New Bellenamine Homologs Inhibiting Human Immunodeficiency Virus Type 1 Infectivity

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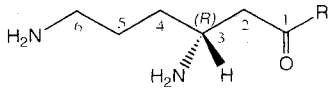
Bellenamine, (*R*)-3,6-diamino-*N*-(aminomethyl)hexanamide which was produced by *Streptomyces nashvillensis* MD743-GF4[1~4] showed a weak antibacterial activity, slightly enhancement of the immune response and potent antiviral activity against human immunodeficiency virus type 1 (HIV-1).[5] The structure has a unique open-chain aldoaminal moiety confirmed by the total synthesis[6] and it is unstable by heating in aqueous solution.[7] In our studies on chemical modification of bellenamine, two stable homologs, (*R*)-3,6-diamino-*N*-(2-aminoethyl)hexanamide (**1**) and (*R*)-3,6-diamino-*N*-(3-aminopropyl)hexanamide (**2**), which inhibit HIV-1 infectivity have been synthesized. In this report synthesis and anti-HIV-1 activity of two new homologs of bellenamine are presented.

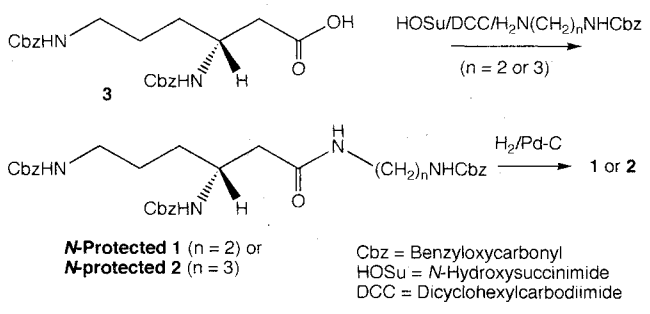


Bellenamine R = $HNCH_2NH_2$

1 R = $HNCH_2\overset{1'}{C}H_2\overset{2'}{N}H_2$

2 R = $HNCH_2\overset{1'}{C}H_2\overset{2'}{C}H_2\overset{3'}{N}H_2$

Scheme 1.



Two homologs **1** and **2** were synthesized by coupling of (*R*)-3,6-bis(benzyloxycarbonylamino)hexanoic acid (3,6-bis(*N*-benzyloxycarbonyl)-D-β-lysine,[6] **3**) with mono-*N*-benzyloxycarbonyl-α,ω-alkanediamines, followed by deprotection in good yields (Scheme 1). Selective mono-*N*-benzyloxycarbonyl protection of 1,2-ethanediamine or 1,3-propanediamine was carried out in aqueous solution at pH 3.5~4.5 by the method of ATWELL and DENNY.[8]

Compounds **1** and **2** (EC_{50} 5.0 and 0.3 μg/ml, respectively) showed potent anti-HIV activity as well as bellenamine (EC_{50} 0.2 μg/ml) using MT-4 cells infected with HTLV-III_B strain of HIV-1. Although bellenamine and two homologs were slightly cytotoxic to MT-4 cells at concentrations of 100 μg/ml, **1** and **2** (IC_{50} 0.012 and 0.028 μg/ml) exhibited stronger cytotoxic activity than bellenamine (IC_{50} 0.36 μg/ml) towards mouse leukemia P388 cells. Antimicrobial activity (MIC on 0.5% peptone agar) of **1** and **2** are as follows: *Staphylococcus aureus* Smith 6.25 and 50, *Bacillus anthrasis*, 100 and 200, *B. subtilis* NRRL B-558, 25 and 100, *B. subtilis* PCI219 25 and 100, *Proteus mirabilis* IFM OM-9 25 and 50 μg/ml, respectively. Single intravenous injection of 250 mg/kg of **1** or **2** did not cause death in female ICR mice (4-weeks old).

Compounds **1** and **2** which were easily synthesized from the *N*-protected D-β-lysine, are more stable than bellenamine in aqueous solution, and have similar biological properties to bellenamine. From the results of some chemical modifications,[1,5] it is concluded that the polyamine-like structure is essential for biological activities of bellenamine.

Experimental

General

Optical rotations were recorded on a JASCO DIP-370 digital polarimeter using 10-cm cell. IR spectra were taken on a Shimadzu FTIR-8100 spectrophotometer. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a JEOL JNM-GSX400 spectrometer. SI-MS were measured on a Hitachi M-80B spectrometer. HR-MS were measured on a JEOL JMS-SX102 mass spectrometer in a FAB mode. *N*-(Benzyloxycarbonyl)-1,2-ethanediamine and *N*-(benzyloxycarbonyl)-1,3-propanediamine were synthesized by the method of ATWELL and DENNY.[8]

Biological Evaluation

Inhibitory activities of HIV-1 infection were determined using MT-4 cells infected with HTLV-III_B (or LAI) strain of HIV-1 according to the method of HOSHINO *et al.*[5,9,10] Cytotoxic activities towards mouse leukemia P388 cells were assayed by the method described in a reference.[11] Antimicrobial activities (MIC) were determined by serial two-fold agar dilution method with 0.5% peptone agar at 37°C for 18 hours.

(*R*)-3,6-Diamino-*N*-(2-aminoethyl)hexanamide (**1**)

To a solution of bis(*N*-benzyloxycarbonyl)-D-β-lysine[6] (**3**, 401 mg, 0.97 mmol) in dioxane (12 ml), *N*-hydroxysuccinimide (124 mg, 1.08 mmol) and dicyclohexylcarbodiimide (225 mg, 1.09 mmol) were added. After being stirred for 21 hours at room temperature, dicyclohexylurea was removed by filtration. To the filtrate a suspension of *N*-(benzyloxycarbonyl)-1,2-ethanediamine (284 mg, 1.46 mmol) in dioxane (6 ml) and an aqueous solution (10 ml) of $NaHCO_3$ (125 mg, 1.49 mmol) were added. After being stirred for 3 hours at room temperature, the precipitate (565 mg) was obtained by filtration and washing with each 5 ml of water and dioxane. The precipitate was purified by column chromatography on silica gel (56 g, C-300, Wako Pure Chemical Industries) developed with $CHCl_3$-MeOH (20:1) to give (*R*)-3,6-bis(benzyloxycarbonylamino)-*N*-(2-benzyloxycarbonylaminoethyl)hexanamide (*N*-protected-**1**) (530 mg, 92.8%) as a colorless solid. SI-MS: *m/z* 591 (MH^+); $[\alpha]_D^{24}$ +3.2° (*c* 1.08, DMSO). *Anal.* Calcd for $C_{32}H_{38}N_4O_7$: C 65.07, H 6.48, N 9.49, O 18.96. Found: C 65.11, H 6.47, N 9.29, O 19.29.

The *N*-protected-**1** (141 mg, 0.24 mmol) in a mixture of MeOH (18 ml) and water (2 ml) was hydrogenated with 10% Pd-carbon (72 mg) as a catalyst for 3 hours under hydrogen stream. After removal of the catalyst by filtration, the reaction product was purified by column chromatography on Amberlite CG-50 (NH_4^+, 5 ml). After being washed with water (20 ml), the column was eluted by stepwise elution with 20 ml each of 0.5%~4.0% aqueous ammonia (0.5%-intervals). The fractions eluted with 2.5%~3.5% aqueous ammonia were collected and concentrated to yield **1** (44.5 mg, 99.2%) as a colorless paste. HR-MS (FAB, positive) Found: *m/z* 189.1729 (MH^+). Calcd for $C_8H_{21}N_4O$: MH, 189.1715. $[\alpha]_D^{24}$ −6.0° (*c* 1.2, H_2O); IR (KBr) cm^{-1} 3300, 2950, 1640, 1570, 1480, 1440, 1390, 1320, 1240, 1160, 1050 and 820; ^1H NMR (400 MHz, D_2O at pD 4) δ 1.80 (4H, m, 4-H_2 and 5-H_2), 2.67 (1H, dd, J=16.4 and 8.5 Hz, 2-H), 2.80 (1H, dd, J=16.4 and 4.6 Hz, 2-H), 3.07 (2H, m, 6-H_2), 3.18 (2H, t, J=5.9 Hz, 2′-H_2), 3.55 (2H, t, J=5.9 Hz, 1′-H_2) and 3.71 (1H, m, 3-H); ^{13}C NMR (100 MHz, D_2O at pD 4) δ 23.8 (C-5), 30.0 (C-4), 37.3 (C-2), 37.7 (C-1′), 39.8 (C-6), 40.0 (C-2′), 49.1 (C-3) and 173.6 (C-1).

(*R*)-3,6-Diamino-*N*-(3-aminopropyl)hexanamide (**2**)

By the similar method described in the synthesis of **1**, (*R*)-3,6-bis(benzyloxycarbonylamino)-*N*-(3-benzyloxycarbonylaminopropyl)hexanamide (*N*-protected-**2**) (183 mg, 90.9%) was prepared from **3** (135 mg, 0.33 mmol) and *N*-(benzyloxycarbonyl)-1,3-propanediamine (103 mg, 0.50 mmol), as a colorless solid. SI-MS: *m/z* 605 (MH^+); $[\alpha]_D^{24}$ +1.3° (*c* 1.06, DMSO). *Anal.* Calcd for $C_{33}H_{40}N_4O_7$: C 65.55, H 6.67, N 9.27, O 18.52. Found: C 65.35, H 6.80, N 9.11, O 18.47.

By hydrogenolysis of *N*-protected-**2** (144 mg, 0.24 mmol), compound **2** (48.2 mg, 100%) was obtained as a colorless paste. HR-MS (FAB, positive) Found: *m/z* 203.1880 (MH^+). Calcd for $C_9H_{23}N_4O$: MH, 203.1872. $[\alpha]_D^{24}$ −3.5° (*c* 0.9, H_2O); IR (KBr) cm^{-1} 3300, 2950, 1640, 1560, 1480, 1440, 1390, 1320, 1210, 1150, 1050 and 820; ^1H NMR (400 MHz, D_2O at pD 4) δ 1.79 (4H, m, 4-H_2 and 5-H_2), 1.92 (2H, tt, J=7.3 and 7.3 Hz, 2′-H_2), 2.65 (1H, dd, J=16.4 and 8.1 Hz, 2-H), 2.76 (1H, dd, J=16.4 and 5.1 Hz, 2-H), 3.05 (2H, t, J=7.3 Hz, 3′-H_2), 3.07 (2H, m, 6-H_2), 3.33 (2H, m, 1′-H_2) and 3.68 (1H, m, 3-H); ^{13}C NMR (100 MHz, D_2O at pD 4) δ 23.7 (C-5), 27.4 (C-2′), 29.9 (C-4), 37.0 (C-1′), 37.4 (C-2), 37.9 (C-3′), 39.7 (C-6), 49.2 (C-3) and 172.8 (C-1).

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