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Supporting Information

Synthesis and Biological Evaluation of 1,3,3,4-Tetrasubstituted Pyrrolidine CCR5 Receptor Antagonists. Discovery of a Potent and Orally Bioavailable Anti-HIV Agent.

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Supporting information

Experimental

3-Dibenzylaminopropionic acid methyl ester (8). To an ice cooled stirring mixture of 3-aminopropionic acid (15.0 g, 0.17 mol) and dry methanol (120 mL) was added dropwise thionyl chloride (30 mL). After being refluxed for 3 h, the mixture was concentrated in vacuo. To the residue was added acetonitrile (300 mL) and benzyl bromide (50 mL, 0.42 mol). After carefully adding potassium carbonate (93.0 g, 0.67 mol), the reaction mixture was stirred at room temperature for 24 h. Water was added and the mixture was extracted with EtOAc for three times. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Chromatography of the residue on silica gel eluting with 20:1 petroleum ether/EtOAc provided ester **8** (42.3 g, 89%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29-7.17 (m, 10 H), 3.56 (s, 3H), 3.51 (s, 4H), 3.74 (t, *J* = 6.6 Hz, 2H), 2.45 (t, *J* = 6.6 Hz, 2H).

1-Ethyl 4-methyl 3-((dibenzylamino)methyl)-2-hydroxy-2-phenylsuccinate (9, 10). To an ice cooled stirring solution of diisopropylamine (15.2 mL, 108.0 mmol) in dry THF (50 mL) was added dropwise *n*-BuLi (4.5 mL, 1.6 M in hexane). The resulting mixture was stirred at 0 ^{o}C for 30 min, then cooled to –78 ^{o}C. A solution of 3-dibenzylaminopropionic acid methyl ester (12.2 g, 43.2 mmol) in THF (100 mL) was added slowly. After being stirred at this temperature for 1 h, ethyl 2-oxo-2-phenylacetate (9.98 g, 56.1 mmol) in THF (40 mL) was added all at once. The reaction mixture was stirred at -78 ^{o}C for 4 h, and then quenched with a saturated

aqueous solution of NH_4Cl (100 mL), the aqueous phase was extracted with EtOAc for two times. The combined organic layers were washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. Chromatography of the residue on silica gel eluting with 15:1 petroleum ether/EtOAc provided ester **9** (7.35 g, 37%) and **10** (7.35 g, 37%). **9**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52-7.45 (m, 2H), 7.25-7.10 (m, 13H), 4.58 (br s, 1H), 4.06 (q, *J* = 6.9 Hz, 2H), 3.68 (s, 3H), 3.56 (dd, *J* = 13.8, 7.2 Hz, 3H), 3.09 (d, *J* = 13.8 Hz, 2H), 2.89 (dd, *J* = 13.5, 10.8 Hz, 1H), 2.20 (dd, *J* = 13.5, 3.6 Hz, 1H), 1.12 (t, *J* = 6.9 Hz, 3H); IR (KBr) 3525, 1736, 1724 cm^{-1}; EI-MS *m/z* 461 (M^+); HRMS Calcd. for $C_{28}H_{31}NO_5$ 461.2202. Found 461.2157. **10**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.51-7.45 (m, 2H), 7.29-7.10 (m, 13H), 4.62 (br s, 1H), 4.13 (q, *J* = 6.7 Hz, 2H), 3.73 (s, 3H), 3.63 (d, J_{AB} = 13.6 Hz, 2H), 3.53 (dd, *J* = 10.7, 3.6 Hz, 1H), 3.22 (d, J_{AB} = 13.6 Hz, 2H), 3.00-2.94 (m, 1H), 2.39-2.35 (m, 1H), 1.19 (t, *J* = 6.7 Hz, 3H); IR (KBr) 3527, 1730 cm^{-1}; EI-MS *m/z* 461 (M^+).

1-Benzyl-4-hydroxy-5-oxo-4-phenyl-pyrrolidine-3-carboxylic acid methyl ester (11). To a solution of **9** (7.15 g, 16.0 mmol) in anhydrous MeOH (100 mL) was added Pd/C (10%, 0.7 g). The resulting mixture was allowed to stir at room temperature under an atmosphere of hydrogen for 1 h. The catalyst was removed by filtration and was washed with MeOH. The solvent was removed under reduced pressure. Chromatography of the residue on silica gel eluting with 2:1 petroleum ether/EtOAc provided ester **11** (3.12 g, 60%) as a white powder. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.25 (m, 10H), 4.56 (q, J_{AB} = 14.7 Hz, 2H), 3.64 (s, 3H), 3.57 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.33-3.30 (m, 2H); IR (KBr) 3304, 3062, 1749, 1685 cm^{-1}; EI-MS *m/z*

325 (M^+); HRMS Calcd. for $C_{19}H_{19}NO_4$ 325.1304, found 325.1296.

1-Benzyl-4-hydroxy-5-oxo-4-phenyl-pyrrolidine-3-carboxylic acid (13). To a solution of **11** (1.49 g, 4.58 mmol) in MeOH (50 mL) was added 1 N NaOH (6.4 mL) at room temperature. The mixture was stirred for 2 h and then the organic solvent was removed under reduced pressure. Water (30 mL) was added, and it was neutralized using 1 N HCl at room temperature. The resulting mixture was extracted with EtOAc for three times. The combined organic layers was washed with brine, dried over Na_2SO_4, and then concentrated in vacou provided acid **13** (1.41 g, 99%) as a white powder. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.42-7.25 (m, 10H), 4.51 (q, J_{AB} = 15.3 Hz, 2H), 3.62-3.56 (m, 1H), 3.40-3.36 (m, 2H); IR (KBr) 3267, 1713, 1688 cm-1; EI-MS *m/z* 311 (M^+); HRMS Calcd. for $C_{18}H_{17}NO_4$ 311.1158 (M^+), found 311.1160.

1-Benzyl-3-hydroxy-3-phenyl-4-(4-phenylpiperidine-1-carbonyl)-pyrrolidin-2-one (14). To an ice cooling solution of **13** (0.20 g, 0.64 mmol) in dry THF (10 mL) was added HOSu (81 mg, 0.71 mmol) and DCC (0.15 g, 0.71 mmol). The reaction mixture was stirred at 0 ^{o}C for 8 h, and the resulting precipitate was filtered. To the residue was added 4-phenylpiperidine (0.11 g, 0.71 mmol) and the reaction mixture was stirred at room temperature for 12 h. Water was added to the mixture, followed by extraction with EtOAc for two times. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacou. Chromatography of the residue on silica gel eluting with 1:1 petroleum ether/EtOAc provided **14** (0.27 g, 92%) as a foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.22 (m, 13H), 7.19-7.01 (m, 2H), 4.87-4.42 (m, 3H), 3.72-3.66 (m, 2H), 3.67-3.58 (m, 1H),

3.52 (m, 1H), 3.34-3.26 (m, 1H), 2.98-2.91 (m, 1H), 2.63-2.49 (m, 2H), 1.85-1.75 (m, 4H); IR (KBr) 3327, 2929, 2852, 1702, 1627, 1612 cm^{-1}; EI-MS *m/z* 354 (M^+); HRMS Calcd. for $C_{29}H_{30}N_2O_3$ 454.2224 (M^+), found 454.2240.

1-Benzyl-3-phenyl-4-(4-phenylpiperidin-1-ylmethyl)pyrrolidin-3-ol (15a). LAH (40 mg, 1.05 mmol) was added to a solution of **14** (60 mg, 0.13 mmol) in dry THF (5 mL). The resulting slurry was heated to reflux for 24 h, and then cooled to room temperature. To the mixture was added 1 N NaOH (0.5 mL), filtered, and the resulting filtrate was concentrated in cacuo. Chromatography of the residue on silica gel eluting with 10:1:0.5 EtOAc/MeOH/NEt_3 afforded **15a** (48 mg, 85%) as a colorless oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.68-7.60 (m, 2H), 7.37-7.13 (m, 13H), 3.66 (q, J_{AB} = 14.7 Hz, 2H), 3.01-2.81 (m, 4H), 2.73-2.58 (m, 1H), 2.57-2.52 (m, 1H), 2.46-2.35 (m, 2H), 2.15-2.02 (m, 2H), 1.80-1.69 (m, 4H), 1.51-1.26 (m, 1H); IR (film) 2933, 2802, 1504 cm^{-1}; EI-MS *m/z* 427 $(M + H)^+$; HRMS Calcd. for $C_{29}H_{34}N_2O$ 426.2671 (M^+), found 426.2657.

[3-Hydroxy-3-phenyl-4-(4-phenylpiperidin-1-ylmethyl)pyrrolidin-1-yl]-phenylmethanone (15b). To a solution of **15a** (122 mg, 0.29 mmol) in anhydrous MeOH (10 mL) was added Pd/C (10%, 20 mg). The resulting mixture was allowed to stir at room temperature under an atmosphere of hydrogen for 20 h. The catalyst was removed by filtration and was washed with MeOH. The solvent was removed under reduced pressure. The resulting residue was dissolved in dry CH_2Cl_2 (1 mL). To the solution was added Et_3N (60 μL, 0.43 mmol) and benzoyl chloride (40 μL, 0.35 mmol) at 0 ^oC. After the mixture had been stirred at 0 ^oC for 3 h, the solvent was concentrated

in vacuo. Chromatography of the residue on silica gel eluting with 2:1 petroleum ether/EtOAc provided **15b** (65 mg, 52%) as an oil. ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.66-7.18 (m, 15H), 4.00 (q, J_{AB} = 15.9 Hz, 1H), 3.87-3.65 (m, 2H), 3.18 (m, 1H), 3.05-2.77 (m, 2H), 2.65-2.61 (m, 2H), 2.59-2.38 (m, 2H), 2.38-2.08 (m, 2H), 1.86-1.73 (m, 4H); IR (KBr) 3269, 2922, 2808, 1723, 1592, 1571 cm^{-1}; EI-MS *m/z* 440 (M^+); HRMS Calcd. for $C_{29}H_{32}N_2O_2$ 440.2464 (M^+), found 440.2448.

The following compounds (**15c**-**15e**) were prepared from **15a** by a method similar to that described for **15b**.

Cyclohexyl-[3-hydroxy-3-phenyl-4-(4-phenylpiperidin-1-ylmethyl)-pyrrolidin-1-yl]methanone (15c). Yield 53% over two steps, colourless powder. ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.56-7.50 (m, 2H), 7.43-7.12 (m, 8H), 3.89-3.70 (m, 4H), 3.10 (m, 1H), 2.85-2.71 (m, 2H), 2.59-2.36 (m, 3H), 2.31-2.13 (m, 3H), 1.82-1.44 (m, 14H); IR (KBr) 3253, 2939, 2850, 1606, 1453 cm^{-1}; EI-MS *m/z* 446 (M^+); HRMS Calcd. for $C_{29}H_{38}N_2O_2$ 446.2956 (M^+), found 446.2945.

Cyclopentyl-[3-hydroxy-3-phenyl-4-(4-phenylpiperidin-1-ylmethyl)-pyrrolidin-1-yl]methanone (15d). Yield 55% over two steps, colourless powder. ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.59-7.53 (m, 2H), 7.43-7.36 (m, 2H), 7.35-7.26 (m, 3H), 7.24-7.20 (m, 3H), 3.90-3.70 (m, 4H), 3.14 (m, 1H), 2.88-2.69 (m, 3H), 2.60-2.44 (m, 3H), 2.30-2.13 (m, 2H), 1.91-1.70 (m, 10H), 1.62-1.43 (m, 2H); IR (KBr) 3276, 2953, 1608, 1453 cm^{-1}; EI-MS *m/z* 432 (M^+);

HRMS Calcd. for $C_{28}H_{36}N_2O_2$ 432.2822 (M^+), found 432.2800.

1-Benzenesulfonyl-3-phenyl-4-(4-phenylpiperidin-1-ylmethyl)-pyrrolidin-3-ol (15e). Yield 50% over two steps, foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.83 (d, *J* = 8.7 Hz, 2H), 7.57-7.47 (m, 3H), 7.33 (d, *J* = 7.5 Hz, 2H), 7.25-7.13 (m, 6H), 7.08 (d, *J* = 7.5 Hz, 2H), 3.60-3.58 (m, 1H), 3.53 (q, J_{AB} = 10.8 Hz, 2H), 3.32-3.27 (m, 1H), 2.76 (d, *J* = 9.9 Hz, 1H), 2.67 (d, *J* = 12.3 Hz, 1H), 2.42-2.35 (m, 4H), 2.07-1.94 (m, 2H), 1.74-1.56 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.6, 132.8, 129.0, 128.5, 128.4, 127.6, 127.3, 126.7, 126.4, 125.0, 81.9, 61.8, 56.2, 55.9, 54.4, 50.4, 45.6, 41.7; IR (KBr) 3062, 2933, 2812, 1737, 1494, 1447 cm^{-1}; EI-MS *m/z* 335 (M^+).

1-(1-Benzyl-3-hydroxy-2-oxo-3-phenylpyrrolidine-4-carbonyl)piperidin-4-one (18). To an ice cooled stirring mixture of **13** (2.18 g, 7.02 mmol) and HOSu (0.89 g, 7.72 mmol) in dry THF (50 mL) was added DCC (1.60 g, 7.72 mmol) slowly, the ice bath was removed after 15 min, and the stirring was continued at room temperature for 24 h. The resulting mixture was filtrated, the precipitate was washed with a small amount of dry THF and discarded. To the filtrate was added triethylamine (2.9 mL, 21.1 mmol) and 4-piperidone monohydrate hydrochloride (1.19 g, 7.72 mmol) respectively, and the resulting mixture was stirred at room temperature for another 20 h. The precipitate was filtrated, and the filtrate was concentrated in vacuo. EtOAc was added and the mixture was washed with water and brine, dried over Na_2SO_4, concentrated in vacuo. Chromatography of the residue on silica gel eluting with 1:1 petroleum ether/EtOAc to provide **18** (2.30 g, 84%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34-7.18 (m, 10H), 4.59 (d, J_{AB} = 15.0 Hz, 2H), 4.10 (s, 1H), 4.03 (d, *J* = 8.4 Hz, 1H),

3.77 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.60-3.52 (m, 1H), 3.44-3.29 (m, 4H), 2.56-2.25 (m, 3H), 2.23 (m, 1H); IR (KBr) 3328, 2929, 2851, 1719, 1691, 1575 cm^{-1}; EI-MS *m/z* 392 (M^+); HRMS Calcd. for $C_{23}H_{24}N_2O_4$ 392.1693 (M^+), found 392.1717.

1-((1-Benzyl-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl acetate (20). To an ice cooled stirring mixture of LAH (1.78 g, 46.9 mmol) and dry THF (30 mL) was added dropwise **18** (2.30 g, 5.87 mmol) in dry THF (50 mL). After being stirred at reflux for 24 h, the reaction was quenched with water (1.8 mL), 15% aqueous NaOH (1.8 mL) and water (1.8 mL) respectively. The slurry was then stirred at room temperature for another 4 h, filtered and the resulting filtrate was concentrated in vacuo to provide **19** (2.11 g, 98%) as an oil. To an ice cooled stirring solution of **19** (1.79 g, 4.88 mmol), triethyl amine (2.0 mL, 14.6 mmol) and DMAP (10 mg) in dry CH_2Cl_2 (100 mL) was added acetic anhydride (1.2 mL, 12.2 mmol). The mixture was stirred at 4 ^oC for 1 h, and water was added. The aqueous layer was extracted with CH_2Cl_2 for three times. The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated in vacuo. Chromatography of the residue on silica gel eluting with 1:20 NEt_3/EtOAc afforded **20** (1.71 g, 86%) as a corlorless foam. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65-7.62 (m, 2H), 7.40-7.18 (m, 8H), 4.69 (m, 1H), 3.68 (s, 2H), 3.00-2.93 (m, 2H), 2.89-2.81 (m, 2H), 2.68-2.58 (m, 2H), 2.45-2.36 (m, 4H), 2.06 (s, 3H), 1.85-1.55 (m, 5H); EI-MS *m/z* 408 (M^+).

1-(((1-Cyclopentanecarbonyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl) piperidin-4-yl acetate (21a). To a solution of **20** (1.71 g, 4.19 mmol) in anhydrous MeOH (60 mL) was added 20% palladium hydroxide on charcoal (0.17 g). The

resulting mixture was allowed to stir at room temperature under an atmosphere of hydrogen for 20 h. The catalyst was removed by filtration and was washed with MeOH. The solvent was removed under reduced pressure. The resulting residue was dissolved in dry CH_2Cl_2 (15 mL). To the solution was added Et_3N (0.87 mL, 6.29 mmol) and cyclopentanecarbonyl chloride (0.67 g, 5.00 mmol) at 0 ^oC. After the mixture had been stirred at 0 ^oC for 3 h, the solvent was concentrated in vacuo. Chromatography of the residue on silica gel eluting with 2:1 petroleum ether/EtOAc provided **21a** (0.99 g, 57%) as a white foam. ^1H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.53-7.12 (m, 5H), 3.87-3.65 (m, 3H), 3.58-3.40 (m, 1H), 2.81-2.25 (m, 6H), 2.06-2.03 (m, 3H), 1.96-1.54 (m, 15H); IR (KBr) 3281, 2949, 2809, 1731, 1639, 1608 cm^{-1}; HRMS Calcd. for $C_{24}H_{35}N_2O_4$ 415.2591 $(M + H)^+$, found 415.2584.

The following compounds (**21b-21e**) were prepared from **20** by a method similar to that described for **21a**.

1-(((1-Cyclohexanecarbonyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl)-piperidin-4-yl acetate (21b). yield 56%, foam. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.54-7.28 (m, 5H), 4.77 (m, 1H), 3.85-3.69 (m, 3H), 3.58-3.40 (m, 1H), 2.80-2.26 (m, 6H), 2.09-2.03 (m, 3H), 1.97-1.43 (m, 13H), 1.30-1.19 (m, 4H); IR (KBr) 2937, 1731, 1606 cm^+; HRMS Calcd. for $C_{25}H_{37}N_2O_4$ 429.2748 $(M + H)^+$, found 429.2745.

1-((1-Benzoyl-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl acetate (21c). yield 62%, foam. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.63-7.24 (m, 10H), 4.76 (m, 1H), 4.15-3.84 (m, 2H), 3.82-3.40 (m, 2H), 2.85-2.20 (m, 6H), 2.03 (m, 3H), 1.94-1.50 (m, 5H); IR (KBr) 3368, 2951, 2814, 1732, 1626 cm^{-1}; HRMS Calcd. for

$C_{25}H_{31}N_2O_4$ 423.2278 $(M + H)^+$, found 423.2273.

1-((4-Hydroxy-1-(2-iodobenzoyl)-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl acetate (21d). yield 59%, foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.88-7.78 (m, 1H), 7.60-7.03 (m, 8H), 4.78-4.74 (m, 1H), 4.14-4.04 (m, 1H), 3.96-3.90 (m, 1H), 3.58-3.15 (m, 2H), 2.95-2.85 (m, 1H), 2.64-2.24 (m, 5H), 2.09-1.97 (m, 3H), 1.90-1.59 (m, 5H); IR (KBr) 3385, 2950, 2814, 1732, 1635 cm^{-1}; HRMS Calcd. for $C_{25}H_{30}N_2O_4I$ 549.1245 $(M + H)^+$, found 549.1240.

1-(((1-Naphthoyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl acetate (21e). yield 61%, foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03-7.80 (m, 3H), 7.61-7.10 (m, 9H), 4.82-4.60 (m, 1H), 4.19-4.05 (m, 2H), 3.49-3.34 (m, 2H), 2.80-2.33 (m, 6H), 2.09 (m, 3H), 1.99-1.60 (m, 5H), 1.56-1.38 (m, 1H); IR (KBr) 3383, 2951, 2814, 1732, 1632 cm^{-1}; HRMS Calcd. for $C_{29}H_{33}N_2O_4$ 473.2435 $(M + H)^+$, found 473.2456.

1-((1-(cyclopentanecarbonyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl) piperidin-4-one (22a). To a stirred solution of **21a** (5.84 g, 14.1 mmol) in (5:1) MeOH/H_2O (100 mL) was added K_2CO_3 (1.95 g, 28.2 mmol). The mixture was stirred at room temperature for 2 h, then the organic solvent was removed under reduced pressure. EtOAc was added, and the aqueous layer was extracted with EtOAc for three times. The combined organic layers were washed respectively with water and brine, dried over Na_2SO_4, concentrated in vacuo to provide a foam (5.26 g). To a stirring solution of oxalyl chloride (1.6 mL, 18.2 mmol) in dry CH_2Cl_2 (20 mL) at –78 ^oC under an atmosphere of argon was added dropwise a solution of dimethyl sulfide

(DMSO, 3.0 mL, 42.0 mmol) in dry CH_2Cl_2 (20 mL). The mixture was stirred at –78 ^oC for 20 min, and then a solution of the residue (5.26 g, 14.1 mmol) provided above in dry CH_2Cl_2 (20 mL) was added dropwise. The mixture was stirred at –78 ^oC for further 1 h, and then triethylamine (5.8 mL, 42.0 mmol) was added dropwise. Once the two additions had been completed, the mixture was wormed to room temperature. After being stirred at room temperature for 30 min, the reaction mixture was then quenched with water. The aqueous layer was extracted with CH_2Cl_2 for two times, and the combined organic layers were washed respectively with water and brine, dried over Na_2SO_4, and concentrated in vacuo to provided a powder material (5.00 g, 96%). mp 138 ^oC; ^1H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.60-7.51 (m, 2H), 7.44-7.33 (m, 2H), 7.32-7.30 (m, 1H), 3.96-3.88 (m, 2H), 3.83-3.79 (m, 1H), 3.73-3.68 (m, 1H), 2.89-2.60 (m, 7H), 2.43-2.36 (m, 4H), 1.92-1.76 (m, 6H), 1.64-1.56 (m, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) (the major amide rotamer) δ 210.0, 175.7, 143.2, 128.8, 127.7, 125.4, 80.0, 61.9, 55.0, 54.5, 48.4, 45.0, 43.1, 30.0, 26.4; (the minor amide rotamer) 210.1, 175.7, 143.0, 128.6, 127.6, 125.3, 82.2, 61.0, 54.8, 54.0, 50.1, 47.5, 42.8, 30.3, 26.3; IR (KBr) 3284, 2958, 2873, 1718, 1608 , 1447 cm^{-1}; ESI-MS *m/z* 371 $(M + H)^+$.

4-Nitrobenzyl allyl(1-((1-(cyclopentanecarbonyl)-4-hydroxy-4-phenyl-pyrrolidin-3-yl)methyl)piperidin-4-yl)carbamate (23a). To a mixture of allylamine (1.0 mL, 13.20 mmol), **22a** (4.90 g, 13.20 mmol) in THF under an atmosphere of argon was added sodium triacetoxyborohydride (4.20 g, 19.80 mmol) and AcOH (0.82 mL, 13.20 mmol) respectively. After being stirred at room temperature for 16 h, The

reaction was quenched by carefully addition of concentrated hydrochloride acid to pH = 2. The solvent was removed in vacuo and the crude product was partition between CH_2Cl_2 and 2 N aqueous sodium hydroxide solution. The aqueous layer was extracted with CH_2Cl_2 for three times, and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to provide a syrup (3.75 g, 69%), which was used without further purification.

To an ice cooled stirring solution of the residue (3.75 g, 9.10 mmol) and triethylamine (2.7 mL, 18.3 mmol) in dry CH_2Cl_2 (25 mL) was added a solution of 4-nitrobenzyl carbonochloridate (2.18 g, 10.10 mmol) in dry CH_2Cl_2 (10 mL). After being stirred at room temperature for 6 h, the reaction mixture was quenched with water, the aqueous layer was extracted with CH_2Cl_2 for three times. The combined organic layers were washed with water and brine, respectively, dried over Na_2SO_4, and concentrated in vacuo to provide yellow syrup. The latter was treated with EtOAc and petroleum ether, and cooled to yield the product as a crystalline material. The material was collected by filtration and washed with cooled EtOAc to give **23a** (3.63 g, 67%). ^{1}H NMR ($CDCl_3$, 400 MHz) (the mixture of amide rotamers) δ 8.20 (d, *J* = 8.1 Hz, 2H), 7.52-7.48 (m, 3H), 7.42-7.36 (m, 2H), 7.32-7.27 (m, 2H), 5.84-5.75 (m, 1H), 5.22 (s, 2H), 5.16-5.11 (m, 2H), 4.03 (m, 1H), 3.88-3.66 (m, 6H), 3.05-2.99 (m, 1H), 2.82-2.63 (m, 3H), 2.53-2.42 (m, 2H), 2.18-2.11 (m, 2H), 1.86-1.25 (m, 13H); IR (KBr) 3364, 2957, 2870, 1717, 1637, 1447 cm^{-1}; HRMS Calcd. for $C_{33}H_{43}N_4O_6$ 591.3177 $(M + H)^+$, found 591.3168; Anal. ($C_{33}H_{42}N_4O_6$): C, H, N.

The following compounds (**23b-23e**) were prepared from **20** by a method

similar to that described for **23a**.

4-Nitrobenzyl allyl(1-((1-(cyclohexanecarbonyl)-4-hydroxy-4-phenyl-pyrrolidin-3-yl)methyl)piperidin-4-yl)carbamate (23b). ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 8.21 (d, *J* = 7.8 Hz, 2H), 7.52-7.46 (m, 3H), 7.42-7.24 (m, 4H), 5.82-5.77 (m, 1H), 5.22 (s, 2H), 5.16-5.10 (m, 2H), 3.92-3.73 (m, 7H), 3.58-3.38 (m, 1H), 3.09-2.60 (m, 5H), 2.60-2.08 (m, 2H), 1.92-1.43 (m, 15H); IR (KBr) 2933, 2855, 1701, 1638 cm^{-1}; ESI-MS *m/z* 605 $(M + H)^+$; HRMS Calcd. for $C_{34}H_{45}N_4O_6$ 605.3334 $(M + H)^+$, found 605.3333. Anal for $C_{34}H_{44}N_4O_6$: C, 67.53; H, 7.33; N, 9.26; Found: C, 68.12; H, 7.70; N, 8.90.

4-Nitrobenzyl allyl(1-((-1-benzoyl-4-hydroxy-4-phenylpyrrolidin-3-yl)-methyl)piperidin-4-yl)carbamate (23c). ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 8.24-8.19 (m, 2H), 7.61-7.21 (m, 12H), 5.82-5.77 (m, 1H), 5.22 (s, 2H), 5.17-5.12 (m, 2H), 4.14-3.61 (m, 8H), 3.07 (m, 1H), 3.00-2.40 (m, 4H), 2.15-2.05 (m, 1H), 1.79-1.57 (m, 5H); IR (KBr) 3375, 2943, 1701, 1627 cm^{-1}; ESI-MS *m/z* 599 $(M + H)^+$; HRMS Calcd. for $C_{34}H_{39}N_4O_6$ 599.2864 $(M + H)^+$; found 599.2879; Anal. ($C_{34}H_{38}N_4O_6 \cdot 0.5H_2O$): C, H, N.

4-Nitrobenzyl allyl(1-((4-hydroxy-1-(2-iodobenzoyl)-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl)carbamate (23d). ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 8.24-8.20 (m, 2H), 7.86-7.80 (m, 1H), 7.55-7.06 (m, 10H), 5.82-5.77 (m, 1H), 5.23 (s, 2H), 5.29-5.12 (m, 2H), 4.08-4.05 (m, 2H), 3.96-3.85 (m, 3H), 3.74-3.29 (m, 3H), 3.08 (m, 1H), 2.80-2.74 (m, 3H), 2.58-2.54 (m, 1H), 2.35-2.01 (m, 1H), 1.74-1.64 (m, 5H); IR (KBr) 3063, 2947, 1701, 1637, 1522 cm^{-1}; HRMS

Calcd. for $C_{34}H_{38}IN_4O_6$ 725.1831 $(M + H)^+$, found 725.1833. Anal. For $C_{34}H_{37}IN_4O_6$: C, 56.36; H, 5.15; N, 7.73; found: C, 57.02; H, 5.11; N, 7.60.

4-Nitrobenzyl allyl(1-((1-(1-naphthoyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)-methyl)piperidin-4-yl)carbamate (23e). ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 8.23-8.18 (m, 2H), 7.96-7.82 (m, 3H), 7.58-7.21 (m, 11H), 5.82-5.77 (m, 1H), 5.21 (s, 2H), 5.19-5.11 (m, 2H), 4.21-4.04 (m, 3H), 3.91-3.57 (m, 3H), 3.54-3.24 (m, 2H), 3.17-3.05 (m, 1H), 2.87-2.54 (m, 4H), 2.33-2.09 (m, 1H), 1.74-1.57 (m, 5H); IR (KBr) 3381, 2943, 1701, 1633, 1522 cm^{-1}; HRMS Calcd. for $C_{38}H_{41}N_4O_6$ 649.3021 $(M + H)^+$; found 649.3016; Anal. ($C_{38}H_{40}N_4O_6$): C, H, N.

1-Ethyl 4-methyl 2-hydroxy-3-oxo-2-phenylsuccinate (24). The mixture of methyl acrylate (18.0 mL, 200 mmol), ethyl 2-oxo-2-phenylacetate (17.80 g, 100 mmol) and DABCO (3.40 g, 30 mmol) was stirred at room temperature for 7 days, then CH_2Cl_2 (300 mL) was added. The resulting solution was washed respectively with 2 N HCl and saturated aqueous $NaHCO_3$, and then dried over Na_2SO_4. After the solution was concentrated in vacuo, the residue was distilled to give **24** (11.50 g, 43%) as an oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (dd, *J* = 8.7, 2.1 Hz, 2H), 7.39-7.36 (m, 3H), 6.39 (s, 1H), 5.38 (s, 1H), 4.29 (q, *J* = 7.2 Hz, 2H), 3.82 (s, 3H), 1.29 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 173.6, 167.1, 143.1, 138.0, 129.4, 128.6, 128.4, 127.0, 78.9, 62.8, 52.5, 14.2.

(3*R*,4*R*)-Methyl 4-hydroxy-5-oxo-4-phenyl-1-((*R*)-1-phenylethyl)pyrrolidine-3-carboxylate (26) and (3*R*,4*S*)-methyl 4-hydroxy-5-oxo-4-phenyl-1-((*R*)-1-phenylethyl)pyrrolidine-3-carboxylate (27). To a solution of **24** (10.0 g, 37.9 mmol)

in anhydrous methanol (100 mL) was added (*R*)-α-methylbenzylamine (5.3 mL, 42.0 mmol), the resulting solution was stirred at room temperature for 12 h. The solvent was removed in vauo to provide 1,4-addition products **25** an oil.

To a solution of **25** afforded above in dioxane (150 mL) was added trifluoroacetic acid (0.5 mL), the resulting mixture was heated to 100 ^{o}C for 6 h, then the solvent was removed in vacuo. Chromatography of the residue on silica gel (petroleum ether/EtOAc 10:1) to provide **26** (4.55 g, 35%) as a solid and **27** (6.44 g, 50%) as an oil. **26**: mp 176 ^{o}C; $[\alpha]_D^{17}$ –155.9 (*c* 0.96, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44-7.34 (m, 5H), 7.26-7.14 (m, 3H), 7.05-7.01 (m, 2H), 5.62 (q, *J* = 5.4 Hz, 1H), 3.58 (s, 1H), 3.59-3.51 (m, 2H), 3.39-3.30 (m, 4H), 1.65 (d, *J* = 5.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 173.5, 170.0, 139.2, 138.8, 129.1, 128.5, 128.3, 127.7, 125.1, 52.0, 51.7, 50.8, 40.8, 16.1; IR (KBr) 3331, 1749, 1689 cm^{-1}; ESI-MS *m/z* 340 $(M + H)^+$; Anal. ($C_{20}H_{21}NO_4$): C, H, N. **27**: $[\alpha]_D^{24}$ –71.1 (*c* 0.73, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.25 (m, 10H), 5.62 (q, *J* = 7.2 Hz, 1H), 4.46 (s, 1H), 3.67 (m, 1H), 3.41 (m, 1H), 3.34 (s, 3H), 3.15 (m, 1H), 1.70 (d, *J* = 7.2 Hz, 3H); IR (KBr) 3304, 1749, 1685 cm^{-1}; ESI-MS *m/z* 340 $(M + H)^+$.

(3*S*,4*R*)-4-Hydroxy-5-oxo-4-phenyl-1-((*R*)-1-phenylethyl)pyrrolidine-3-carboxylic acid (28). To an ice cooling solution of **26** (4.30 g, 12.7 mmol) in methanol (20 mL) was added 1 N NaOH (17.7 mL), the resulting solution was stirred at room temperature for 6 h. The solvent was removed in vacuo, then water was added to the residue. The resulting mixture was neutralized using 1 N HCl under ice cooling, and extracted with EtOAc for three times. The combined organic layers were washed with

water and brine, dried over Na_2SO_4, and concentrated in vacuo to provide **28** (4.12 g, 100%) as a white powder. mp 155 ^oC. $[\alpha]_D^{23}$ +108.9 (*c* 1.0, MeOH); ^1H NMR (DMSO-*d6*, 300 MHz) δ 12.20 (br s, 1H), 7.39-7.26 (m, 10H), 5.31 (q, *J* = 5.4 Hz, 1H), 3.50-3.43 (m, 1H), 3.35-3.29 (m, 2H), 1.56 (d, *J* = 5.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 172.9, 171.3, 143.7, 140.6, 129.1, 128.2, 128.0, 127.8, 127.7, 126.8, 80.1, 51.2, 49.9, 41.8, 17.6; IR (KBr) 3331, 1749, 1689 cm^{-1}; ESI-MS *m/z* 326 $(M + H)^+$; Anal. ($C_{19}H_{19}NO_4$): C, H, N.

((3*S*,4*R*)-4-((4-(Allylamino)piperidin-1-yl)methyl)-3-hydroxy-3-phenyl-pyrrolidin-1-yl)(cyclopentyl)methanone (29). Following the same procedure from the acid **13** to carbamate **23**, **29** was synthesized from **28**. mp 146 ^oC. $[\alpha]_D^{23}$ +2.5 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.48-7.36 (m, 2H), 7.34-7.30 (m, 2H), 7.26-7.23 (m, 1H), 5.89-5.82 (m, 1H), 5.16-5.04 (m, 2H), 3.81-3.61 (m, 4H), 3.22 (d, *J* = 5.6 Hz, 2H), 2.90-2.78 (m, 1H), 2.73-2.46 (m, 3H), 2.44-2.36 (m, 3H), 2.14-2.05 (m, 2H), 1.84-1.72 (m, 8H), 1.56-1.51 (m, 2H), 1.42-1.33 (m, 2H); ^{13}C NMR ($CDCl_3$, 300 MHz) (the major amide rotamer) δ 175.5, 143.9, 137.1, 128.6, 127.4, 125.3, 116.1, 82.3, 61.4, 56.1, 53.8, 53.5, 49.5, 48.1, 44.4, 43.0, 32.8, 30.0, 26.4; (the minor amide rotamer) 175.4, 144.4, 137.1, 128.5, 127.3, 125.5, 116.1, 80.3, 60.3, 54.0, 53.6, 49.5, 46.7, 44.4, 42.7, 32.9, 30.2, 26.3; IR (KBr) 3276, 2948, 2882, 1608 , 1454 cm^{-1}; ESI-MS *m/z* 412 $(M + H)^+$.

4-Nitrobenzyl allyl(1-(((3*S*,4*R*)-1-(cyclopentanecarbonyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl)carbamate (30). To an ice cooled stirring solution of **29** (3.75 g, 9.10 mmol) and triethylamine (2.7 mL, 18.3 mmol) in

dry CH_2Cl_2 (25 mL) was added a solution of 4-nitrobenzyl chloroformate (2.18 g, 10.10 mmol) in dry CH_2Cl_2 (10 mL). After being stirred at room temperature for 6 h, the reaction mixture was quenched with water, the aqueous layer was extracted with CH_2Cl_2 for three times. The combined organic layers were washed with water, brine respectively, dried over Na_2SO_4, and concentrated in vacuo to provide yellow syrup. The latter was treated with EtOAc and petroleum ether, and cooled to yield the product as a crystalline material. The material was collected by filtration and washed with cooled EtOAc to give **30** (3.63 g, 67%). $[\alpha]_D^{26}$ +11.1 (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) (the mixture of amide rotamers) δ 8.20 (d, *J* = 8.1 Hz, 2H), 7.52-7.48 (m, 3H), 7.42-7.36 (m, 2H), 7.32-7.27 (m, 2H), 5.84-5.75 (m, 1H), 5.22 (s, 2H), 5.16-5.11 (m, 2H), 4.03 (m, 1H), 3.88-3.66 (m, 6H), 3.05-2.99 (m, 1H), 2.82-2.63 (m, 3H), 2.53-2.42 (m, 2H), 2.18-2.11 (m, 2H), 1.86-1.25 (m, 13H); IR (KBr) 3364, 2957, 2870, 1717, 1637, 1447 cm^{-1}; HRMS Calcd. for $C_{33}H_{43}N_4O_6$ 591.3177 $(M + H)^+$.

The following compounds (**32-34**) were prepared from **29** by a method similar to that described for **30**.

Benzyl allyl(1-(((3*S*,4*R*)-1-(cyclopentanecarbonyl)-4-hydroxy-4-phenyl-pyrrolidin-3-yl)methyl)piperidin-4-yl)carbamate (32). $[\alpha]_D^{24}$ +10.8 (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.55-7.43 (m, 2H), 7.41-7.27 (m, 8H), 5.84-5.74 (m, 1H), 5.13 (br s, 2H), 5.08 (br s, 2H), 4.13-3.97 (m, 1H), 3.87-3.64 (m, 6H), 3.03-2.93 (m, 1H), 2.82-2.59 (m, 3H), 2.52-2.39 (m, 2H), 2.17-2.04 (m, 2H), 1.86-1.76 (m, 6H), 1.69-1.67 (m, 4H), 1.61-1.53 (m, 2H); ^{13}C NMR ($CDCl_3$, 300 MHz) (the major amide rotamer) δ 175.2, 155.9, 143.5, 136.7, 128.4,

127.7, 127.3, 125.0, 115.8, 82.1, 67.0, 61.3, 55.4, 55.2, 54.0, 53.4, 47.7, 44.2, 42.7, 30.1, 29.7, 26.1; (the minor amide rotamer) 175.0, 155.9, 143.5, 136.7, 135.4, 128.3, 127.8, 127.1, 125.1, 115.8, 80.3, 67.0, 60.0, 55.8, 54.7, 54.1, 53.4, 49.0, 46.3, 42.5, 30.3, 29.9, 26.0; IR (KBr) 3276, 2946, 2884, 1698 , 1623, 1608, 1454 cm^{-1}; HRMS Calcd. for $C_{33}H_{44}N_3O_4$ 546.3326 $(M + H)^+$, found 546.3347; Anal. ($C_{33}H_{43}N_3O_4$): C, H, N.

4-Methoxybenzyl allyl(1-(((3*S*,4*R*)-1-(cyclopentanecarbonyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl)carbamate (33). $[\alpha]_D^{24}$ +12.2 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.52-7.47 (m, 2H), 7.41-7.34 (m, 2H), 7.29-7.27 (m, 3H), 6.88 (d, *J* = 7.5 Hz, 2H), 5.80-5.77 (m, 1H), 5.10 (br m, 2H), 5.06 (br m, 2H), 4.04 (br s, 1H), 3.87 (s, 3H), 3.80-3.64 (m, 6H), 3.02-2.98 (m, 1H), 2.82-2.63 (m, 3H), 2.51-2.41 (m, 2H), 2.20-2.05 (m, 2H), 1.84-1.75 (m, 6H), 1.67-1.55 (m, 6H); ^{13}C NMR ($CDCl_3$, 300 MHz) (the major amide rotamer) δ 175.3, 159.4, 156.0, 143.6, 135.5, 129.6, 128.9, 127.3, 125.1, 113.8, 82.2, 66.9, 61.3, 60.0, 55.5, 55.2, 54.1, 47.7, 44.2, 42.8, 30.2, 29.8, 26.1; (the minor amide rotamer) 175.1, 159.4, 156.0, 143.6, 135.5, 128.9, 128.3, 127.2, 125.2, 115.9, 80.4, 66.9, 60.3, 55.8, 55.3, 54.1, 48.2, 46.3, 42.5, 30.2, 30.0, 26.0; IR (KBr) 3311, 2954, 1694, 1607, 1516 cm^{-1}; HRMS Calcd. for $C_{34}H_{46}N_3O_5$ 576.3432 $(M + H)^+$, found 576.3448; Anal. ($C_{34}H_{45}N_3O_5$): C, H, N.

(Benzo[*d*][1,3]dioxol-5-yl)methyl allyl(1-(((3*S*,4*R*)-1-(cyclopentanecarbonyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl)carbamate (34). $[\alpha]_D^{24}$ +12.1 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) (the mixture of amide

rotamers) δ 7.52-7.46 (m, 2H), 7.41-7.34 (m, 2H), 7.31-7.26 (m, 2H), 6.83-6.75 (m, 2H), 5.96 (m, 2H), 5.81-5.75 (m, 1H), 5.19 (br d, *J* = 7.2 Hz, 2H), 5.01 (br s, 2H), 4.07 (br m, 1H), 3.87-3.64 (m, 6H), 3.03-2.98 (m, 1H), 2.82-2.64 (m, 3H), 2.52-2.40 (m, 2H), 2.18-2.05 (m, 2H), 1.86-1.73 (m, 6H), 1.67-1.53 (m, 6H); ^{13}C NMR ($CDCl_3$, 300 MHz) (the major amide rotamer) δ 175.3, 155.9, 147.7, 147.4, 143.6, 135.5, 128.5, 127.4, 125.1, 121.8, 116.0, 108.7, 101.0, 82.2, 67.1, 61.3, 55.7, 55.4, 54.1, 47.7, 44.3, 42.8, 30.4, 29.7, 26.2; (the minor amide rotamer) 175.1, 155.9, 147.7, 147.4, 143.6, 135.5, 128.4, 127.3, 125.2, 121.8, 116.0, 80.5, 67.1, 61.0, 55.9, 54.9, 54.1, 47.7, 46.3, 42.6, 30.4, 29.8, 26.0; IR (KBr) 3345, 2947, 1694, 1622, 1606 cm^{-1}; HRMS Calcd. for $C_{34}H_{44}N_3O_6$ 590.3225 $(M + H)^+$, found 590.3230; Anal. ($C_{34}H_{45}N_3O_5$): C, H, N.

((3*R*,4*S*)-4-((4-((4-Methoxybenzyl)(allyl)amino)piperidin-1-yl)methyl)-3-hydroxy-3-phenylpyrrolidin-1-yl)(cyclopentyl)methanone (35). To a solution of **29** (100 mg, 0.24 mmol) and 4-methoxybenzylchloride (46 mg, 0.29 mmol) in dry DMF (2 mL) was added anhydrous K_2CO_3 (40 mg, 0.29 mmol) and tetrabutyl ammonium bromide (20 mg, 0.048 mmol) respectively. The resulting mixture was heated at 70 ^oC for 18 h, and then water was added. The mixture was extracted with EtOAc for three times, and the combined organic layers were washed with brine, dried over Na_2SO_4, concentrated in vacuo. Chromatography of the residue on silica gel eluting with 1:2 petroleum ether/EtOAc provided **35** (85 mg, 66%) as a white powder. $[\alpha]_D^{25}$ +11.2 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.52-7.47 (m, 2H), 7.40-7.34 (m, 2H), 7.31-7.21 (m, 3H), 6.83 (d, *J* = 8.7 Hz, 2H), 5.83-5.74 (m, 1H), 5.17-5.04 (m, 2H), 3.86-3.65 (m, 7H), 3.54 (s, 2H), 3.09 (d, *J* = 6.0 Hz, 2H),

3.02-2.98 (m, 1H), 2.79-2.37 (m, 6H), 2.06-1.95 (m, 2H), 1.86-1.71 (m, 8H), 1.67-1.53 (m, 4H); Anal. ($C_{33}H_{45}N_3O_3$): C, H, N.

((3*R*,4*S*)-4-((4-((4-Aminobenzyl)(allyl)amino)piperidin-1-yl)methyl)-3-hydroxy-3-phenylpyrrolidin-1-yl)(cyclopentyl)methanone (36). To an ice cooled stirring solution of **37** (40 mg, 0.65 mmol) in dry CH_2Cl_2 (1 mL) under an atmosphere of argon was added TFA (0.25 mL). The ice bath was removed, and the resulting mixture was stirred at rt over night. The solvents were removed in vacuo, and saturated aqueous $NaHCO_3$ solution was added. The mixture was extracted with EtOAc for three times, the combined organic layers were washed with water and brine, dried over Na_2SO_4, concentrated in vacuo. Chromatography of the residue on silica gel eluting with ethyl acetate afforded **36** (33 mg, 98%) as a yellow foam. $[\alpha]_D^{25}$ +8.8 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.44-7.39 (m, 2H), 7.33-7.28 (m, 2H), 7.23-7.20 (m, 1H), 7.03 (d, *J* = 7.8 Hz, 2H), 6.56 (d, *J* = 8.1 Hz, 2H), 5.76-5.74 (m, 1H), 5.12-4.99 (m, 2H), 3.76-3.56 (m, 4H), 3.45 (br s, 2H), 3.05 (br s, 2H), 2.95-2.91 (m, 1H), 2.75-2.30 (m, 7H), 2.02-1.87 (m, 2H), 1.78-1.70 (m, 8H), 1.66-1.53 (m, 4H). IR (KBr) 3349, 1624, 1517, 1447 cm^{-1}.

***tert*-Butyl 4-((allyl(1-(((3*S*,4*R*)-1-(cyclopentanecarbonyl)-4-hydroxy-4-phenylpyrrolidin-3-yl)methyl)piperidin-4-yl)amino)methyl)phenylcarbamate (37).** To an ice cooled stirring solution of *tert*-butyl 4-(hydroxymethyl)phenyl-carbamate (0.50 g, 2.24 mmol) in dry THF (10 mL) was added PPh_3 (0.76 g, 2.91 mmol) and carbon tetrabromide (0.97 g, 2.91 mmol) respectively. The ice bath was removed, and the resulting mixture was stirred at room temperature for 1 h. The

reaction was quenched with addition of saturated aqueous $Na_2S_2O_3$. Hexane and ether were added, and the organic layer was washed with saturated aqueous $CuSO_4$ solution and brine, dried over Na_2SO_4, concentrated in vacuo to provide *tert*-butyl 4-(bromomethyl)phenylcarbamate (0.64 g) as a brown oil, which was used without further purification.

To a solution of **29** (200 mg, 0.49 mmol) and *tert*-butyl 4-(bromomethyl)-phenylcarbamate (0.17 g, 0.58 mmol) in dry DMF (3 mL) was added anhydrous K_2CO_3 (0.08 g, 0.58 mmol) and tetrabutyl ammonium bromide (40 mg, 0.096 mmol) respectively. The resulting mixture was heated at 70 ^oC for 18 h, and then water was added. The mixture was extracted with EtOAc for three times, and the combined organic layers were washed with brine, dried over Na_2SO_4, concentrated in vacuo. Chromatography of the residue on silica gel eluting with 1:2 petroleum ether/EtOAc provided **36** (200 mg, 67%) as a white powder. $[\alpha]_D^{25}$ +8.9 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.45-7.40 (m, 2H), 7.33-7.26 (m, 2H), 7.22-7.13 (m, 5H), 6.42 (br s, 1H), 5.75-5.66 (m, 1H), 5.10-4.96 (m, 2H), 3.75-3.57 (m, 4H), 3.47 (br s, 2H), 3.01 (d, *J* = 5.4 Hz, 2H), 2.94-2.91 (m, 1H), 2.75-2.29 (m, 6H), 2.01-1.84 (m, 2H), 1.79-1.73 (m, 8H), 1.62-1.50 (m, 4H), 1.47 (s, 9H). IR (KBr) 3294, 1724, 1625, 1529, 1448 cm^{-1}.

***N*-Allyl-*N*-(1-(((3*S*,4*R*)-1-(cyclopentanecarbonyl)-4-hydroxy-4-phenyl-pyrrolidin-3-yl)methyl)piperidin-4-yl)-4-methylbenzenesulfonamide (38).** To a solution of **29** (0.10 g, 0.24 mmol) in CH_2Cl_2 (3 mL) was added 1 N NaOH (0.3 mL) and TsCl (49 mg, 0.25 mmol). The resulting mixture was stirred at room temperature

for 10 h, and water was added. The aqueous layer was extracted with CH_2Cl_2 for two times, and the combined organic layers were washed with brine, dried over Na_2SO_4, concentrated in vacuo. Chromatography of the residue on silica gel eluting with 1:2 petroleum ether/EtOAc afforded **38** (98 mg, 71%) as a white powder. $[\alpha]_D^{25}$ +8.9 (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ 7.68 (d, *J* = 8.4 Hz, 2H), 7.48-7.43 (m, 2H), 7.39-7.30 (m, 3H), 7.28-7.25 (m, 3H), 5.84-5.73 (m, 1H), 5.25 (br d, *J* = 16.5 Hz, 1H), 5.15 (d, *J* = 9.9 Hz, 1H), 3.85-3.59 (m, 7H), 2.95-2.85 (m, 1H), 2.80-2.54 (m, 3H), 2.48-2.41 (m, 2H), 2.37 (s, 3H), 2.14-1.97 (m, 2H), 1.83-1.45 (m, 13H); ^{13}C NMR ($CDCl_3$, 300 MHz) (the major amide rotamer) δ 175.3, 143.4, 143.3, 136.0, 129.6, 128.4, 127.3, 126.9, 125.0, 82.1, 61.3, 55.7, 55.4, 55.3, 54.1, 47.7, 46.0, 44.3, 42.8, 30.5, 29.8, 26.1; (the minor amide rotamer) 175.1, 143.5, 143.1, 138.3, 129.6, 128.3, 127.2, 126.9, 125.1, 80.3, 60.0, 55.6, 55.4, 54.8, 54.1, 49.0, 46.3, 44.3, 42.5, 30.8, 30.0, 26.0; IR (KBr) 3276, 2941, 1623, 1608, 1449 cm^{-1}; HRMS Calcd. for $C_{32}H_{43}N_3O_4S$ 566.3047 $(M + H)^+$, found 566.3058; Anal. ($C_{32}H_{43}N_3O_4S$): C, H, N.

1-Allyl-1-(1-(((3*S*,4*R*)-1-(cyclopentanecarbonyl)-4-hydroxy-4-phenyl-pyrrolidin-3-yl)methyl)piperidin-4-yl)-3-phenylurea (39). To a solution of **29** (0.10 g, 0.24 mmol) in CH_2Cl_2 (3 mL) was added 1-isocyanatobenzene (34 mg, 0.29 mmol). The resulting mixture was stirred at room temperature overnight, then the solvent was removed in vacuo. Chromatography of the residue on silica gel eluting with 1:2 petroleum ether/EtOAc afforded **39** (102 mg, 80%) as an white powder. $[\alpha]_D^{25}$ –28.4 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) (the mixture of amide rotamers) δ

7.57-7.46 (m, 1H), 7.41-7.23 (m, 8H), 7.08-6.99 (m, 1H), 6.53-6.50 (m, 1H), 5.96-5.85 (m, 1H), 5.46-5.34 (m, 2H), 4.42-4.26 (m, 1H), 3.86-3.59 (m, 6H), 3.05-2.96 (m, 1H), 2.83-2.62 (m, 3H), 2.54-2.39 (m, 2H), 2.30-2.12 (m, 2H), 1.87-1.62 (m, 10H), 1.61-1.45 (m, 2H); IR (KBr) 3248, 1734, 1630 cm^{-1}; HRMS Calcd. for $C_{32}H_{43}N_4O_3$ 531.3330 $(M + H)^+$, found 531.3350; Anal. ($C_{32}H_{42}N_4O_3 \cdot 0.25H_2O$): C, H, N.

Molecular Modeling. A structural model of human CCR5 has been obtained in our previous studies. [14,15] In brief, the initial model was constructed through homology modeling by using the crystal structure of bovine rhodopsin (Protein Data Bank entry 1F88) as a template. This task was carried out by using the InsightII software (version 2000.1). This initial model was minimized in a two-step procedure. Firstly, backbones of the seven trans-membrane helices were held fixed to release steric clashes among side chains. Next, alpha-carbon atoms on backbone were held fixed to maintain the helical structures while all of the other atoms were relaxed. The model was then subjected to further refinement through molecular dynamics (MD) simulation. The entire system was equilibrated at 300K for a total length of 400 *ps*. The final snapshot on the MD trajectory was minimized to provide a structural model of antagonist-bound CCR5 for the molecular docking studies in this work. All of the above minimizations and MD simulation was carried out by using the AMBER program (version 7.0).

Binding modes of compound **30** as well as one of the Merck's compounds (compound **6**) were predicted based on this CCR5 model through molecular docking. The Glide program [19] was applied for this purpose. For each compound, a total of 8

initial binding poses were selected by this program from an exhaustive sampling of conformations. Each binding pose was further subjected to a local Monte Carlo search for nearby minima with lower energies in torsional space. During the above procedures, steric energy of each pose was computed by the OPLS-AA force field in conjunction with a distance-dependent dielectric model. The fitness between each binding pose and CCR5 was evaluated by GlideScore, an empirical scoring function implemented in the Glide program. The best-scored binding pose of each compound was used in our analysis.

HIV-1 Replication in PBMC Cultures. All HIV-1 strains were obtained from NIH AIDS Research and Reference Reagent Program except for HNz2, which was obtained from Wuhan University, China. PBMCs was obtained from healthy volunteers were isolated by Ficoll- Hypaque gradient density centrifugation and stimulated with 5 μg/ml phytohemagglutinin (PHA) in RPMI1640 supplemented with 20% FBS, 100 U/ml recombinant human IL-2, and antibiotics for 3 days. Stocks of viruses were prepared by limited passage through phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC).

Human PBMC were stimulated *in vitro* by PHA and IL-2 for 2 days. Cells were resuspended in medium and 100 μL cells suspend were seeded into 96-well plate (10^5/well). 50 μL of medium containing the appropriate concentration of the compound **30** and 50 μl of virus solution in the final concentration of 100$TCID_{50}$/well were added and incubated at 37 ^oC and 5% CO_2 for 1 day. The cells were washed three times by using medium to remove residual virus and cultured in fresh medium with

different concentration of **30** for 6 days. HIV-1 replication was quantitated by measurement of extracellular p24 antigen by ELISA.

Pharmacokinetics Evaluation. Groups of 12 h fasted rats were dosed with the compound **30** by i.v. injection at 10 mg/kg (n = 8) or also by oral gavage at 10 mg/kg (n = 8). For the dog studies, fasted beagle dogs were dosed at 3 mg/kg (n = 8) similarly by i.v. injection or oral gavage. Serial blood samples (0.25 mL for rats, 0.5 mL for dogs) were collected predose and between 5 min and 24 h postdose from the retro-orbital plexus of rats or femoral small saphenous vein of dogs into heparinized tubes. Blood was processed to plasma and stored at –20 °C until pharmacokinetic analysis. The plasma samples were gotten rid of protein before injected into the LC/MS/MS system (ThermoFinnigan, San Jose, CA, USA.) and plasma concentration was determined by this system. Pharmacokinetic parameters were calculated by using the Topfit 2.0 software. oral bioavailability was calculated from the relative AUC ratios of the i.v. and oral gavage total concentration equivalents versus time profiles.