JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 37, Number 21 October 14, 1994

Expedited Articles

L-735,524: The Design of a Potent and Orally Bioavailable HIV Protease Inhibitor

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Received June 16, 1994®

A series of HIV protease inhibitors possessing a hydroxylaminepentanamide transition state isostere have been developed. Incorporation of a basic amine into the backbone of the L-685,434 (**2**) series provided antiviral potency combined with a highly improved pharmacokinetic profile in animal models. Guided by molecular modeling and an X-ray crystal structure of the inhibited enzyme complex, we were able to design L-735,524. This compound is potent and competitively inhibits HIV-1 PR and HIV-2 PR with K_i values of 0.52 and 3.3 nM, respectively. It also stops the spread of the HIV-1_{IIIb}-infected MT4 lymphoid cells at concentrations of 25−50 nM. To date, numerous HIV-PR inhibitors have been reported, but few have been studied in humans because they lack acceptable oral bioavailability. L-735,524 is orally bioavailable in three animals models, using clinically acceptable formulations, and is currently in phase II human clinical trials.

Introduction

The alarming spread of human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS),[1] has initiated an urgent pursuit to comprehend and control this disease. The World Health Organization (WHO) now estimates that by the end of 1993 almost fourteen million adults and one million children were infected with the virus, and the pandemic continues unabated. Advances in molecular, viral, and cell biology have defined numerous targets for potential drug intervention. The virally encoded homodimeric aspartyl protease,[2] which is responsible for processing the *gag* and *gag/pol* gene products that allow for the organization of core structural proteins and release of viral enzymes, is one such target. Inhibition of this protease enzyme prevents the maturation and replication of the virus in cell culture.[3] Recently, we and others have described antiviral effects of protease inhibitors in human clinical trials.[4] These results confirm the importance of HIV protease (HIV-PR) inhibitors as another weapon in the arsenal needed to confront AIDS.

A number of reports have described very potent HIV-PR inhibitors based on the transition state isostere concept.[5] In general, these peptidomimetic compounds

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® Abstract published in *Advance ACS Abstracts*, September 15, 1994.

0022-2623/94/1837-3443$04.50/0

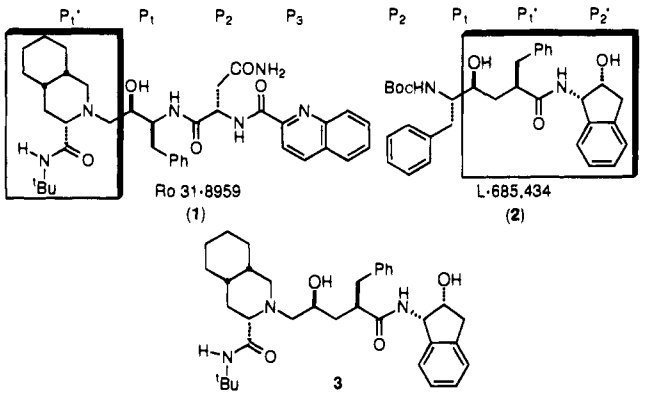


Figure 1. Design concept.

retain a substantial amount of peptide character, and as a result, they possess poor aqueous solubility and inadequate oral bioavailability in animal models. These difficulties have limited their usefulness as therapeutic agents.[6] To circumvent these difficulties, Lam and coworkers[7] have taken a different tack. Through a series of 3D chemical database searches in combination with molecular modeling, they have developed a series of nonpeptide cyclic ureas which possess reasonable pharmacokinetic properties. On the other hand, we have chosen to optimize a peptidomimetic lead structure by manipulating its physical properties. This has provided a novel series of potent HIV-PR inhibitors which maintain desirable pharmacokinetics. Herein we disclose the design concepts that generated this novel class of HIV-PR inhibitors, ultimately providing L-735,524, a potent and orally bioavailable protease inhibitor.[8,9]

Design Rationale. A series of hydroxyethylene dipeptide isostere inhibitors of HIV-PR, represented by L-685,434 (**2**) in Figure 1, have been previously described from these laboratories.[10] Although very potent, the optimized molecules of this series lacked aqueous solubility and an acceptable pharmacokinetic profile.[11] Researchers at Hoffmann-La Roche published a series of potent (hydroxyethyl)amine HIV-PR inhibitors, exemplified by Ro 31-8959 (1).[12] We hypothesized that incorporation of a basic amine into the backbone of the L-685,434 series might improve the bioavailability of this series of compounds. Therefore, replacement of the P_2/P_1 ligands, the *tert*-butyl carbamate and Phe moieties, of compound **2** with the P_2'/P_1' ligands, the decahydroisoquinoline *tert*-butylamide, of 1 would generate a novel class of *h*ydroxyl*a*mine*p*entan*a*mide (HAPA) isosteres. Incorporation of the decahydroisoquinoline *tert*-butylamide should provide two advantages. The amine would provide much needed aqueous solubility, and enclosure of the amine into a ring should limit the conformational freedom of this P_1/P_2 ligand, thereby decreasing the entropy change upon binding to HIV-PR. This unique class of transition state inhibitors is represented by compound **3**.

To support the concept that compound **3** could serve as a novel HIV-PR inhibitor, computer-assisted modeling studies were initiated. These results are shown in Figures 2 and 3. Figure 2 illustrates the X-ray crystal structure of the compound 1 (green)–HIV-1 protease complex[13] overlapped with the energy-minimized structure **2**[14] (magenta)–HIV-1 protease complex. Only one orientation of inhibitor binding is shown, and the HIV-1 protease dimer is deleted for clarity. As shown, both inhibitors fill the same hydrophobic binding pockets from S_2 through S_2' with compound 1 extending into the S_3 domain of the HIV-PR with the quinaldic amide group. The P_2 and P_1' carbonyl moieties in both inhibitors are in position to maintain a critical hydrogen bond to a water molecule which is found in most reported X-ray crystal structures of inhibitors bound to HIV-1 PR.[15] Replacement of the Boc and Phe ligands of compound **2** with the decahydroisoquinoline amide then generates our hybrid structure, **3**. Figure 3 illustrates an energy-minimized structure for compound **3**[14] (yellow) vs **2** (magenta). The P_1' and P_2' groups of each of these inhibitors almost superimpose. The P_1 (Phe) moiety and the P_2 (Boc) group of compound **2** occupy the same three dimensional space as the P_1 decahydroisoquinoline and P_2 *tert*-butylamide in **3**. The positions of the P_2 and P_1' carbonyl moieties of compound **3** maintain the proper alignment to hydrogen bond to water, which in turn hydrogen bonds to Ile_{50} and Ile_{250} on the flaps of the HIV-1 PR. With these promising molecular modeling results in mind, we turned our attention to the synthesis of compound **3**.

Chemistry

As illustrated in Scheme 1, commercially available (*S*)-(+)-dihydro-5-(hydroxymethyl)-2(3*H*)-furanone was converted into the *tert*-butyldimethylsilyl ether under standard conditions.[16] The protected lactone was deprotonated with LDA and alkylated with benzyl bromide to afford a 6:1 mixture of diastereomers that were separated via flash chromatography. The major diastereomer was then treated with HF, and resulting alcohol **4** was treated with methanesulfonyl chloride and triethylamine to provide the activated lactone. Displacement of the mesylate moiety with *tert*-butyl (3(*S*),-4a(*R*),8a(*S*))-decahydroisoquinoline-3-carboxamide in hot xylenes afforded lactone **5** in 35% yield. Hydrolysis of the lactone with LiOH was followed by treatment with excess TBSCl. Upon workup with water, the silyl ester hydrolyzed to provide protected carboxylic acid **6**. Standard coupling with EDC, HOBt, and 1(*S*)-amino-2(*R*)-hydroxyindan[10b] yielded the amide which was followed by silyl ether deprotection with tetrabutylammonium fluoride to provide target molecule **3**. This sequence provided the desired product in nine steps in 12% overall yield.

Besides the efficiency, another major advantage of this synthetic route was the flexibility to introduce various P_1, P_2, P_1', and P_2' ligands onto target molecules. For example, modification of the P_1 and P_2 ligands could be effected by substituting a variety of cyclic secondary amino *tert*-butyl carboxylic amides for the decahydroisoquinoline carboxamide. Similarly, P_1' ligands could be modified by replacement of electrophiles other than benzyl bromide, and the P_2' moieties could be varied by the coupling of amines other than 1(*S*)-amino-2(*R*)-hydroxyindan. Initially, L-proline, L-pipecoline, *cis*-4-(2-naphthyloxy)-L-proline, and N-4-protected L-piperazine *tert*-butylamides were explored as replacements for the decahydroisoquinoline *tert*-butylamide, as illustrated in Table 1. These targets were obtained following the procedure outlined in Scheme 1. However, because of the low yield in the mesylate displacement/coupling step, it was difficult to obtain gram quantities of these materials needed for biological testing. There-

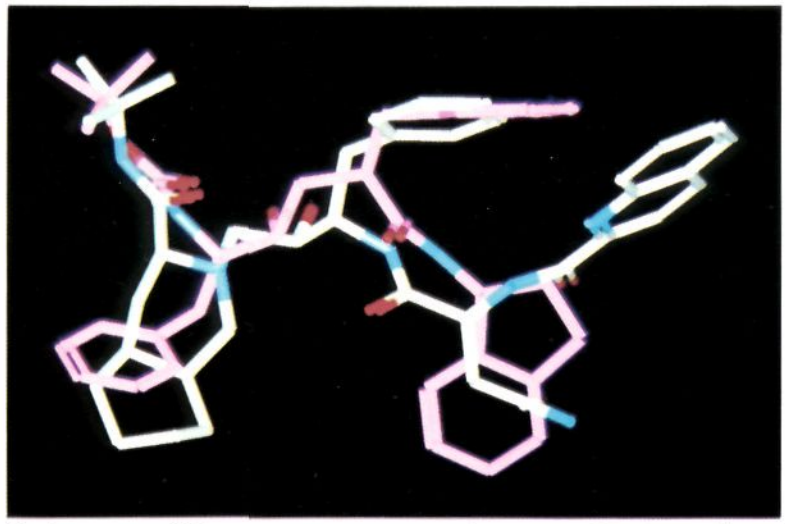


Figure 2. Energy-minimized structure of **2** (magenta) vs X-ray crystal structure of Ro 31-8959 (green) bound in the HIV-1 PR.

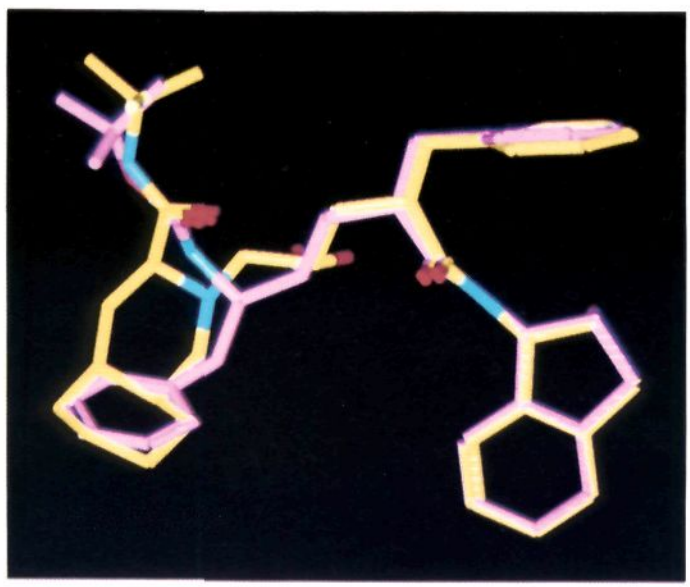
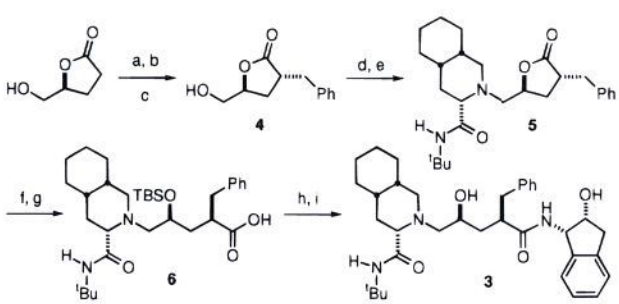


Figure 3. Superposition of the energy-minimized structure for **3** (yellow) vs the energy-minimized structure for **2** (magenta).

Scheme 1. Representative Synthesis of an HIV-PR Inhibitor[a]

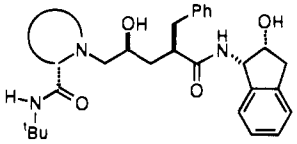


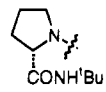
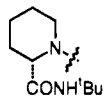
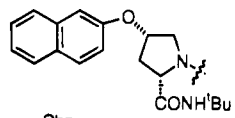
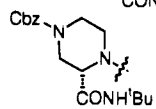
[a] Reagents and conditions: (a) *tert*-butyldimethylsilyl chloride, imidazole, DMF, rt; (b) *n*-BuLi, diisopropylamine, THF, lactone (−78 °C), benzyl bromide; (c) HF, CH_3CN, rt, 1 h; (d) MsCl, CH_2Cl_2, Et_3N (66% yield for four steps); (e) xylenes, K_2CO_3, decahydroisoquinoline *tert*-butylamide, 140 °C (35% yield); (f) 1 M LiOH, DME, rt, 3 h; (g) TBSCl, imidazole, DMF, rt; (h) EDC, HOBt, aminohydroxyindan, pH = 8−9, DMF (61% for three steps); (i) tetrabutylammonium fluoride, THF, rt, 20 h (85% yield).

fore, an optimized procedure was developed, as illustrated in Scheme 2 with the synthesis of L-735,524.

The known (*S*)-2-piperazinecarboxylic acid bis[(*S*)-(+)-camphorsulfonic acid] salt (**7**)[17] was converted into the differentially protected piperazine following the procedure of Bigge and Hays.[18] The resulting acid was then coupled with *tert*-butylamine following standard peptide coupling procedures. Hydrogenolysis then removed the N1 benzyloxycarbonyl protecting group to provide amine **8** in 78% yield for the three steps. At this point, all attempts made to optimize the mesylate displacement reaction by varying solvents, temperature, and bases were unsuccessful. However, modification of the leaving group provided a significant improvement in yield. Reaction of lactone **4** with triflic anhydride and 2,6-lutidine provided the very stable, crystalline lactone **9** in 96% yield. Displacement of triflate with amine **8** at room temperature in isopropyl alcohol with *N,N*-diisopropylethylamine now provided the desired coupled lactone in 83% yield. Hydrolysis, protection, amine coupling, and final deprotection then provided penultimate amine **10**. This was converted into L-735,524 through the reaction with 3-picolyl chloride in DMF with triethylamine. This sequence could provide the desired target molecules in 10 steps, from (*S*)-(+)-dihydro-5-(hydroxymethyl)-2(3*H*)-furanone, generally in 35% over-

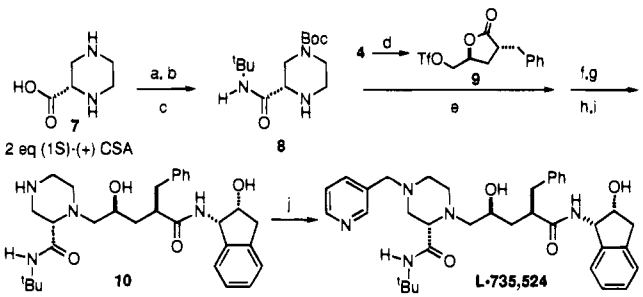
Table 1. Exploration of the S_1 and S_3 Domains



Compd	R	IC_{50} (nM)[a]	CIC_{95} (nM)[a]
3	DIQ[b]	7.8	400
11		347	1500
12		80	nd[c]
13		15	>400
14 L-732,747		0.35	100

[a] For each determination, $n = 1$. [b] DIQ—*N*-*tert*-butyl-(4a(*S*),8a(*S*))-decahydroisoquinoline-3(*S*)-carboxamide. [c] Not determined.

Scheme 2. Synthesis of L-735,524[a]



[a] Reagents and conditions: (a) BocON, H_2O, pH = 11.0, CbzCl, pH = 9.5 (96% yield); (b) EDC, HOBt, *tert*-butylamine, Et_3N, DMF (85% yield); (c) H_2, Pd/C 10%, MeOH (96% yield); (d) triflic anhydride, 2,6-lutidine, CH_2Cl_2 (96% yield); (e) isopropyl alcohol, diisopropylethylamine (83% yield); (f) 1 M LiOH, DME, rt, 3 h; (g) TBSCl, imidazole, DMF, rt (96% for two steps); (h) EDC, HOBt, hydroxyaminoindane, pH = 8–9, DMF; (i) 8 N HCl, isopropyl alcohol (78% yield); (j) 3-picolyl chloride hydrochloride, Et_3N, DMF (76% yield).

all yield. Most importantly, 10 g batches could be conveniently processed in this manner.

Results and Discussion

Despite the relatively high intrinsic potency of compound **3** (IC_{50} = 7.6 nM), the inhibition of the spread of viral infection in MT4 human T-lymphoid cells infected with the IIIb isolate was relatively weak (CIC_{95} = 400 nM). However, because **3** did possess a favorable pharmacokinetic profile,[22] when compared to L-685,434, other analogs were pursued. Initially, L-proline, *cis*-4-substituted L-proline, and L-pipecolinic *tert*-butylamides were explored as replacements for the decahydroisoquinoline *tert*-butylamine, as shown in Table 1. These N-terminal analogs provided no improvement in potency. Next, 2-*tert*-butylcarboxamide 4-substituted piperazines were examined. The piperazine analogs would provide two potential advantages over the decahydroisoquinoline *tert*-butylamide or the substituted L-proline *tert*-butylamides. First, the nitrogen in the 4 position of the piperazine ring could be easily functionalized. This would allow for the introduction and optimization of a P_3 ligand which could balance both the hydrophobic and hydrophilic requirements of our target molecules. Second, the additional amine in the piperazine ring should provide improved aqueous solubility which might improve oral bioavailability.

One of the first compounds prepared in the piperazine series (**14**) possessed a benzyloxycarbonyl moiety attached to the N4 position of the piperazine ring. This compound showed an improvement in both intrinsic potency and in the ability to inhibit viral spread in infected cells. To better understand the significant increase in potency of compound **14** (L-732,747), a cocrystallization with HIV-1 PR was undertaken (Figure 4). Consistent with the modeling observations, the ligands from P_2 to P_2' tightly bind into the S_2 to S_2' region of the HIV-1 PR. Also observed is the critical water molecule bridging the two carbonyl moieties of the P_2 and P_1' ligands. The most important observation was that the benzyloxycarbonyl moiety fills the lipophilic S_3 domain of HIV-1 PR. These interactions combined to generate the first subnanomolar compound in the HAPA isostere series.

Replacement of the benzyloxycarbonyl moiety with an acyl or sulfonyl moiety resulted in, for most examples, compounds with subnanomolar potency (Table 2). However, this did not always translate into increased potency in the cell-based assay, as exemplified by **17** and **18**. One critical factor in cell-based potency is the ability of the inhibitors to cross a cell membrane. In these examples, this ability to penetrate the cell membrane appears to have been severely restricted.

Concurrently with the above series, alkylated piperazine analogs were also pursued. Structure–activity data from this set of examples revealed that a variety of arylmethyl substitutions increased potency in the cell-based assay over the previously presented acylated or sulfonylated piperazines by 2–3-fold. The ability to modify the P_3 ligands without adversely affecting potency proved crucial in our search for an orally bioavailable inhibitor. Large and highly lipophilic P_3 ligands, i.e., phenylmethyl and 2-(benzyloxy)ethyl, were effective at increasing potency but also significantly decreased aqueous solubility. Smaller P_3 ligands improved solubility but lost potency (**10** and **21**). The 3-pyridylmethyl group provided both lipophilicity for binding to the protease and a weakly basic nitrogen which increased aqueous solubility. This combination of the 3-pyridylmethyl and the piperazine basic amines proved successful in improving oral bioavailability.

Pharmacokinetics. A representative set of compounds which possessed a range of aqueous solubilities and log *P* values was examined for oral bioavailability in dogs, and the results are shown in Table 3. Compound **14**, which was very insoluble at pH 7.4, showed no appreciable plasma levels when administered to dogs as a citric acid solution (10 mg/kg). The same result was obtained for sulfonamide **16**. Acylation or sulfonylation completely removes the basic character of the N4 nitrogen and also decreases the basicity of the N1 nitrogen of the piperazine ring. This lack of basicity translated into a lack of aqueous solubility which was found to be detrimental to bioavailability. Adequate

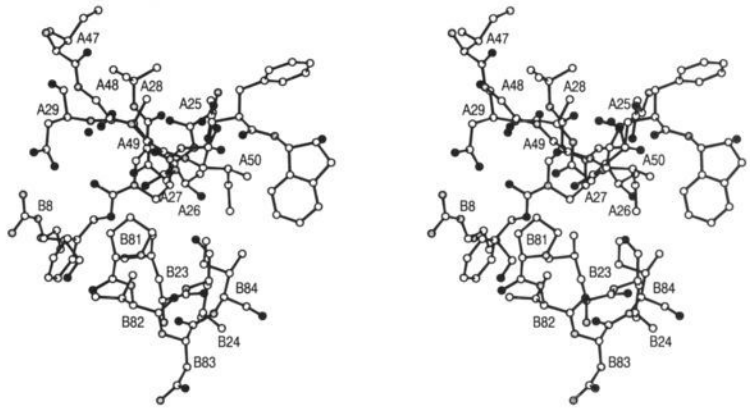
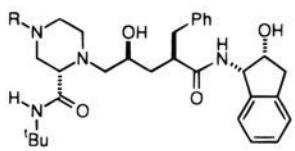


Figure 4. X-ray cocrystal structure of L-732,747 (**14**) and HIV-1 PR.

Table 2. Acylated, Sulfonylated, and Alkylated Piperazines



compd	R	IC_{50} (nM)[a]	CIC_{95} (nM)[a]
10	hydrogen	38	3000
14	benzyloxycarbonyl	0.36	100
15	coumarin-3-ylcarbonyl	0.13	50
16	8-quinolinylsulfonyl	0.013 ± 0.03 $n = 2$	12.5–50 $n = 2$
17	(2,5-dimethylthiazol-4-yl)sulfonyl	0.16	200
18	3-pyridylsulfonyl	0.30	200
19	2-(benzyloxy)ethyl	1.03	100
20	[(*tert*-butyloxy)-carboxyl]methyl	0.81	100
21	ethyl	6.1	>400
L-735,524	3-pyridylmethyl	0.56 ± 0.2 $n = 89$	25–100[b] $n = 59$
22	4-pyridylmethyl	0.39	50–100 $n = 2$
23	phenylmethyl	0.30 ± 0.05 $n = 2$	50 $n = 2$
24	(2,4-difluoro-3-phenyl)methyl	0.31 ± 0.05 $n = 2$	25–50 $n = 2$
25	(4-bromo-2-thio-pheneyl)methyl	0.06 ± 0.02 $n = 2$	12–25 $n = 2$
26	2-thiopheneylmethyl	0.54	25

[a] All entries are for $n = 1$ except where noted. [b] Some variability was observed in the antiviral potency for L-735,524 due to inherent difficulties in the cell-based assay. However, for $n = 59$, the average determination was 50.4 nM.

plasma levels were obtained with the slightly soluble (difluorophenyl)methyl **24**. Still further improvement occurred when the lipophilic aromatic moiety was replaced by a more soluble pyridine derivative. Of the variety of ligands explored, the most exciting results were obtained with L-735,524. The maximum plasma concentration levels achieved with an oral dose of 10 mg/kg in 0.05 M citric acid solution for dogs ($n = 4$) was 11.4 ± 2.3 μM. The C_{max} levels for rat ($n = 4$; 20 mg/kg) and monkey ($n = 4$; 10 mg/kg) after oral dosing as a citric acid solution was found to be 2.80 ± 1.05 and 0.71 ± 0.24 μM, respectively (not shown). The oral bioavailability for this compound in the three animal species was 70%, 22%, and 13%, respectively, when compared to iv studies. It should be noted that in all animal models examined the plasma concentrations after 6 h were twice the levels needed to completely stop viral growth in the cell-based assay. Solid dosage formulations with the crystalline free base, although more variable, gave comparable levels to the solution formulations. An improvement in formulation was found with the sulfate salt of L-735,524. A crystalline sulfate salt was prepared with both improved aqueous solubility (>450 mg/mL) and consistency of bioavailability in the solid dosage formulation studies.

Conclusion

In summary, a novel series of HIV-PR transition state isosteres have been developed. Starting from an initial peptide renin screening lead, Vacca[10a] and Lyle[10b] developed the highly potent and selective hydroxyethylene isostere L-685,434 (**2**). On the basis of this achievement, we were able to incorporate a basic amine into the backbone of this series to provide a novel HAPA isostere series of HIV-PR inhibitors. By modifying the physical properties of this series of inhibitors (i.e., solubility and lipophilicity) and concurrently maintaining potency, we were able to design L-735,524.

L-735,524 is potent and competitively inhibits HIV-1 PR and HIV-2 PR with K_i values of 0.52 and 3.3 nM, respectively. This compound effectively halts the spread of HIV-1_{IIIb}-infected MT4 lymphoid cells at concentrations of 50 nM. Also, this compound prevented the spread of viral infection in the genetically diverse SIV_{mac251}-infected cells at concentrations of less than 100 nM. L-735,524 is a selective inhibitor of HIV-1 PR, showing no inhibition against a variety of proteases including human renin, human cathepsin D, porcine pepsin, and bovine chymosin at concentrations exceeding 10 μM. No serious safety liability was observed in several animal experiments.

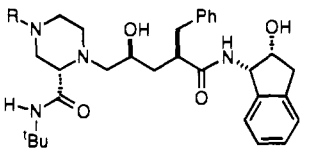
Initial safety, tolerability, and pharmacokinetic studies of L-735,524 free base and sulfate salt in 48 healthy HIV-1 seronegative and 24 HIV-1-infected human subjects have been undertaken.[4b] The multidose studies of 100 and 400 mg of sulfate salt q6h and of 200 mg free base q6h were well tolerated, with the sulfate salt formulation providing the most consistent pharmacokinetic profile. L-735,524 is presently undergoing extensive human clinical studies (phase II), and these results will be disclosed in due course.

Experimental Section

Biological Methods. Protease inhibition assays, acute infection assays, and animal pharmacokinetic studies have all been previously described.[8d]

Crystallography. Crystals of a complex between HIV-PR and L-732,747 (**14**) were grown as described previously.[11] Data

Table 3. Bioavailability in Dogs



compd	R	C_{max} (μM)[a]	sol, pH = 7.4 (5.2) (mg/mL)	log P ($\pm$0.05)
14	benzyloxycarbonyl	<0.10	<0.001	4.67
16	8-quinolinylsulfonyl	<0.10	<0.001	3.70
24	(2,4-difluoro-3-phenyl)methyl	0.73 $\pm$ 0.15	0.0012 (0.03)	3.69
L-735,524	3-pyridylmethyl	11.4 $\pm$ 2.3	0.07 (0.69)	2.92

[a] Each compound was delivered orally in 0.05 M citric acid. For all cases, $n = 2$ except for L-735,524 ($n = 4$). C_{max}, maximum plasma concentration.

were measured at room temperature from a crystal measuring 0.55 × 0.55 × 0.03 mm^3 on a Siemens multiwire area detector and processed with the XENGEN software.[19] The space group was found to be $P2_12_12$, with a = 58.23, b = 87.05, and c = 47.04 Å; 11 478 unique reflections were deduced from 28 575 observations, yielding a data set with an R_{merge} of 0.041 for data from infinity to 2.15 Å (84.3% complete for reflections with $I > \sigma(I)$). The structure was refined against the data from 20.0 to 2.15 Å, using the PROTIN/PROLSQ refinement programs,[20] beginning with the model for the isomorphous structure of an actylpepstatin–HIV-1 protease complex.[21] The electron density clearly indicated that the inhibitor binds in only one direction. The final model contains 1552 non-hydrogen atoms in the protein (mean B = 16.28), 48 non-hydrogen atoms in the inhibitor (mean B = 15.50), and 182 oxygen atoms in the solvent model (mean B = 16.26). The overall R value for the final model is 0.184 (0.195 in the outermost shell of 2.24–2.15 Å) for a model with deviations of 0.018, 0.038, 0.041, and 0.015 Å and 0.167 Å^3 from ideal bond distances, bond angles, 1–4 distances, planarity, and chirality, respectively. Coordinates for this structure will be deposited with the Protein Data Bank.

Chemical Methods. Reactions were carried out under an argon atmosphere, using solvents and reagents from commercial suppliers which were used as received. THF was distilled under nitrogen from sodium and benzophenone, and dichloromethane was distilled from calcium hydride. All reactions were monitored by thin-layer chromatography (TLC) using E. Merck silica gel 60 F_{254} plates (0.25 mm thickness). Flash column chromatography was performed with solvents indicated using E. Merck silica gel 60 (230–400 mesh). All melting points were obtained on a Thomas-Hoover apparatus and are uncorrected. Unless specified otherwise, IR and ^{1}H NMR spectra were obtained in $CHCl_3$ and $CDCl_3$ solutions, respectively. Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer, and optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Proton magnetic resonance spectra were obtained on either a Varian XL-300 (300 MHz) or a Varian VXR-400 (400 MHz) spectrometer using TMS as an internal standard. The abbreviations DME, DMF, THF, HOBt, EDC, LDA, TBSCl, BocON, CbzCl, and CSA refer to 1,2-dimethoxyethane, *N*,*N*-dimethylformamide, tetrahydrofuran, 1-hydroxybenzotriazole hydrate, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride, lithium diisopropylamine, [[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile, benzyl chloroformate, and camphorsulfonic acid, respectively.

Preparation of Dihydro-5(*S*)-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-3(2*H*)-furanone. To a solution of dihydro-5-(*S*)-(hydroxymethyl)-3(2*H*)-furanone (16.9 g, 145 mmol) in 160 mL of CH_2Cl_2 at 0 °C were added imidazole (29.77 g, 437 mmol) and TBSCl (32.9 g, 218 mmol). After 1 h, the reaction was warmed to room temperature and after an additional 3 h was quenched by the addition of 30 mL of methanol. The solvents were removed *in vacuo*, and the residue was redissolved in 1 L of EtOAc. Following the standard workup, the residue was purified by flash column chromatography (120 × 150 mm column; gradient elution Hex:EtOAc, 5:1–4:1) to afford 32.49 g (96% yield) of the product as a clear oil: TLC (EtOAc:Hex, 2:1) R_f = 0.62; $[\alpha]^{22}_D$ +13.1° (c = 0.047, $CHCl_3$); FTIR 1778, 1472, 1416, 1389 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.001 (s, 3H), 0.009 (s, 3H), 0.82 (s, 9H), 2.10–2.22 (m, 2H), 2.35–2.55 (m, 2H), 3.62 (dd, 1H, J = 3, 11.4 Hz), 3.80 (dd, 1H, J = 3.0, 11.4 Hz), 4.51–4.55 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.9, −5.8, 17.8, 23.1 (3C), 25.5, 28.2, 64.5, 79.7, 177.1; MS (FAB-POSI) m/z 321 (M + 1).

Preparation of Dihydro-5(*S*)-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-3(*R*)-(phenylmethyl)-3(2*H*)-furanone. A solution of LDA was generated by the addition 1.55 mL of *n*-BuLi (2.5 M in hexane) to 0.55 mL (3.9 mmol) of diisopropylamine in 10 mL of THF at −78 °C. After 30 min, a solution of dihydro-5-(*S*)-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-3(2*H*)-furanone (1.38 g, 3.89 mmol) in 5 mL of THF was added. After the solution was stirred for an additional 30 min, benzyl bromide (0.68 g, 3.9 mmol) was added and stirring was continued for 3 h, after which time the reaction was quenched with the addition of a 10% aqueous citric acid solution. Standard workup provided an oil which was purified by flash column chromatography (30 × 150 mm column; Hex:EtOAc, 4:1) to afford 1.07 g of a colorless oil (86% yield): TLC (EtOAc:Hex, 1:1) R_f = 0.64; $[\alpha]^{22}_D$ +7.5° (c = 0.020, $CHCl_3$); FTIR 3528, 3084, 3026, 1602, 1470, 1304 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.034 (s, 3H), 0.041 (s, 3H), 0.86 (s, 9H), 2.01–2.07 (m, 1H), 2.15–2.21 (m, 1H), 2.69–2.81 (m, 1H), 3.07–3.11 (m, 1H), 3.17–3.25 (m, 1H), 3.59 (dd, J = 2.7, 11.4 Hz, 1H), 3.82 (dd, J = 3.0, 11.2 Hz, 1H), 4.39–4.42 (m, 1H), 7.18–7.33 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −3.9, −3.1, 17.96, 25.58 (3C), 29.17, 36.67, 41.08, 64.82, 77.80, 126.37, 128.40 (2C), 128.60 (2C), 138.35, 178.65; HRMS (FAB-POSI; M + 1) calcd 321.1885, found 321.1875.

Preparation of Dihydro-5(*S*)-(hydroxymethyl)-3(*R*)-(phenylmethyl)-3(2*H*)-furanone (4). To 5.26 g of dihydro-5(*S*)-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-3(*R*)-(phenylmethyl)-3(2*H*)-furanone (16.43 mmol) in 40 mL of acetonitrile was added 1.34 mL of a 49% aqueous HF solution. After 18 h at room temperature, the reaction was concentrated to dryness and the residue subjected to the standard workup. Flash column chromatigraphy (50 × 150 mm column; gradient elution Hex:EtOAc, 4:1–3:1) afforded 3.30 g of the title compound as a white solid (97% yield): TLC (EtOAc:Hex, 1:1) R_f = 0.21; $[\alpha]^{22}_D$ −2.22° (c = 0.006, $CHCl_3$); FTIR 3604, 3018, 1604, 1454, 1350, 701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03–2.08 (m, 1H), 2.14–2.21 (m, 1H), 2.65 (br s, 1H), 2.79 (dd, J = 9.1, 13.7 Hz, 1H), 3.06–3.09 (m, 1H), 3.18 (dd, J = 4.4, 13.7 Hz, 1H), 3.55–3.60 (m, 1H), 3.79–3.82 (m, 1H), 4.38–4.41 (m, 1H), 7.18–7.32 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.72, 36.71, 41.23, 64.25, 78.69, 126.71, 128.62 (2C), 128.84 (2C), 138.06, 179.19; HRMS (FAB-POSI; M + 1) calcd 207.1021, found 207.1028.

Preparation of 4-(1,1-Dimethylethyl)-1-(phenylmethyl)-1,2(*S*),4-piperazine-2(*S*)-carboxylic Acid. To a solution of the bis[(+)-CSA] carboxylic acid salt **7** (11.47 g, 19.28 mmol) in dioxane:water (64 mL, 1:1) was added a 40% NaOH solution until pH 11. BosON (5.22 g, 21.21 mmol) was added in two portions maintaining the pH at 11. After 2.5 h, the pH was lowered to 9.5 with 10% HCl and CbzCl was added. Again, the pH was maintained at 9.5 with 40% NaOH. After 14 h, the mixture was washed with diethyl ether (3 × 100 mL) and the aqueous layer acidified to pH 3 with 6 N HCl, extracted

with EtOAc (2 × 100 mL), washed with brine, dried over $MgSO_4$, filtered, and concentrated to provide 6.75 g of the title compound (96% yield): TLC (EtOAc:Hex:AcOH, 69:30:1) R_f = 0.30; $[\alpha]^{22}_D$ −21.5° (c = 0.011, $CHCl_3$); FTIR 3660, 3015, 1694, 1497, 1257 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.75−3.40 (m, 3H), 2.80−4.15 (m, 2H), 4.45−4.82 (m, 2H), 5.18 (d, J = 8.4 Hz, 2H), 7.31−7.47 (m, 5H), 9.53 (br s, 1H); HRMS (FAB-POSI; M + 1) calcd 3651713, found 365.1727.

Preparation of *N*-*tert*-Butyl-4-[(1,1-dimethylethoxy)carbonyl]-1-[(phenylmethyl)carbonyl]piperazine-2(*S*)-carboxamide. To a solution of 4-(1,1-dimethylethyl)-1-(phenylmethyl)-1,2(*S*),4-piperazine-2(*S*)-carboxylic acid (14.0 g, 38.42 mmol) dissolved in 150 mL of DMF and cooled to 0 °C were added EDC (8.83 g, 46.10 mmol), HOBt (6.22 g, 46.10 mmol), *tert*-butylamine (4.84 mL, 46.10 mmol), and triethylamine (6.96 mL, 49.94 mmol). The reaction mixture was stirred for 18 h, and the reaction volume was concentrated by half. The mixture was diluted with 600 mL of EtOAc and after standard workup provided a solid. This solid was triturated with EtOAc:Hex (1:2) and filtered to provide 13.68 g of the product as a white solid (85% yield): mp 134−135 °C; TLC (EtOAc:Hex, 1:1) R_f= 0.38; $[\alpha]^{22}_D$ −19.47° (c = 0.010, $CHCl_3$); FTIR 3017, 2975, 1685, 1419, 1340, 1251 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (s, 9H), 1.46 (s, 9H), 2.95 (br s, 1H), 3.11−3.25 (m, 2H), 3.80−4.08 (m, 2H), 4.32−4.61 (m, 2H), 5.10−5.29 (m, 2H), 5.70 (br s, $^1/_2$H), 6.05 (br s, $^1/_2$H), 7.35 (s, 5H); HRMS (FAB-POSI; M + 1) calcd 420.2498, found 420.2491.

Preparation of *N*-*tert*-Butyl-4-[(1,1-dimethylethoxy)carbonyl]piperazine-2(*S*)-carboxamide (8). To a solution of *N*-*tert*-butyl-4-[(1,1-dimethylethoxy)carbonyl]-1-[(phenylmethyl)carbonyl]piperazine-2(*S*)-carboxamide (13.68 g, 32.61 mmol) in 250 mL of methanol was added 10% Pd/C (6.8 g). The vessel was charged with hydrogen, and after 3 h, the mixture was filtered through Celite and washed with methanol. The solvents were removed *in vacuo* to provide **8** as a foam. Purification by column chromatography (30 × 150 mm column; gradient elution MeOH:$CHCl_3$ saturated NH_3:CH_2Cl_2, 1:30:69, 2:30:68, 3:30:67) afforded 9.00 g (96% yield) of a white solid: mp 102−104 °C; TLC (MeOH:$CHCl_3$ saturated NH_3:CH_2Cl_2, 5:30:65) R_f = 0.48; $[\alpha]^{22}_D$ +14.1° (c = 0.020, $CHCl_3$); FTIR 3018, 2979, 1677, 1521, 1270, 1170 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.50 (s, 9H), 2.05 (br s, 1H), 2.70−3.02 (m, 4H), 3.21 (dd, J = 18, 7 Hz, 1H), 3.81 (br s, 1H), 4.10 (m, 1H), 6.65 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.93, 28.25, 28.33 (3C), 28.64 (3C), 44.11, 50.81, 58.85, 79.99, 154.63, 170.16. Anal. ($C_{14}H_{27}N_3O_3$) C, H, N.

Preparation of Dihydro-5(*S*)-[[[(trifluoromethyl)sulfonyl]oxy]methyl]-3(*R*)-(phenylmethyl)-3(2*H*)-furanone (9). To a solution of dihydro-5(*S*)-(hydroxymethyl)-3(*R*)-(phenylmethyl)-3(2*H*)-furanone (18.4 g, 89.2 mmol) in 350 mL of methylene chloride cooled to 0 °C was added 2,6-lutidine (13.51 mL, 115.98 mmol) followed by a dropwise addition of trifluoromethanesulfonic anhydride (16.51 mL, 98.1 mmol). After 1.5 h at 0 °C, the reaction mixture was poured into a mixture of 300 mL of ice/brine and stirred for 0.5 h. The aqueous layer was then extracted with methylene chloride (3 × 150 mL), and the standard workup gave a solid residue. Purification via flash column chromatography (120 × 150 mm column; gradient elution Hex:EtOAc, 4:1−3:1) afforded 29.0 g (96% yield) of **9** as a waxy solid: mp 53−54 °C; TLC (Hex:EtOAc, 2:1) R_f = 0.36; $[\alpha]^{22}_D$ −2.91° (c = 0.018, $CHCl_3$); FTIR 3026, 1604, 1454, 1365, 1246 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.15−2.22 (m, 2H), 2.85 (dd, J = 8.6, 13.8 Hz, 1H), 3.03−3.06 (m, 1H), 3.17 (dd, J = 4.7, 13.8 Hz, 1H), 4.43−4.52 (m, 2H), 4.60 (dd, J = 2.5, 10.7 Hz, 1H), 7.18−7.34 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.20, 36.40, 40.33, 73.93, 75.78, 116.84, 120.02, 128.82 (2C), 128.85 (2C), 137.24, 177.06; HRMS (FAB-POSI; M + 1) calcd 338.9919, found 339.0514.

Preparation of Dihydro-5(*S*)-[[4-[(1,1-dimethylethoxy)carbonyl]-2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]methyl]-3(*R*)-(phenylmethyl)-3(2*H*)-furanone. To a solution of **9** (22.40 g, 66.2 mmol) and **8** (18.0 g, 63.0 mmol) dissolved in 180 mL of isopropyl alcohol was added *N*,*N*-diisopropylethylamine (11.53 mL, 66.2 mmol). After 3.5 h, the solution was concentrated to a thick oil and triturated with EtOAc:Hex (1:2; 200 mL) providing a white solid which was filtered and discarded. The filtrate was concentrated, and the oil was purified by flash column chromatography (120 × 150 mm column; gradient elution EtOAc:Hex, 1:1, 2:1, 3:1 to all EtOAc) to afford 25.53 g (83% yield) of a white foam: mp 48−51 °C; TLC (EtOAc:Hex, 2:1) R_f = 0.31; $[\alpha]^{22}_D$ −4.2° (c = 0.030, $CHCl_3$); FTIR 3018, 2979, 1772, 1682, 1515, 1167 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (s, 9H), 1.45 (s, 9H), 1.92−1.99 (m, 1H), 2.04−2.32 (m, 1H), 2.32−2.38 (m, 1H), 2.49−2.58 (m, 1H), 2.61−2.67 (m, 1H), 2.78 (m, 1H), 2.80 (dd, J = 13.5, 8.9 Hz, 1H), 2.82−2.90 (m, 1H), 2.99−3.08 (m, 3H), 3.16 (dd, J = 13.6, 4.4 Hz, 1H), 3.79 (m, 1H), 3.92−3.96 (m, 1H), 4.38 (m, 1H), 6.31 (br s, 1H), 7.17−7.34 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.65 (3C), 28.93 (3C), 31.20, 36.76, 40.93, 45.87, 51.12, 51.28, 56.45, 60.35, 67.06, 77.48, 80.53, 127.29, 129.11 (2C), 128.19 (2C), 138.10, 154.65, 170.16, 178.34; HRMS (FAB-POSI; M + 1) calcd 474.2968, found 474.2956. Anal. ($C_{26}H_{39}N_3O_5$·0.2$CHCl_3$) C, H, N.

Preparation of 2(*R*)-(Phenylmethyl)-4(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5-[1-[4-[(1,1-dimethylethoxy)carbonyl]-2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]]pentanamide. To a solution of dihydro-5(*S*)-[[4-[(1,1-dimethylethoxy)carbonyl]-2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]methyl]-3(*R*)-(phenylmethyl)-3(2*H*)-furanone (25.50 g, 52.50 mmol) dissolved in 120 mL of DME cooled to 0 °C was added a solution of 60 mL of water and lithium hydroxide (1.51 g, 63.01 mmol). After 0.5 h, the reaction was quenched with the addition of 10% HCl until pH 6 and the solution was concentrated *in vacuo*. The residue was dissolved in 50 mL of water and extracted with EtOAc (4 × 75 mL), and the organic layers were washed with water (1 × 20 mL) and brine (1 × 20 mL). The aqueous was back-extracted with EtOAc (2 × 75 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated to provide a yellow solid. This crude product was dissolved in 100 mL of DME, and imidazole (17.87 g, 0.262 mol) was added. The mixture was cooled to 0 °C, and then *tert*-butyldimethylsilyl chloride (31.50 g, 0.21 mol) was added. After 20 h (0 °C to room temperature), the reaction was quenched with 10 mL of methanol and the solution concentrated to half the volume; 100 mL of pH 7 buffered water was added, and the aqueous layer was extracted with EtOAc (4 × 100 mL), followed by standard workup to afford a yellow foam (30.76 g, 96% yield). This material was used directly in the next step. An analytical sample could be obtained from trituration with EtOAc:Hex (1:1) to provide a white solid: mp 50−54 °C; TLC (EtOAc:Hex:AcOH, 66:33:1) R_f = 0.53; $[\alpha]^{22}_D$ −31.51° (c = 0.026, $CHCl_3$); FTIR 3016, 2957, 2931, 1677, 1256 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.102 (s, 3H), 0.098 (s, 3H), 0.70 (s, 9H), 1.18 (s, 9H), 1.30 (s, 11H), 2.00−2.10 (m, 2H), 2.52−3.12 (m, 7H), 3.59−3.98 (m, 4H), 7.03−7.11 (m, 6H), 9.55 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.14, −4.49, 17.47, 25.45 (3C), 25.53, 27.96 (3C), 28.11, 28.22 (3C), 31.30, 36.39, 36.70, 38.53, 42.60, 77.21, 80.21, 126.08, 126.16, 128.11 (2C), 128.65 (2C), 128.76, 138.37, 153.66, 162.83, 177.43; HRMS (FAB-POSI; M + 1) calcd 606.3875, found 606.3868.

Preparation of *N*-(2(*R*)-Hydroxy-1(*S*)-indanyl)-2(*R*)-(phenylmethyl)-4(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5-[1-[4-[(1,1-dimethylethoxy)carbonyl]-2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]]pentanamide. To a solution of acid (27.0 g, 44.6 mmol), above, dissolved in 180 mL of DMF were added EDC (8.98 g, 46.8 mmol), HOBt (6.32 g, 46.8 mmol), and 2(*R*)-hydroxy-1(*S*)-aminoindan (7.31 g, 49 mmol), and it was cooled to 0 °C. Triethylamine (6.52 mL, 46.8 mmol) was added and the reaction mixture stirred at 0 °C for 2 h and then at room temperature for 16 h. The reaction was quenched by diluting the mixture with 500 mL of EtOAc. Standard workup then afforded a foam. Purification by column chromatography (120 × 150 mm column; gradient elution EtOAc:Hex, 1:2, 1:1, 2:1) afforded 30.2 g (92% yield) of a white solid: TLC (EtOAc:Hex, 2:1) R_f = 0.45; $[\alpha]^{22}_D$ −10.89° (c = 0.022, $CHCl_3$); FTIR 3019, 2956, 2399, 1670, 1367, 1248 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 0.08 (s, 3H), 0.12 (s, 3H), 0.84 (s, 9H), 1.10 (s, 1H), 1.35 (s, 9H), 1.44 (s, 9H), 1.79 (br s, 1H), 2.09−2.17 (m, 1H), 2.29 (dd, J = 12.0, 4.5 Hz, 1H), 2.44−2.56 (m, 2H), 3.65 (dd, J = 9.6, 4.1 Hz, 1H), 2.78−3.07 (m, 8H), 3.84−3.96 (m, 2H), 4.06 (m, 1H), 4.23 (br s, 1H), 5.23 (m, 1H), 5.79 (br s, 1H), 6.51 (br s, 1H), 7.17−7.4 (m, 9H); ^{13}C NMR (100

MHz, $CDCl_3$) δ −4.40, −4.00, 13.94, 17.83, 22.46, 25.67 (3C), 28.22 (3C), 28.55 (3C), 31.39, 38.81, 39.20, 40.21, 45.78, 50.50, 50.78, 57.03, 62.46, 67.61, 72.82, 79.99, 124.18, 125.02, 126.42, 126.65, 127.92, 128.51 (2C), 128.76 (2C), 139.63, 139.91, 140.39, 154.11, 170.15, 174.67. Anal. ($C_{41}H_{64}N_4O_6Si$) C, H, N.

Preparation of *N*-(2(*R*)-Hydroxy-1(*S*)-indanyl)-2(*R*)-(phenylmethyl)-4(*S*)-hydroxy-5-[1-[2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]]pentanamide (10). To a solution of *N*-(2(*R*)-hydroxy-1(*S*)-indanyl)-2(*R*)-(phenylmethyl)-4(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5-[1-[4-[(1,1-dimethylethoxy)carbonyl]-2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]]pentanamide (30.20 g, 40.97 mmol) dissolved in 275 mL of isopropyl alcohol at 0 °C was added 8 N HCl (300 mL) over 1 h. The reaction mixture was stirred for an additional 6 h at room temperature and cooled to 0 °C. A 5 N NaOH solution was carefully added until pH 10, and the mixture was partitioned between 700 mL of EtOAc and 200 mL of water. The aqueous layer was then extracted with EtOAc (2 × 200 mL), and the combined organic layers were washed with water (1 × 50 mL) and brine (1 × 75 mL), dried over $MgSO_4$, and concentrated. The residue was purified via column chromatography (120 × 150 mm column; gradient elution CH_2Cl_2:$CHCl_3$ saturated NH_3:MeOH, slowly increasing methanol 2%, 3%, 4%, 5%, 6% to 10%). This provided 14.75 g (69% yield) of the product as a white foam: mp 68−77 °C; TLC (MeOH:$CHCl_3$ saturated NH_3:CH_2Cl_2, 10:30:60) R_f = 0.38; $[\alpha]^{22}_D$ + 15.2° (*c* = 0.010, $CHCl_3$); FTIR 3018, 2398, 1669, 1476, 1364, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 9H), 1.51 (m, 1H), 1.98 (m, 1H), 2.29−2.32 (m, 1H), 2.45 (d, *J* = 6.2 Hz, 2H), 1.60−2.50 (br s, 2H), 2.73−2.82 (m, 7H), 2.88−3.03 (m, 4H), 3.81−3.83 (m, 1H), 4.00−4.25 (br s, 1H), 4.23 (dd, *J* = 4.7, 4.0 Hz, 1H), 5.24 (dd, *J* = 8.2, 4.9 Hz, 1H), 6.52 (d, *J* = 8.3 Hz, 1H), 7.09−7.29 (m, 9H), 7.53 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 16.35, 28.87 (3C), 38.13, 39.19, 39.52, 45.34, 46.39, 47.95, 50.96, 57.43, 62.12, 64.75, 65.87, 72.93, 124.02, 125.07, 126.41, 126.72, 127.86, 128.42 (2C), 129.01 (2C), 139.86, 140.33, 140.55, 170.34, 175.02; HRMS (FAB-POSI; M + 1) calcd 523.3284, found 523.3277. Anal. ($C_{30}H_{42}N_4O_4$·0.35$CHCl_3$) C, H, N.

Preparation of *N*-(2(*R*)-Hydroxy-1(*S*)-indanyl)-2(*R*)-(phenylmethyl)-4(*S*)-hydroxy-5-[1-[4-(3-pyridylmethyl)-2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]]pentanamide (L-735,524). To a solution of *N*-(2(*R*)-hydroxy-1(*S*)-indanyl)-2(*R*)-(phenylmethyl)-4(*S*)-hydroxy-5-[1-[2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]]pentanamide (10.0 g, 19 mmol) and 3-picolyl chloride hydrochloride salt (3.45 g, 21 mmol) dissolved in 40 mL of DMF was added triethylamine (5.85 mL, 42 mmol). After 12 h, the reaction mixture was diluted with 400 mL of EtOAc and standard workup afforded a white foam. The residue was purified via column chromatography (80 × 150 mm column; gradient elution CH_2Cl_2:$CHCl_3$ saturated NH_3:MeOH, slowly increasing methanol 2%, 3%, 4%, 5% to 6%). Further recrystallization from EtOAc provided 8.85 g of white crystals (76% yield): mp 167.5−168 °C; TLC (MeOH:$CHCl_3$ saturated NH_3:CH_2Cl_2, 10:30:60) R_f = 0.19; $[\alpha]^{22}_D$ +24.1° (*c* = 0.0133, $CHCl_3$); FTIR 3019, 1664, 1513, 1366 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 9H), 1.53−1.58 (m, 2H), 1.94−1.99 (m, 1H), 2.34 (s, 2H), 2.37−2.48 (m, 1H), 2.51−3.13 (m, 11H), 3.48 (s, 2H), 3.80 (s, 1H), 3.95 (s, 1H), 4.26 (s, 1H), 5.26 (dd, *J* = 4.9, 8.4 Hz, 1H), 6.22 (d, *J* = 8.6 Hz, 1H), 7.09−7.31 (m, 10H), 7.58−7.61 (m, 1H), 7.67 (br s, 1H), 8.51−8.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.97 (3C), 38.05, 39.12, 39.58, 46.50, 47.82, 51.09, 52.63, 54.60, 57.42, 60.13, 61.39, 64.13, 65.76, 72.92, 123.36, 123.92, 125.10, 126.43, 126.70, 127.87, 128.44 (2C), 129.04 (2C), 132.40, 136.77, 139.91, 140.34, 140.44, 149.00, 150.43, 169.33, 174.94. Anal. ($C_{36}H_{47}N_5O_4$·0.65H_2O) C, H, N.

Preparation of *N*-(2(*R*)-Hydroxy-1(*S*)-indanyl)-2(*R*)-(phenylmethyl)-4(*S*)-hydroxy-5-[1-[4-(3-pyridylmethyl)-2(*S*)-(*N*-*tert*-butylcarbamoyl)piperazinyl]]pentanamide Sulfate Hydrate. To a solution of L-735,524 (1.77 g, 2.805 mmol) in 4 mL of ethanol (95%) was added concentrated H_2SO_4 (0.149 mL, 2.805 mmol), and the mixture was concentrated to a foam. The residue was redissolved in hot isopropyl alcohol, slowly cooled to room temperature, aged at 0 °C for 1 h, and filtered under a stream of argon. The white crystals were dried under high vacuum at 40 °C to afford a crystalline sulfate hydrate salt: mp 150−153 °C (softened at 135 °C); ^1H NMR (400 MHz, $CDCl_3$/CD_3OD, 9/1) δ 1.19 (d, $(CH_3)_2CHOH$, *J* = 6.05 Hz), 1.30 (s, 9H), 1.51−1.57 (m, 1H), 1.90 (t, *J* = 11.2 Hz, 1H), 2.77−3.17 (m, 15H), 3.27 (dd, *J* = 9.1, 9.5 Hz, 1H), 3.60 (d, *J* = 12.1 Hz, 1H), 3.83 (d, *J* = 13.9 Hz, 1H), 3.90 (d, *J* = 13.9 Hz, 1H), 3.97−4.04 (m, $(CH_3)_2CHOH$), 4.05−4.11 (m, 2H), 4.30−4.33 (m, 1H), 5.19 (d, *J* = 4.9 Hz, 1H), 7.12−7.32 (m, 9H), 7.49 (dd, *J* = 2.3, 5.3 Hz, 1H), 8.01 (d, *J* = 7.7 Hz, 1H), 8.55 (d, *J* = 4.4 Hz, 1H), 8.76 (s, 1H). Anal. ($C_{36}H_{47}N_5O_4$·1.0H_2SO_4·1.0H_2O·0.2C_3H_8O) C, H, N.

Acknowledgment. The authors would like to thank Process Chemistry (Rahway, NJ) for generous supplies of 2(*R*)-hydroxy-1(*S*)-aminoindan. Also, the authors gratefully acknowledge the contributions of Mr. John Moreau (elemental analyses), Mr. Art Coddington (mass spectrometry), Mr. Matthew Zrada (solubility and log *P* determinations), and Ms. Jean Kaysen (manuscript preparation).

Supplementary Material Available: Complete experimental description for Scheme 1 and analytical data for compounds **11**−**25** (8 pages). Ordering information is given on any current masthead page.

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