.98 g, 18.2 mmol) in methanol (60 mL) was stirred at room temperature under nitrogen for 1.5 h and was then evaporated to dryness *in vacuo*. The residual solid was taken up in ethyl acetate (500 mL) and the mixture washed with water (2 × 50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$) and evaporated *in vacuo* to a colorless oil which was purified by chromatography on silica gel. Elution with cyclohexane/ethyl acetate (3:1) gave the title compound as a colorless oil (955 mg, 63%): $[\alpha]^{21}_D$ +5.1° (*c* 1.4, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.60 (m, 1H), 2.78 (m, 1H), 3.10 (m, 1H), 3.24 (m, 1H), 3.55 (br d, J = 11.0 Hz, 1H), 4.77 (br s, 1H); HRMS *m/z* 174.112973 $(M + H)^+$ (calcd for $C_8H_{16}NO_3$, 174.113 019).

Molecular Modeling. The molecular models described were constructed by modification of the crystallographic structure of the penicillin dimer–HIV proteinase complex.[6] Construction of the models was achieved with the aid of INSIGHT *II*[22] modeling system, using where possible units from within the fragment library. Each modeled ligand was assumed to bind as half of a "dimer", *i.e*, with the thiazolidine ring system in the S_1 pocket and the phenylacetamido group in the S_2 pocket so as to maintain hydrogen bonding contacts. The resulting models were then minimized in the static enzyme structure (including crystallographic water molecules) using DISCOVER.

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References

(1) (a) Barre-Sinoussi, F.; Checmann, J. C.; Rey, F.; Nugeyre, M. T.; Chamoret, S.; Gruest, J.; Daugnet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for acquired Immune Deficiency Syndrome (AIDS). *Science* **1983**, *220*, 868–871. (b) Gallo, R. C.; Salahuddin, S. Z.; Popovic, M.; Shearer, G. M.; Kaplan, M.; Haynes, B. F.; Palker, T. J.; Redfield, R.; Oleske, J.; Safai, B.; White, G.; Foster, P.; Markham, P. D. Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for Aids. *Science* **1984**, *224*, 500–503.

(2) (a) Seelmeier, S.; Schmidt, H.; Turk, V.; von de Helm, K. Human Immunodeficiency Virus has an Aspartic-type Protease that can be Inhibited by Pepstatin A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6612–6616. (b) Mous, J.; Heimer, E. P.; Le Grice, S. F. J. Processing Protease and Reverse Transcriptase from Human Immunodeficiency Virus Type I polyprotein in *Escherichia coli*. *J. Virol.* **1988**, *62*, 1433–2314.

(3) Huff, J. R. HIV Protease: A Novel Chemotherapeutic Target for AIDS. *J. Med. Chem.* **1991**, *34*, 2305–2314.

(4) Norbeck, D.; Kempf, D.; Marsh, K.; Paul, D.; Knigge, M.; Vasavanonda, S.; Clement, J.; Kohlbrenner, W.; Sham, H.; Wideburg, N.; Plattner, J. Discovery of Potent, Orally Bioavailable Inhibitors of HIV Proteinase. VIII International Conference on AIDS, Amsterdam, Netherlands, 19–24 July, 1992.

(5) Humber, D. C.; Cammack, N.; Coates, J. A. V.; Cobley, K. N.; Orr, D. C.; Storer, R.; Weingarten, G. G.; Weir, M. P. Penicillin Derived C_2-Symmetric Dimers as Novel Inhibitors of HIV-1 Proteinase. *J. Med. Chem.* **1992**, *35*, 3080.

(6) Wonacott, A.; Cooke, R.; Hayes, F. R.; Hann, M. M.; Jhoti, H.; McMeekin, P.; Mistry, A.; Murray-Rust, P.; Singh, O. M. P.; Weir, M. P. A Series of Penicillin-Derived C_2-Symmetric Inhibitors of HIV-1 Proteinase: Structural and Modeling Studies. *J. Med. Chem.* **1993**, *36*, 3113.

(7) Holmes, D. S.; Clemens, I. R.; Cobley, K. N.; Humber, D. C.; Kitchin, J.; Orr, D. C.; Patel, B.; Paternoster, I. L.; Storer, R. Novel Dimeric Penicillin Derived Inhibitors of HIV-1 Proteinase: Interaction with the Catalytic Aspartates. *Bioorg. Med. Chem. Lett.* **1993**, *3* (4), 503–508.

(8) Holmes, D. S.; Bethell, R. C.; Cammack, N.; Clemens, I. R.; Kitchin, J.; McMeekin, P.; Mo. C. L.; Orr, D. C.; Patel, B.; Paternoster, I. L.; Storer, R. Synthesis and Structure Activity Relationships of a Series of Penicillin Based HIV Proteinase Inhibitors containing a Stereochemically Unique Peptide Isostere. *J. Med. Chem.* **1993**, *36*, 3129.

(9) (a) Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Kröhn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. Rational Design of Peptide-Based HIV Proteinase Inhibitors. *Science* **1990**, *248*, 358. (b) Martin, J. A. *Drugs Future* **1991**, *16* (3), 210.

(10) Perseo, G.; Piani, S.; De Castiglione, R. Preparation and analytical data of some unusual t-butyloxycarbonyl amino acids. *Int. J. Pept. Protein Res.* **1983**, *21*, 227.

(11) Freed, M. E.; Potoski, J. R. U.S. Pat. 1978, US 4089958.

(12) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. Synthesis of 9-fluorenylmethyloxycarbonyl protected N-Alkyl Amino Acids by Reduction of Oxazolidinones. *J. Org. Chem.* **1983**, *48*, 77.

(13) Edwards, M. L.; Snyder, R. D.; Stemerick, D. M. Synthesis and DNA-Binding Properties of Polyamine Analogues. *J. Med. Chem.* **1991**, *34*, 2414.

(14) Parkes, K. E. B.; Redshaw, S.; Thomas, G. J. Eur. Pat. A-0432694, 1991.

(15) Herak, J. J.; Kovocevic, M.; Gaspert, B. Preparation and Isomerisation of 3S-Benzyl-Penicilloamides. *Croat. Chem. Acta* **1978**, *51* (3), 265.

(16) Humber, D. C.; Bamford, M. J.; Bethell, R. C.; Cammack, N.; Cobley, K.; Evans, D. N.; Gray, N. M.; Orr, D. C.; Saunders, J.; Shenoy, B. E. V.; Storer, R.; Weingarten, G. G.; Wyatt, P. G. A Series of Penicillin Derived C_2-Symmetric Inhibitors of HIV-1 Proteinase: Synthesis, Mode of Interaction and Structure - Activity Relationships. *J. Med. Chem.* **1994**, *36*, 3120.

(17) Gigot, D.; Penninckx, M. New Compounds: Peptide Derivatives of the Antitumour Agent N-Phosphonoacetyl-L-aspartic acid. *J. Pharm. Sci.* **1984**, *73* (2), 275.

(18) (a) Rich, D. H.; Green, J.; Toth, M. V.; Marshall, G. R.; Kent, S. B. H. Hydroxyethyl amine Analogues of the p17/p24 Substrate Cleavage Site are Tight-Binding Inhibitors of HIV Protease. *J. Med. Chem.* **1990**, *33*, 1285–1288. (b) Swain, A. L.; Miller, M. M.; Green, J.; Rich, D. H.; Schneider, J.; Kent, S. B. H.; Wlodawer, A. X-Ray crystallographic structure of a complex between a synthetic protease of human immunodeficiency virus-1 and a substrate-based hydroxyethylamine inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8805. (c) Miller, M.; Swain, A. L.; Jaskolski, M.; Sathyanarayana, B. K.; Marshall, G. R.; Rich, D. H.; Kent, S. B. H.; Wlodawer, A. In *Retroviral Proteases: Control of Maturation and Morphogenesis*; Pearl, L., Ed.; MacMillan Press: New York, 1990; pp 93–106.

(19) Krohn, A.; Redshaw, S.; Ritchie, J. C.; Graves, B. J.; Hatada, M. H. Novel Binding Mode of Highly Potent HIV-Proteinase Inhibitors Incorporating the (R)-Hydroxyethylamine Isostere. *J. Med. Chem.* **1991**, *34*, 3340.

(20) Said, H. M.; Redha, R.; Nylander, W. Biotin Transport in the human intestine: site of maximum transport and effect of pH. *Gastroenterology* **1988**, *95* (5), 1312–7.

(21) (a) The assay procedure employed was essentially as described by Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; DeClercq, E. Rapid and Automated Tetrazolium-Based Colorimetric Assay for the Detection of Anti-HIV Compounds. *J. Virol. Methods* **1988**, *20*, 309–321. (b) Assays for inhibition of syncytium formation and expression of p24 core antigen were as previously detailed: Coates, J. A. V.; Inggall, J. J.; Pearson, B. A.; Penn, C. R.; Storer, R.; Williamson, C.; Cameron, J. M. Carbovir: The (−) Enantiomer is a Potent and Selective Antiviral Agent Against Human Immunodeficiency Virus In Vitro. *Antiviral Res.* **1991**, *15*, 161–168.

(22) Computational results obtained using software programs from Biosym Technologies of San Diego. Minimizations were done with the DISCOVER program, using the CVFF force field, and graphical displays were printed out from the Insight *II* molecular modeling system running on a silicon graphics 4D35TG.