Viracept (Nelfinavir Mesylate, AG1343): A Potent, Orally Bioavailable Inhibitor of HIV-1 Protease

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Using a combination of iterative structure-based design and an analysis of oral pharmacokinetics and antiviral activity, AG1343 (Viracept, nelfinavir mesylate), a nonpeptidic inhibitor of HIV-1 protease, was identified. AG1343 is a potent enzyme inhibitor (K_i = 2 nM) and antiviral agent (HIV-1 ED_{50} = 14 nM). An X-ray cocrystal structure of the enzyme−AG1343 complex reveals how the novel thiophenyl ether and phenol−amide substituents of the inhibitor interact with the S1 and S2 subsites of HIV-1 protease, respectively. In vivo studies indicate that AG1343 is well absorbed orally in a variety of species and possesses favorable pharmacokinetic properties in humans. AG1343 (Viracept) has recently been approved for marketing for the treatment of AIDS.

Introduction

The human immunodeficiency virus protease (HIVPr) has emerged as a promising therapeutic target for the treatment of AIDS due to the essential role this enzyme plays in virus maturation. Inhibitors of HIVPr are presently being used in therapy for the treatment of AIDS.[1] The best of these inhibitors combine potent antiviral activity with good pharmacokinetics.[2] Inhibitor development has been facilitated by the use of HIVPr−ligand cocrystal structure information.[2−4] We have previously described the application of protein crystallography to the design and optimization of novel nonpeptidic HIVPr inhibitors.[4] In the course of this structure-based work, inhibitor **1** was discovered (Figure 1). Compound **1**, containing a novel 2-methyl-3-hydroxybenzamide group, was found to be a tight binding inhibitor of HIVPr (Figure 1).[4] While compound **1** was considered interesting by virtue of its small nonpeptide P2 group, it was not viewed as a viable drug candidate due to its suboptimal antiviral activity (ED_{50} = 970 nM) and poor aqueous solubility. However, its relatively low molecular weight (556 Da) and nonpeptidic structure prompted further structure−activity studies in this series. Using the crystal structure of compound **1** in complex with HIVPr and other structural information as a guide, we identified a compound with potent in vitro antiviral activity and superior pharmacokinetics in a variety of species.[4,5] We report herein the identification of **11a** (Viracept, nelfinavir mesylate, AG1343), an orally bioavailable inhibitor of HIVPr which is now being marketed for the treatment of AIDS.[6]

Chemistry

The syntheses of compounds **11a**−**c** are summarized in Scheme 1. The unnatural amino acids **2a** and **2b** were prepared from *N*-Cbz-L-serine by formation of the corresponding β-lactone followed by in situ opening with the desired sodium aryl thiolate as previously described.[4c,7] Protected amino acids **2a**−**c** were converted to the chloromethyl ketones **4a**−**c** by treatment of the respective diazoketone intermediates with hydrochloric acid. Sodium borohydride reduction of the ketone yielded the corresponding chloromethyl alcohols as a mixture of diastereomers. The desired products were obtained as the more polar isomers by radial chromatography. Base-induced cyclization of the intermediate chloro alcohols produced the corresponding amino epoxides **6a**−**c**. Terminal opening of the epoxides with the known secondary amine **7**[8] afforded the *N*-Cbz-protected amino alcohols. Treatment with HBr−acetic acid liberated the primary amines **9a**−**c** in high yield. 2-Methyl-3-hydroxybenzoic acid was obtained by treatment of the diazonium salt of 2-methyl-3-aminobenzoic acid with aqueous sulfuric acid. Carbodiimide-mediated coupling of amines **9a**−**c** with the benzoic acid followed by mesylate salt formation yielded inhibitors **11a**−**c**.

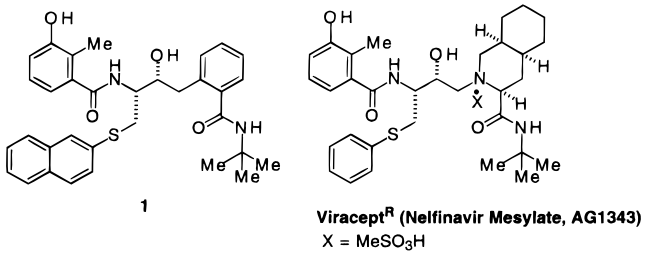

Figure 1. Structures of the lead HIV-1 protease inhibitor **1** and the optimized drug Viracept (nelfinavir mesylate, AG1343).

Discussion

Initially, we focused on enhancing the aqueous solubility and antiviral activity of **1**. Superposition of the enzyme bound conformations of **1** and Ro31-8959,[2a] derived from their respective cocrystal structures, suggested that the known tertiary amine-containing dipeptide isostere **9c**[8] could be combined with 2-methyl-3-hydroxybenzoic acid to afford **11c**. This resulted in a slightly weaker enzyme inhibitor but markedly improved antiviral agent when compared to compound **1**

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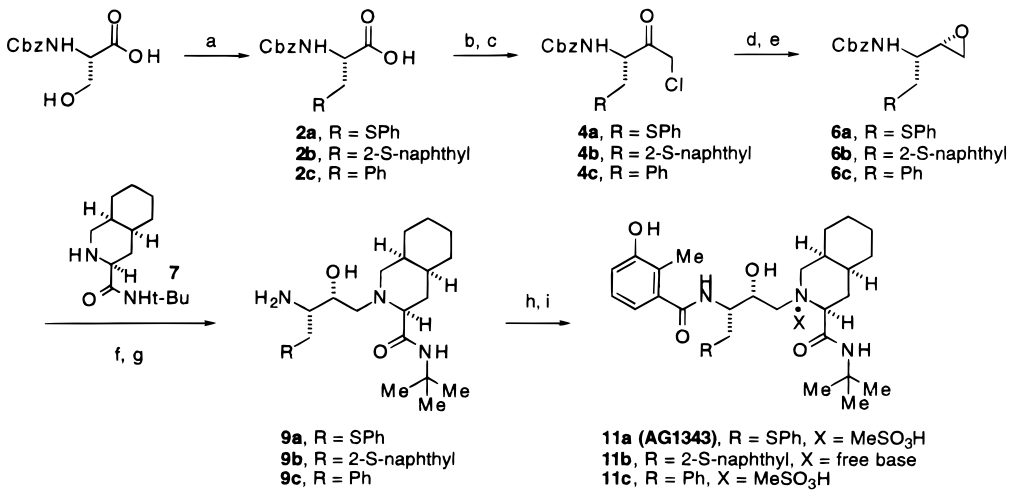
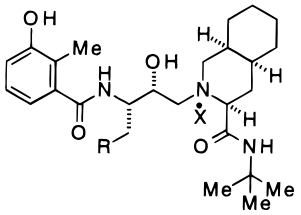
Scheme 1[a]



[a] Reaction conditions: (a) PPh_3, DMAD, THF; Na-aryl thiolate, THF; (b) *i*-BuOCOCl, Et_3N, THF; CH_2N_2; (c) HCl, Et_2O; (d) $NaBH_4$, THF/H_2O; (e) KOH, EtOH; (f) **7**, EtOH (80 °C); (g) HBr, AcOH; (h) 3-hydroxy-2-methylbenzoic acid, DCC, HOBt, DMF; (i) $MeSO_3H$, CH_2Cl_2 (step i omitted for **11b**).

Table 1. Representative Biological Data for HIV-1 Protease Inhibitors



entry	R	X	K_i (nM)[a]	ED_{50} (nM)[a,b]
1			3	970
11a (AG1343)	SPh	$MeSO_3H$	2.0	14
11b	2-*S*-naphthyl	free base	98	100
11c	Ph	$MeSO_3H$	21	33

[a] Reference 9. [b] Concentration required to inhibit 50% HIV-induced cell death.

(Table 1).[9] Additional optimization work focused on the P_1 phenylalanine portion of **11c**. We had previously demonstrated that *S*-aryl substituents effectively span from the S1 to S3 subsites of HIV-1 protease, providing compounds with substantially improved enzyme inhibitory activity relative to their phenylalanine counterparts.[4c] As illustrated in Table 1, the *S*-phenyl analog **11a** (Nelfinavir) is a potent inhibitor of HIV-1 protease (K_i = 2.0 nM), which binds with approximately 10-fold better affinity than phenyl analog **11c**, whereas the corresponding 2-*S*-naphthyl derivative **11b** is a weaker enzyme inhibitor. In an effort to understand this unanticipated trend in binding affinities, the cocrystal structure of nelfinavir complexed with HIV-1 protease was studied.[10] As predicted from the structure of **1**, nelfinavir binds to the enzyme in an extended conformation (Figure 2).[4c] The *tert*-butylcarboxamide moiety occupies the S2′ subsite of HIV-1 protease, the lipophilic dodecahydroisoquinoline ring system fits into the hydrophobic S1′ pocket, and the central hydroxyl group binds to the catalytic aspartates of the enzyme. The *S*-phenyl group resides in the S1 site and partially extends into the S3 region. The 2-methyl-3-hydroxybenzamide portion of the inhibitor occupies the S2 pocket, with the *o*-methyl substituent making hydrophobic interactions with Val-32 and Ile-84, and the *m*-phenol group hydrogen bonding to the Asp-30 carboxylate. A tightly bound water molecule serves to relay hydrogen bonds from the two amide carbonyls of the inhibitor into the flap region of the enzyme in a manner analogous to that observed in other HIV-1 protease−inhibitor complexes.[2,3] The RMS deviation between the backbone atoms of nelfinavir and Ro31-8959 was 0.49 Å, confirming that they adopt similar binding modes. From the cocrystal structure it is not clear why the *S*-phenyl substituent of nelfinavir is favored 50-fold over the *S*-naphthyl in **11b**, although it is apparent that the second ring found in the *S*-naphthyl analog makes at best one favorable interaction with Val-182, the remainder of the S3 pocket being relatively hydrophilic in nature.

Compounds **11a** and **11c** were potent antiviral agents in cell culture (Table 1), with ED_{50} values of 14 and 33 nM, respectively, against the HIV strain IIIB in CEM cells, and exhibited minimal cellular toxicity (TD_{50}s >5000 nM).[9] Although both compounds showed encouraging oral bioavailability results in a preliminary animal screen, nelfinavir was selected for more extensive antiviral and pharmacokinetic studies due to its superior antiviral activity. Additional antiviral testing demonstrated that nelfinavir was effective against the replication of several laboratory and clinical HIV-1 and HIV-2 isolates with ED_{50} values ranging from 9 to 60 nM.[11]

A summary the pharmacokinetics of nelfinavir in different species is provided in Table 2.[12] Initial investigation of the compound in fed rats indicated an oral bioavailability of 43%. In contrast, the oral bioavailability was significantly reduced in fasted rats to 29%.[13] Therefore, all subsequent in vivo pharmacokinetic studies were conducted in fed animals. As shown in Table 2, nelfinavir demonstrated significant oral bioavailability across a range of species including dogs (47%), marmosets (17%), and cynomolgus monkeys (26%). In addition, a single oral dose of nelfinavir exhibited plasma levels exceeding the in vitro antiviral ED_{95} (58 nM, 40 ng/mL) for more than 6 h in three of the four species (Figure 3). The observed long plasma half-life after oral dosing is likely due to a combination of slow dissolution and absorption. The preclinical results of nelfinavir demonstrated potential antiviral and/or pharmacokinetic advantages when compared to most known

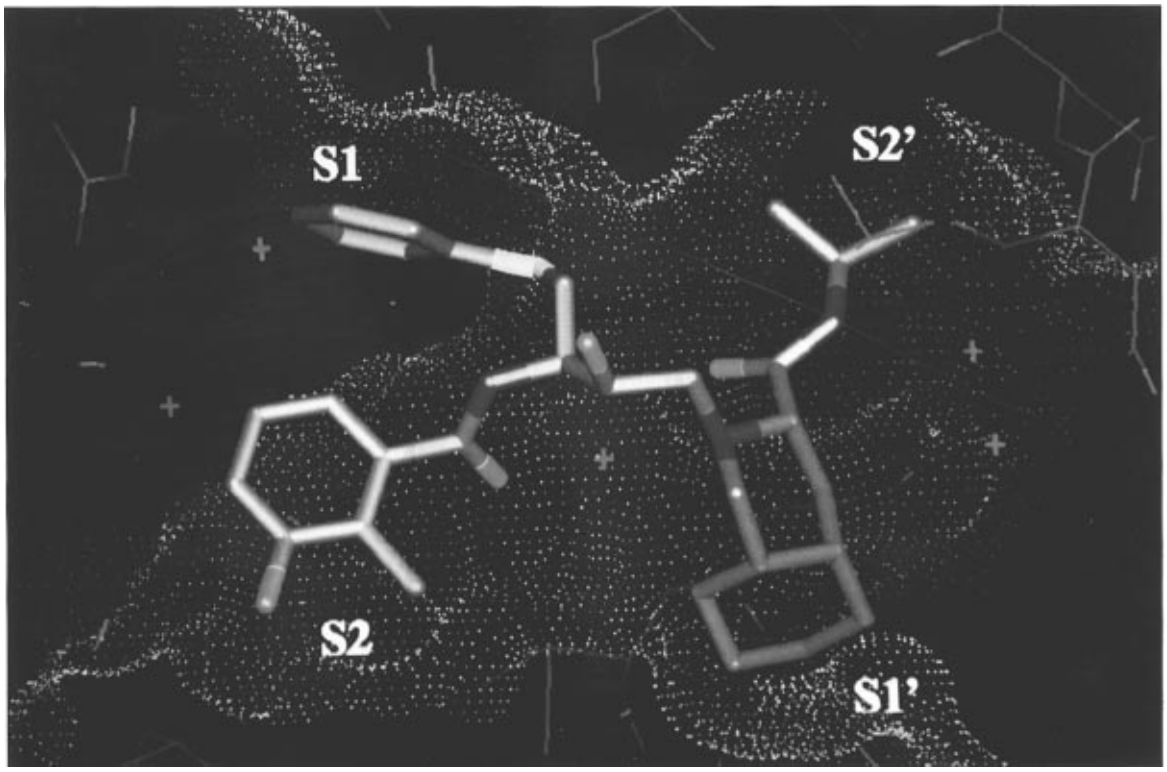


Figure 2. View of AG1343 in complex with the HIV-1 protease. Atom colors are green (carbon) red (oxygen), blue (nitrogen), and yellow (sulfur). A solvent accessible surface for the protein active site is shown as white dots. Water molecules are represented as red crosses.

Table 2. Mean[a] Pharmacokinetic Parameters for AG1343 in Different Species

species	dose[b] (mg/kg)	route	C_{max} (μg/mL)	T_{max} (h)	$T_{1/2-el}$ (h)	$AUC_{0-\infty}$ (μg h/mL)	F (%)
rat	25	iv	12.72 ± 3.95	0.06 ± 0.01	1.26 ± 0.09	7.01 ± 0.89	
rat	50	oral	1.34 ± 0.63	2.82 ± 2.79	*d*	6.09 ± 2.62[e]	43
rat	50[c]	oral	1.47 ± 0.43	1.39 ± 1.42	1.07 ± 0.18	4.04 ± 2.38	29
dog	15	iv	8.79, 11.92	0.08, 0.08	0.92, 1.18	8.78, 11.23	
dog	30	oral	1.18, 1.22	2.00, 3.00	1.94, 7.72	6.19, 13.28	35, 59
monkey	12.5	iv	7.07, 7.99	0.08, 0.08	1.38, 1.47	7.45, 10.35	
monkey	25	oral	0.26, 1.59	2.00, 3.00	1.58, 3.00	1.84, 6.29	9, 42
marmoset	12.5	iv	3.45, 5.67	0.08, 0.08	0.88, 1.23	6.02, 6.63	
marmoset	25	oral	0.38, 0.84	0.50, 1.00	*d*, 2.04	1.04,[e] 2.89	10, 24

[a] Values shown are mean ± SD of data from three rats and data from two animals each for dogs, monkeys, and marmosets. [b] Delivered in 5% dextrose to all animals except marmosets where propylene glycol:water (50:50) was used. [c] Animals were fasted overnight prior to AG1343 administration. [d] Elimination rate constant could not be determined due to prolonged absorption. [e] AUC_{0-t} value presented.

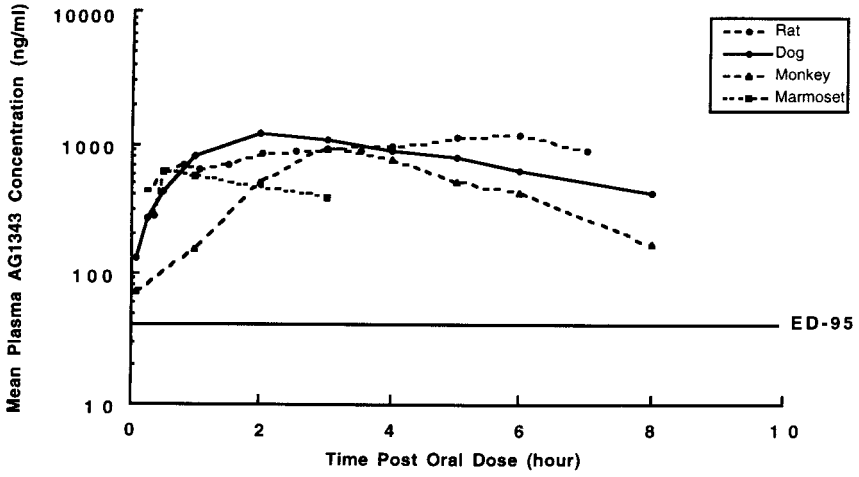


Figure 3. Plasma concentrations of AG1343 (ng/mL) plotted versus time (hours) for four animal species.

HIV protease inhibitors and thus warranted additional development of the compound for testing in humans.

Initially, the safety and pharmacokinetics of orally administered nelfinavir were evaluated in a phase I clinical study.[14] Single doses of 100−800 mg and multiple doses of 800−900 mg per day were well tolerated. In addition, peak plasma levels up to 100 times the antiviral ED_{95} and trough levels up to 10 times the ED_{95} were achieved in these studies. Additional clinical studies in HIV-infected individuals have

Table 3. Mean[a] Pharmacokinetic Parameters of AG1343 When Administered Orally with Food to Healthy Human Volunteers

dose (mg)	C_{max} (ng/mL)	T_{max}[b] (h)	$T_{1/2-el}$ (h)	$AUC_{0-\infty}$ (ng h/mL)
100	313 ± 91	3.00	1.72 ± 0.35	1250 ± 230
200	439 ± 124	3.00	2.21 ± 0.75	1828 ± 372
400	1577 ± 965	4.00	2.28 ± 0.36	7555 ± 4175
800	3163 ± 584	3.50	3.37 ± 1.23	22208 ± 6306

[a] Values shown are mean ± SD of data from four subjects. [b] Median values for T_{max} are presented.

demonstrated that nelfinavir is well absorbed in humans particularly when administered with food and possesses minimal and easily managed side effects (Table 3).

In conclusion, we have identified nelfinavir (AG1343), a potent, nonpeptidic inhibitor of the HIV-1 protease with a desirable combination of potent in vitro antiviral activity and excellent pharmacokinetics in a variety of species. AG1343 (Viracept) has recently been approved for marketing for the treatment of AIDS, providing a useful drug in the fight against this deadly disease.

Experimental Section

3-Hydroxy-2-methylbenzoic Acid. To a cooled (−10 °C) solution of 45 g (0.30 mol) of 3-amino-2-methylbenzoic acid and 106 g (58 mL, 1.08 mol) of concentrated sulfuric acid in 400 mL of water was added 22.6 g (0.33 mol) of sodium nitrite in small portions so that the reaction temperatures did not exceed 7 °C. The resulting blood red solution was stirred 30 min at −10 °C, poured into a solution of 1.2 L water and 240 mL concentrated sulfuric acid, and then slowly heated to 80 °C over 1 h (heavy gas evolution occurred between the temperatures of 40−60 °C). After gas evolution had stopped, the reaction mixture was cooled to room temperature and extracted five times with 600 mL of ethyl acetate. The combined organic phases were extracted four times with 500 mL of saturated sodium carbonate, and then the combined aqueous phases were made acidic (pH 2) by addition of concentrated hydrochloric acid. The resulting solution was extracted four times with 500 mL of ethyl acetate, and the combined organic phases were extracted once with brine and dried over sodium sulfate. Two recrystallizations from ethyl acetate/chloroform gave 23.2 g of the product as a light orange powder: yield 52%; ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H), 3.28−3.35 (br s, 1H), 6.92 (d, 1H, J = 8.0 Hz), 7.02 (t, 1H, J = 7.9 Hz), 7.14 (d, 1H, J = 7.9 Hz), 9.45−9.63 (br s, 1H); IR ($CHCl_3$) 3600−2100 (br), 3602, 2983, 1696, 1588, 1462, 1406, 1338, 1279, 1174, 1154, 1075, 1038, 920, 892, 854, 816 cm^{-1}; MS (FD) *m/e* 152 (M^+, 100). Anal. ($C_8H_8O_3$) C, H.

(*R*)-*N*-[(Phenylmethoxy)carbonyl]-3-(phenylthio)alanine (2a). To a chilled (−55 °C) solution of triphenylphosphine (110 g, 0.418 mol) in CH_2Cl_2 was added dimethyl azodicarboxylate (61.1 g, 0.418 mol) in 100 mL of CH_2Cl_2 dropwise by addition funnel over 15 min. After 30 min, *N*-Cbz-L-serine (100 g, 0.418 mol) was introduced dropwise as a solution in 100 mL of THF over 20 min. The reaction mixture was slowly warmed to room temperature and stirred overnight. In a separate reaction flask, thiophenol (45.8 g, 0.417 mol) in THF (800 mL) under nitrogen atmosphere was treated at room temperature with 60% NaH (16.7 g of NaH, 0.417 mol). After 15 min, the *N*-[(benzyloxy)carbonyl]serine β-lactone solution was added slowly by cannula over 20 min. The resulting mixture was stirred for 2.5 h and then concentrated in vacuo to a semisolid. This material was dissolved in water and extracted twice with CH_2Cl_2. Ethyl acetate was added to the aqueous layer, followed by sufficient 1 M $NaHSO_4$ to adjust to pH 2.5. After separation, the aqueous phase was extracted once more with ethyl acetate, and the combined ethyl acetate layers were in turn washed with brine and dried over sodium sulfate. Upon concentration, a thick solid was obtained which crystallized on standing (54.1 g, 39%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55−7.18 (m, 10H), 5.55 (d, J = 7 Hz, 1H), 5.08 (s, 2H), 4.73−4.60 (m, 1H), 3.55−3.30 (m, 2H); IR (KBr) 3304, 3035, 1687, 1532, 736 cm^{-1}; MS (FD) *m/e* 332, 288, 271, 181. Anal. ($C_{17}H_{17}NO_4S$) C, H, N.

(*R*)-[3-Diazo-2-oxo-1-[(phenylthio)methyl]propyl]carbamic Acid Phenylmethyl Ester (3a). Compound **2a** (12.1 g, 0.037 mol) dissolved in EtOAc (200 mL) under nitrogen was cooled by a dry ice/CCl_4 bath as triethylamine (5.09 mL, 0.037 mol) was added followed by slow addition of isobutyl chloroformate (7.13 mL, 0.055 mol). By long-stem funnel an ether solution of diazomethane (0.146 mol) was added in one portion. The diazomethane solution was prepared as follows: In a 500 mL Erlenmeyer flask free of flaws behind a blast shield was placed diethyl ether (100 mL) and 5 N NaOH (150 mL). The mixture was cooled by an ice bath and stirred as 1-methyl-3-nitro-1-nitrosoguanidine (21.0 g, 0.146 mol) was carefully added. After evolution of gas had ceased, the ether was decanted and dried over KOH. Filtration of the ether solution of diazomethane into the reaction flask by long-stem funnel was made in one portion. The reaction was stirred for 30 min before warming to room temperature for 40 min. The solution was swept with nitrogen and evaporated to dryness. The residue was taken up in EtOAc, washed twice with water, twice with aqueous $NaHCO_3$, and once with brine, and dried over Na_2SO_4. A yellow oil was isolated by flash chromatography (gradient 0−5% EtOAc in CH_2Cl_2) in 73% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−7.19 (m, 10H), 5.62 (d, J = 7 Hz, 1H), 5.47 (br s, 1H), 5.11 (s, 2H), 4.50−4.32 (m, 1H), 3.33 (d, J = 6 Hz, 1H); IR (KBr) 3012, 2115, 1720, 1501, 1367, 1228 cm^{-1}; MS (FD) *m/e* 356, 328, 242.

(*R*)-[3-Chloro-2-oxo-1-[(phenylthio)methyl]propyl]carbamic Acid Phenylmethyl Ester (4a). To compound **3a** (22.3 g, 0.063 mol) in diethyl ether (400 mL) at −20 °C under nitrogen atmosphere was added HCl gas in small portions, while the disappearance of starting material by TLC (CH_2Cl_2) was closely monitored. After 4 h, the solvent was removed under reduced pressure, leaving a white solid (23.0 g). No further purification was made: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−7.15 (m, 10H), 5.56 (dd, J = 2.0, 6.7 Hz, 1H), 5.11 (s, 2H), 4.78−4.67 (m, 1H), 4.20 (d, J = 15.9 Hz, 1H), 4.12 (d, J = 15.9 Hz, 1H), 3.48−3.23 (m, 2H); IR (KBr) 3349, 1732, 1684, 1515, 1266; MS (FD) *m/e* 363. Anal. ($C_{18}H_{18}ClNO_3S$) C, H, N.

[*R*-(*R,*S**)]-[3-Chloro-2-hydroxy-1-[(phenylthio)methyl]propyl]carbamic Acid Phenylmethyl Ester (5a).** Ketone **4a** (21 g, 0.058 mol) dissolved in THF (300 mL) was cooled by an ice water bath as $NaBH_4$ (2.4 g, 0.063 mol) was added in one portion. After 30 min, TLC (5% EtOAc/$CHCl_3$) indicated no starting material present, but two closely moving products at lower R_f. The reaction was quenched with aqueous NH_4Cl and the mixture extracted with ether. The organic phase was washed sequentially with water and brine and then dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue separated by two flash chromatographies (column 1, 0−2% MeOH in CH_2Cl_2; column 2, 0−2% EtOAc in $CHCl_3$) and recrystallized from CH_2Cl_2 at −78 °C. The slower moving alcohol was the major product (8.3 g, 39% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.19 (m, 10H), 5.22−5.03 (m, 1H), 5.09 (s, 2H), 4.01−3.89 (m, 2H), 3.75−3.58 (m, 2H), 3.32 (d, J = 4 Hz, 2H); IR (KBr) 3321, 2951, 1688, 1542, 1246, 738 cm^{-1}; MS (FD) *m/e* 366, 119. Anal. ($C_{18}H_{20}ClNO_3S$) C, H, N.

[*S*-(*R,*S**)]-[1-Oxiranyl-2-(phenylthio)ethyl]carbamic Acid Phenylmethyl Ester (6a).** Chloro alcohol **5a** (8.3 g, 0.023 mol) was dissolved in EtOH (400 mL) and cooled by an ice bath as KOH (1.4 g, 0.025 mol) was added. The ice water bath was removed and the mixture allowed to warm to room temperature. After 2 h, the solvent was removed by reduced pressure and the residue separated by flash chromatography (gradient, 0−2% EtOAc in CH_2Cl_2). Removal of solvent yielded a white solid (6.4 g, 85% yield): ^{1}H NMR (300 MHz,$CDCl_3$) δ 7.45−7.15 (m, 10H), 5.12 (s, 1H), 5.08 (s, 2H), 3.77−3.62 (m, 1H), 3.21 (d, J = 6 Hz, 2H), 2.99 (m, 1H), 2.77 (m, 2H); IR (KBr) 3303, 3067, 1694, 1538, 1257, 741; MS (FD) *m/e* 329. Anal. ($C_{32}H_{45}N_3O_4S$) C, H, N.

[*R*-(1*R,2*R**,3′*s**,4a′*S**,8a′*S**)]-[3-[3′-[[(1,1-Dimethylethyl)amino]carbonyl]octahydro-2′(1*H*)-isoquinolinyl]-2-hydroxy-1-[(phenylthio)methyl]propyl]carbamic acid Phenylethyl Ester (8a).** Compound **7** (3-[[(1,1-dimethylethyl)amino]carbonyl]octahydro-2′(1*H*)-isoquinoline, 5.00 g, 0.021

mol) was dissolved in EtOH (300 mL), and epoxide **6a** (6.31 g, 0.019 mol) was added. The solution was refluxed for 8 h. The solvent was removed under reduced pressure and the product isolated by flash chromatography (gradient of 0−20% EtOAc in CH_2Cl_2). Removal of solvent yielded **8a** as a white solid (4.3 g, 40%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.11 (m, 10H), 5.90 (d, J = 5 Hz, 1H), 5.64 (s, 1H), 5.05 (d, J = 4 Hz, 2H), 4.08−3.90 (m, 2H), 3.40 (d, J = 6 Hz, 2H), 3.05 (s, 1H), 2.95−2.85 (m, 1H), 2.62−2.45 (m, 2H), 2.28−2.15 (m, 2H), 2.05−1.88 (m, 2H), 1.78−1.10 (m, 7H), 1.29 (s, 9H); IR (KBr) 3330, 2925, 2862, 1706, 1661, 1520, 1454, 1246, 738, 694 cm^{-1}; MS (FD) *m/e* 568, 467. Anal. ($C_{32}H_{45}N_3O_4S$) C, H, N.

[*R*-(2*R,3*R**,3′*S**,4a′*S**,8a′*S**)]-2-[3-Amino-2-hydroxy-4-(phenylthio)butyl]-*N*-(1,1-dimethylethyl)decahydro-3′-isoquinolinecarboxamide (9a).** Cbz-protected amine **8a** (1.00 g, 0.0018 mol) was dissolved in 30% HBr/AcOH (40 mL) at room temperature. The reaction mixture was stirred for 1 h, and then the solvent was removed under reduced pressure. The residue was azeotroped with toluene three times. The residue was then dissolved in methanol (30 mL), diethylamine (2 mL) and concentrated NH_4OH (2 mL) were added, and the solvent was removed under reduced pressure. The residue was extracted from water with EtOAc. The organic phase was washed with aqueous $NaHCO_3$ and then with brine. The extract was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was separated by flash chromatography (gradient, $CHCl_3$ (3 drops of NH_4OH/1000 mL) with 0−10% MeOH). Removal of solvent under reduced pressure gave a white foam (0.54 g, 71% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.16 (m, 5H), 6.07 (s, 1H), 3.78−3.70 (m, 1H),3.45−3.38 (m, 1H), 3.03−2.84 (m, 3H), 2.38−2.20 (m, 3H), 2.00−1.05 (m, 12H), 1.33 (s, 9H); IR (KBr) 2924, 2862, 1660, 1517, 1454, 1439, 737, 691; MS (FD) *m/e* 434, 293.

[*S*-(2*R,1′*S**,2′*s**,3″*R**,4a″*R**,8a″*R**]-N-[3′-[3″-[[(1,1-Dimethylethyl)amino]carbonyl]octahydro-2″(1*H*)-isoquinolinyl]-2′-hydroxy-1′-[(phenylthio)methyl]propyl]-2-[((2″-methyl-3″-hydroxyphenyl)carbonyl)amino]butanediamide (10a).** To a cold (−10 °C) solution containing 70 mg (0.16 mmol) of amine **9a**, 24.6 mg (0.160 mmol) of 2-methyl-3-hydroxybenzoic acid, and 22 mg (0.16 mmol) of 1-hydroxybenzotriazole hydrate in 4 mL of THF was added 33 mg (0.16 mmol) of 1,3-dicyclohexylcarbodiimide. The reaction mixture was gradually warmed to room temperature overnight. After a total reaction time of 48 h, the mixture was concentrated under reduced pressure and then redissolved in ethyl acetate. The mixture was filtered through Celite, and the resulting clear solution was washed sequentially with saturated sodium bicarbonate and brine, dried over sodium sulfate, filtered, and concentrated. The crude product was purified by radial chromatography (1 mm plate, eluent of 3% methanol in CH_2Cl_2), yielding 54 mg (59%) of **10a** as a white foam: ^{1}H NMR (300 MHz,$CDCl_3$) δ 7.45 (m, 2H), 7.32−7.17 (m, 4H), 7.06 (m, 2H), 6.84 (m, 1H), 5.51 (br s, 1H), 5.37 (m, 1H), 4.47 (m, 1H), 4.07 (m, 1H), 3.78 (m, 1H), 3.42 (m, 1H), 2.91 (m, 1H), 2.61−2.43 (m, 2H), 2.31 (s, 3H), 2.30−2.17 (m, 2H), 2.02−1.93 (m, 2H), 1.79−1.12 (m, 12H), 1.09 (s, 9H); IR (KBr) 3297, 2925, 2862, 1627, 1586, 1530, 1482, 1466, 1366, 1287, 1221, 1156, 1119, 1026, 801, 735, 689 cm^{-1}; HRMS (FAB) *m/e* calcd for $C_{32}H_{46}N_3O_4S$ 568.3209, found 568.3182.

[*S*-(2*R,1′*S**,2′*s**,3″*R**,4a″*R**,8a″*R**]-*N*-[3′-[3″-[[(1,1-dimethylethyl)amino]carbonyl]octahydro-2″(1*H*)-isoquinolinyl]-2′-hydroxy-1′-[(phenylthio)methyl]propyl]-2-[((2″-methyl-3″-hydroxyphenyl)carbonyl)amino]butanediamide Methanesulfonate Salt (11a, AG1343).** To a stirring cooled (0 °C) solution of 50 mg (0.088 mmol) of amine **10a** in anhydrous methylene chloride (4 mL) was added dropwise over 5 min 88 μL (0.088 mmol) of a 1.0 M solution of methanesulfonic acid in methylene chloride. Solvent was removed from the precipitated salt by concentration under reduced pressure. On standing overnight under high vacuum (0.2−0.1 Torr) 58 mg (99%) of salt **11a** was obtained as an off white foam: ^{1}H NMR (DMSO-d_6) δ 9.18−9.02 (br s, 1H), 8.10 (s, 2H), 7.33−7.25 (m, 4H), 7.19−7.14 (m, 1H), 7.00-6.94 (m, 1H), 6.80 (m, 2H), 5.80−5.70 (m, 1H), 4.08−3.78 (m, 4H), 3.59−2.88 (m, 4H), 2.27 (s, 3H), 2.12 (s, 3H), 1.97−1.81 (m, 5H), 1.74−1.60 (m, 4H), 1.56−1.45 (m, 2H), 1.40−1.23 (m, 3H), 1.19 (s, 9H). Anal. ($C_{33}H_{49}N_3O_7S_2$) C, H, N.

(*R*)-*N*-[(Phenylmethoxy)carbonyl]-3-(2-naphthylthio)-alanine (2b). A 250 mL round bottom flask, equipped with stir bar and an addition funnel, was charged with 1.28 g (8.00 mmol) of naphthalene-2-thiol and 30 mL of THF. Under a nitrogen atmosphere, 1.77 g (8.16 g) of 60% NaH was added in portions to produce a yellow solution. After 15 min of stirring, the β-lactone (formed by the dehydration of Cbz-L-serine) was added over 5 min through the addition funnel as a solution in 20 mL of THF. The resultant solution was stirred for an additional hour and then concentrated in vacuo. The residue was dissolved in ethyl acetate and washed once with 0.5 N $NaHSO_4$ and once with brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography provided the title compound (2.08 g, 68%) as a pale yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.42−3.61 (br m, 2H), 5.53−5.76 (br s, 1H), 4.85−5.08 (br m, 2H), 5.54−5.76 (br s, 1H), 7.06−7.97 (m, 12H); $[\alpha]_D$ −55.72° (*c* 1.0, MeOH); IR (KBr) 3348, 3048, 1746, 1715, 1674, 1560, 1550, 1269, 1200, 1060 cm^{-1}; MS (FD) *m/e* 381 (M^+), 381 (100). Anal. ($C_{20}H_{19}NO_4S$) C, H, N.

(*R*)-[3-Diazo-2-oxo-1-[(2-naphthylthio)methyl]propyl]-carbamic Acid Phenylmethyl Ester (3b). A 1 L round bottom flask was charged with 15.4 g (40.3 mmol) of (*R*)-*N*-[(phenylmethoxy)carbonyl]-3-(2-naphthylthio)alanine and 230 mL of ethyl acetate. The solution was cooled to −30 °C in dry ice/carbon tetrachloride, and 5.62 mL (4.08 g, 40.3 mmol) triethylamine was added dropwise via syringe. Next, 7.84 mL (8.26 g, 60.5 mmol) of isobutyl chloroformate was added by syringe. The resulting solution was stirred under nitrogen in the cold. As the mixed anhydride solution was stirring, a bilayer of 170 mL of diethyl ether and 170 mL of 5 N NaOH was prepared in a 500 mL Erlenmeyer flask. While the flask was kept behind a blast shield, 10 g of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine was added carefully. When the copious gas evolution ceased, the ether layer was decanted from the aqueous layer onto KOH, dried for a few minutes, and poured through a long-stemmed funnel into the mixed anhydride solution. The above diazomethane formation and addition was repeated an additional time using identical quantities of ether and NaOH and 30 g of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine. After the solution was stirred in the cold for 20 min, nitrogen was bubbled through the solution using a fire-polished Pasteur pipet for approximately 2 min to remove any excess diazomethane, and then the solution was concentrated in vacuo. The residue was purified by flash chromatography to yield 13.62 g (83%) of the title compound as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.32−3.46 (m, 2H), 4.40−4.67 (m, 1H), 5.00−5.09 (m, 2H), 5.44 (s, 1H), 5.76 (d, J = 7.8 Hz, 1H), 7.25−7.86 (m, 12H).

(*R*)-[3-Chloro-2-oxo-1-[(2-naphthylthio)methyl]propyl]-carbamic Acid Phenylmethyl Ester (4b). In a 500 mL round bottom flask was placed 13.62 g (33.59 mmol) of the above diazo ketone in 230 mL of diethyl ether. The solution was cooled to −20 °C in dry ice/CCl_4. A short burst (about 2 s) of anhydrous HCl was passed through the solution, and gas evolution was immediately apparent. The reaction was stirred and monitored by TLC, care being taken not to add excess HCl. When the reaction was complete by TLC, the solution was concentrated in vacuo and purified by flash chromatography to afford 12.05 g (87%) of the title compound as a pale tan solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41 (dd, J = 12, 6 Hz, 1H), 3.53 (dd, J = 12, 6 Hz, 1H), 4.18 (AB q, $\Delta\nu$ = 41.9 Hz, J = 15.9 Hz, 2H), 4.77 (dd, J = 9, 3 Hz, 1H), 5.04 (AB q, J = 12 Hz, $\Delta\nu$ = 10.4 Hz, 2H), 5.59 (d, J = 7 Hz, 1H), 7.24−7.85 (complex, 12H); $[\alpha]_D$ −80.00° (*c* 1.0, MeOH); IR ($CHCl_3$) 3426, 3031, 3012, 1717, 1502, 1340, 1230, 1228, 1045 cm^{-1}; MS (FD) *m/e* 413 (M^+), 413(100). Anal. ($C_{22}H_{20}NO_3SCl$) C, H, N.

[*R*-(*R,*S**)]-[3-Chloro-2-hydroxy-1-[(2-naphthylthio)-methyl]propyl]carbamic Acid Phenylmethyl Ester (5b).** A 25 mL round bottom flask was charged with 530 mg (1.28 mmol) of the above chloro ketone, 10 mL of THF, and 1 mL of water. The solution was cooled in an ice bath, and 73 mg (1.92 mmol) of $NaBH_4$ was added in one portion. The reaction was stirred in the cold, and was found to be complete by TLC in 30 min. The pH was carefully adjusted to 3 with about 10 mL of saturated NH_4Cl and 500 μL of 5 N HCl. The acidic solution was extracted twice with methylene chloride. The

combined organic layers were washed once with water, dried over Na_2SO_4, filtered, and concentrated in vacuo. TLC analysis showed the presence of two diastereomers, with the lower R_f compound being the desired product. Purification was accomplished by radial chromatography using 100% methylene chloride and provided 212 mg (40%) of the title compound as a tan solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (apparent s, 2H), 3.61−3.71 (m, 2H), 3.97−3.99 (m, 2H), 4.99 (apparent s, 2H), 5.16 (br s, 1H), 7.21−7.83 (complex, 12H); FDMS *m/e* 415 (M^+), 415 (100); $[\alpha]_D$ −47.67° (*c* 0.86, MeOH); IR ($CHCl_3$) 3630, 3412, 3011, 1720, 1502, 1236, 1044 cm^{-1}. Anal. ($C_{22}H_{22}NO_3ClS$) C, H, N.

[*S*-(*R,*S**)]-[1-Oxiranyl-2-(2-naphthylthio)ethyl]carbamic Acid Phenylmethyl Ester (6b).** A mixture of 31 mg (0.55 mmol) of KOH in 1 mL of ethanol was prepared in a 10 mL round bottom flask and allowed to stir until all of the KOH was in solution. Next, a solution of 190 mg (0.46 mmol) of the above chloro alcohol in 4 mL of ethyl acetate and 2 mL of ethanol was added via Pasteur pipet. The resultant solution was stirred at room temperature and followed by TLC. The reaction was complete in 15 min, at which time the reaction mixture was poured into a bilayer of water and methylene chloride. The layers were separated, and the aqueous layer was extracted once more with methylene chloride. The combined organic layers were washed once with water, dried over Na_2SO_4, and concentrated in vacuo. The product was purified by radial chromatography to provide 172 mg (99%) of the title compound as a light tan solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.76 (br s, 2H) 3.01 (br s, 1H), 3.31 (d, J = 5 Hz, 2H), 3.77 (br s, 1H), 5.05 (s, 2H), 5.22 (d, J = 6 Hz, 1H), 7.25−7.85 (complex, 12H); $[\alpha]_D$ −125.42° (*c* 0.59, MeOH); FDMS *m/e* 379 (M^+), 379 (100); IR ($CHCl_3$) 3640, 3022, 2976, 1720, 1502, 1235, 1045 cm^{-1}. Anal. ($C_{22}H_{21}NO_3S$) C, H, N.

[*R*-(1*R,2*R**,3′*s**,4a′*S**,8a′*S**)]-[3-[3′-[[(1,1-dimethylethyl)amino]carbonyl]octahydro-2′(1*H*)-isoquinolinyl]-2-hydroxy-1-[(2-naphthylthio)methyl]propyl]carbamic Acid Phenylethyl Ester (8b).** A 10 mL round bottom flask was charged with 165 mg (0.40 mmol) of the above epoxide, 94 mg (0.43 mmol) of 3-[[(1,1-dimethylethyl)amino]carbonyl]-octahydro-2′(1*H*)-isoquinoline, and 5 mL of ethanol. The flask was equipped with a reflux condenser and heated to 80 °C for 19 h. The solution was then cooled to room temperature and concentrated in vacuo. Radial chromatography of the residue afforded 103 mg (42%) of the title compound as an off-white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.098−1.732 (m, 20H), 2.13−2.31 (m, 2H), 2.44−2.53 (m, 1H), 2.56−2.68 (m, 1H), 2.86−2.97 (m, 1H), 3.52 (br s, 2H), 4.02 (br s, 2H), 4.98 (apparent s, 2H), 5.65 (s, 1H), 5.94 (s, 1H), 7.25−7.83 (complex, 13H); FDMS *m/e* 629 (M^+), 138 (100); $[\alpha]_D$ −92.45° (*c* 1.06, MeOH); IR ($CHCl_3$) 3429, 3010, 2929, 1713, 1670, 1514, 1455, 1047 cm^{-1}. Anal. ($C_{35}H_{47}N_3O_4S$) C, H, N.

[*R*-(2*R,3*R**,3′*S**,4a′*S**,8a′*S**)]-2-[3-Amino-2-hydroxy-4-(2-naphthylthio)butyl]-*N*-(1,1--dimethylethyl)decahydro-3′-isoquinolinecarboxamide (9b).** In a 10 mL round bottom flask under nitrogen were placed 50 mg (0.081 mmol) of the above Cbz-amino alcohol and 1 mL of 38% HBr in acetic acid. The red solution was stirred at room temperature for 1 h and concentrated in vacuo. The residue was azeotroped once with toluene and dried on the vacuum pump. The residue (61 mg, quantitative) was used crude in the next coupling: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (s, 1H), 1.17−2.07 (complex, 15H), 2.66, 2.87 (m, 2H), 3.21−3.25 (m, 2H), 3.75 (d, J = 12 Hz, 1H), 3.85 (d, J = 6 Hz, 1H), 4.36−4.47 (m, 1H), 6.73 (s, 1H), 7.39−7.90 (complex, 7H); FDMS 483 (M^+), 483 (100).

[*S*-(2*R,1′*S**,2′*s**,3″*R**,4a″*R**,8a″*R**]-*N*-[3′-[3″-[[(1,1-Dimethylethyl)amino]carbonyl]octahydro-2″(1*H*)-isoquinolinyl]-2′-hydroxy-1′-[(2-naphythylthio)methyl]propyl]-2-[((2″-methyl-3″-hydroxyphenyl)carbonyl)amino]-butanediamide (10b).** To a cold (−10 °C) solution containing 70 mg (0.145 mmol) of amine **9b**, 22 mg (0.145 mmol) of 2-methyl-3-hydroxybenzoic acid, and 19 mg (0.145 mmol) of 1-hydroxybenzotriazole hydrate in 4 mL of THF was added 29 mg (0.145 mmol) of 1,3-dicyclohexylcarbodiimide. The reaction mixture was gradually warmed to room temperature overnight. After a total reaction time of 48 h, the mixture was concentrated under reduced pressure and then redissolved in ethyl acetate. The mixture was filtered through Celite, and the resulting clear solution was washed sequentially with saturated sodium bicarbonate and brine, dried over sodium sulfate, filtered, and concentrated. The crude product was purified by radial chromatography (2 mm plate, gradient eluent of 5−15% acetone in CH_2Cl_2), yielding 65 mg (73%) of **10b** as a white solid: ^{1}H NMR (300 MHz,$CDCl_3$) δ 7.90 (s, 1H), 7.73 (m, 3H), 7.53−7.40 (m, 3H), 7.21 (d, J = 8.8 Hz, 1H), 6.93 (m, 2H), 6.79 (m, 1H), 5.53 (br s, 1H), 5.36 (br s, 1H), 4.10 (m, 1H), 3.92−3.83 (m, 1H), 3.51 (m, 1H), 2.94 (d, 1H), 2.61−2.42 (m, 2H), 2.29 (s, 3H), 2.28−2.17 (m, 2H), 2.06−1.93 (m, 1H), 1.80−1.15 (m, 12H), 1.10 (s, 9H); IR (KBr) 3427, 3311 (br), 2929, 2864, 1703, 1661, 1587, 1514, 1456, 1393, 1366, 1276, 1200, 1177, 1146, 1119, 1070, 1042 cm^{-1}; $[\alpha]_D$ −112° (*c* 0.25, MeOH). MS(FD) *m/e* 618 (M + 1, 100). Anal. ($C_{36}H_{47}N_3O_4S$) C, H, N.

[3*S*-(3*R,4a*R**,8a*R**,2′*S**,3′*R**)]-2-[2′-Hydroxy-3′-(phenylmethyl)-4′-aza-5′-oxo-5′-(2″-methyl-3″-hydroxyphenyl)pentyl]decahydroisoquinoline-3-*N*-*tert*-butylcarboxamide (10c).** To a cold (−10 °C) solution containing 261 mg (0.65 mmol) of amine **9c**, 100 mg (0.65 mmol) of 2-methyl-3-hydroxybenzoic acid, and 88 mg (0.65 mmol) of 1-hydroxybenzotriazole hydrate in 6 mL of THF and 0.2 mL of DMF was added 134 mg (0.65 mmol) of 1,3-dicyclohexylcarbodiimide. The reaction mixture was gradually warmed to room temperature overnight. After a total reaction time of 48 h, the mixture was concentrated under reduced pressure and then redissolved in ethyl acetate. The mixture was filtered through Celite, and the resulting clear solution was washed sequentially with saturated sodium bicarbonate and brine, dried over sodium sulfate, filtered, and concentrated. Purification of the crude product by radial chromatography (2 mm plate, gradient eluent of 1%-5% methanol/methylene chloride) gave 304 mg of a white solid: yield 87%; $[\alpha]_D$ −75.00° (*c* = 2.00, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 9H), 1.19−2.05 (m, 18H), 2.20−2.35 (m, 2H), 2.50−2.70 (m, 2H), 2.90−3.05 (m, 2H), 3.22−3.35 (m, 1H), 3.96−4.05 (m, 1H) 4.45−4.55 (m, 1H), 5.77 (s, 1H), 6.53 (d, J = 7.4 Hz, 2H), 6.75 (d, J = 7.8 Hz, 1H), 6.85−6.90 (m, 1H), 7.15−7.35 (m, 6H); IR ($CDCl_3$) 3606, 3600−3100 (br), 3429, 3011, 2929, 2865, 1663, 1604, 1587, 1514, 1455, 1367, 1277, 1200, 1156, 1046, 910 cm^{-1}; HRMS (FAB) *m/e* calcd for $C_{32}H_{46}N_3O_4$ 536.3488, found 536.3488.

[3*S*-(3*R,4a*R**,8a*R**,2′*S**,3′*R**)]-2-[2′-Hydroxy-3′-(phenylmethyl)-4′-aza-5′-oxo-5′-(2″-methyl-3″-hydroxyphenyl)pentyl]decahydroisoquinoline-3-*N*-*tert*-butylcarboxamide Monomethanesulfonate Salt (11c).** To a stirring cooled (0 °C) solution of 125 mg (0.23 mmol) of 3*S*-(3*R**,4a*R**,8a*R**,2′*S**,3′*R**)]-2-[2′-hydroxy-3′-(phenylmethyl)-4′-aza-5′-oxo-5′-(2″-methyl-3″-hydroxyphenyl)pentyl]-decahydroisoquinoline-3-*N*-*tert*-butylcarboxamide in anhydrous methylene chloride (5 mL) was added dropwise over 5 min 240 μL (0.24 mmol) of a 1.0 M solution of methane sulfonic acid in methylene chloride. After the mixture was pumped overnight under high vacuum (0.2−0.1 torr) 136 mg of the crude salt was obtained as an off-white foam: yield 95%; ^{1}H NMR (methanol-d_4) δ 1.12 (s, 9H), 1.10−2.20 (m, 16H), 2.60−2.75 (m, 4H), 3.10−3.50 (m, 6H), 3.60−3.70 (m, 1H), 3.90−4.30 (m, 3H), 6.53 (d, J = 7.35 Hz, 1H), 6.55 (d, J = 7.87 Hz, 1H), 6.89 (t, J = 7.82 Hz, 1H). Anal. ($C_{33}H_{49}N_3O_7S$) C, H, N.

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(10) The HIV-1 protease cloning, expression, and purification has been described previously (Hostomsky, Z.; Appelt, K.; Ogden, R. C. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 1056−1063). Crystals of the inhibited enzyme were grown by the hanging drop vapor diffusion method from 1.2−1.6 M ammonium sulfate, 5% (vol/vol) dimethyl sulfoxide, 3% (vol/vol) isopropyl alcohol, and 0.05 M citrate−tartrate buffered at pH = 5.8. The enzyme crystallized in space group P2(1)2(1)2(1) with cell dimensions of $a = 52$ Å, $b = 59$ Å, and $c = 62$ Å. A 2.1 Å X-ray diffraction data set was collected from one crystal using dual area detectors from Area Detector Systems, Inc. Refinement of the AG1343 complex was initiated using the crystal structure of a previously solved inhibited complex as a starting point. Using the X-PLOR program (Brunger, A. T.; Kuriyan, J.; Karplus, M. *Science* **1987**, *235*, 458−460), the structure was refined to a crystallographic R-factor of 0.20 using all data between 6 and 2.1 Å. Details of the crystallographic work will be published elsewhere (J. F. Davies and K. Appelt). Atomic coordinates have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratories, Long Island, NY 11973. Accession number: 10HR.

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(12) Animal studies: Pharmacokinetics of AG1343 were determined after intravenous and oral administration of AG1343 to male Sprague−Dawley rats ($n = 3$), beagle dogs (one male and one female), female cynomolgus monkeys, and marmosets (one male and one female). Intravenous dose of AG1343 ranged from 12.5 to 25 mg/kg, and oral dose of AG1343 ranged from 25 to 50 mg/kg. AG1343 was delivered as a solution in 5% dextrose to rats, dogs, and monkeys or in propylene glycol:water (50:50) to marmosets. Blood (0.25−2 mL) was sampled from the jugular, femoral, or cephalic vein before dosing and at time points up to 48 h after dosing. Plasma was separated immediately after sampling by centrifugation and stored at −20 °C until analyzed. AG1343 was extracted from plasma and quantified using high performance liquid chromatography (HPLC) and UV detection. Details of preclinical pharmacokinetics will be published in appropriate forum (B. Shetty).

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