TOTAL SYNTHESES OF BELLENAMINE† AND ITS ISOMERS

YOKO IKEDA, DAISHIRO IKEDA and SHINICHI KONDO*

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

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The total synthesis of bellenamine was achieved by a modified CURTIUS procedure starting with D-β-lysine. Bis(*N*-benzyloxycarbonyl)-D-β-lysylglycine was converted to tris(*N*-benzyloxycarbonyl)bellenamine which was catalytically hydrogenated to yield bellenamine. D-β-Lysine was synthesized from D-ornithine by the ARNDT-EISTERT homologation sequence. Three isomers, L-β-lysyl, D- and L-lysyl congeners synthesized by a similar method, showed no antibacterial activities.

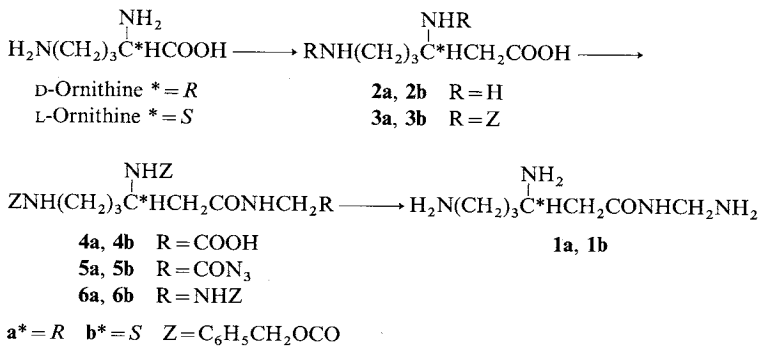
A water-soluble, basic antibiotic, bellenamine[1] produced by *Streptomyces nashvillensis* MD743-GF4 inhibits weakly growth of Gram-positive bacteria and strongly infection of human immunodeficiency virus,[2] and enhances both delayed-type hypersensitivity to sheep red blood cells and antibody formation in the mouse spleen. It has a unique open-chain aldoaminal structure, and D-β-lysine has been first found as the natural amino acid. The absolute structure, (*R*)-*N*-aminomethyl-3,6-diaminohexanamide was confirmed by the total synthesis which was preliminary reported.[1] In this report, the syntheses of bellenamine and its three isomers with the full experimental details are presented.

Total Synthesis of Bellenamine

The starting compound for the total synthesis of bellenamine (**1a**), D-β-lysine (**2a**) has been synthesized from D-ornithine by the method of L-β-lysine synthesis of VAN TAMELEN and SMISSMAN.[3,4] L-β-Lysine (isolysine, **2b**) which was found as a component in the streptothricin and viomycin antibiotics was synthesized by the ARNDT-EISTERT homologation sequence starting with L-ornithine.

As shown in Scheme 1, the total synthesis of **1a** was achieved by starting with bis(*N*-benzyloxycarbonyl)-D-β-lysine (**3a**) which was obtained by the amino protection of **2a** with benzyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate. Compound **3a** was coupled with glycine by the active ester method using *N*-hydroxy-5-norbornene-2,3-dicarboximide[5] and *N*,*N*′-dicyclohexylcarbodiimide to yield bis(*N*-benzyloxy-

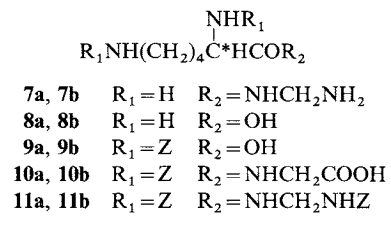
Scheme 1.



† Bellenamine was formerly called D-β-lysylmethanediamine.[1]

carbonyl)-D-β-lysylglycine (**4a**). Acylamine formation from **4a** through the azide **5a** by a modified CURTIUS procedure[6] yielded tris(*N*-benzyloxycarbonyl)bellenamine (**6a**). Removal of the amino protective groups of **6a** by catalytic hydrogenation, followed by column chromatography on Amberlite CG-50 resin gave **1a**.

Fig. 1. The lysyl congeners.



Synthesis of Three Isomers

L-β-Lysylmethanediamine (**1b**), D- and L-lysylmethanediamines (**7a** and **7b**, Fig. 1) were synthesized from L-β-lysine (**2b**), D- and L-lysines (**8a** and **8b**), respectively, by the similar methodology as described above. They showed no antibacterial activities at concentrations of 100 μg/ml.

Experimental

General

MP's were determined in capillary tubes with a Yamato MP-21 apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra in KBr tablets were recorded on a Hitachi 260-10 spectrometer. MS were taken on Hitachi RMU-6M (EI) and M80H (SI) spectrometers. ^{1}H and ^{13}C NMR spectra (400 and 100 MHz) were measured on a JEOL GX-400 spectrometer.

D-β-Lysine (**2a**)

Compound **2a** was synthesized from D-ornithine by a method similar to L-β-lysine synthesis.[4] MP 168~172°C (dec); $[\alpha]_D^{22}$ −24° (*c* 0.8, 1 N HCl); ^{1}H NMR (D_2O, pD 4.0) δ 1.78 (4H, m, 4-H_2 and 5-H_2), 2.48 (1H, dd, J=8, 17 Hz, 2-H), 2.61 (1H, dd, J=5.6, 17 Hz, 2-H), 3.06 (2H, br t, J=7 Hz, 6-H_2), 3.56 (1H, m, 3-H); ^{13}C NMR (D_2O, pD 4.0) δ 178.6 (C-1), 49.7 (C-3), 39.8 (C-6), 39.0 (C-2), 30.0 (C-4), 23.8 (C-5).

Anal Calcd for $C_6H_{14}N_2O_2$: C 49.30, H 9.65, N 19.17.
Found: C 48.75, H 9.52, N 19.29.

Bis(*N*-benzyloxycarbonyl)-D-β-lysine (**3a**)

To a solution of **2a** (68.7 mg, 0.47 mmol) in 50% aqueous MeOH (4 ml), benzyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate (399.5 mg, 1.46 mmol, Kokusan Chemical Works) and triethylamine (0.135 ml, 0.96 mmol) were added. After being stirred overnight at room temperature, the mixture was concentrated to dryness and the residue was washed with aqueous MeOH to yield **3a** (150.0 mg, 77% yield) as a colorless powder. MP 148~151°C (dec); $[\alpha]_D^{19}$ +8.0° (*c* 1.0, dioxane); IR cm^{-1} 3370, 3320, 1730, 1700, 1655, 1555, 1310, 1290, 1280, 700.

Bis(*N*-benzyloxycarbonyl)-D-β-lysylglycine (**4a**)

To a solution of **3a** (120.9 mg, 0.29 mmol) in dioxane (5 ml), *N*-hydroxy-5-norbornene-2,3-dicarboximide (52.9 mg, 0.30 mmol, Peptide Institute, Inc.) and *N*,*N*'-dicyclohexylcarbodiimide (60.9 mg, 0.30 mmol) were added.[5] After being stirred overnight at room temperature, dicyclohexylurea was removed by filtration. The filtrate was added to an aqueous solution (4 ml) of glycine (34.4 mg, 0.46 mmol) and $NaHCO_3$ (37.5 mg, 0.45 mmol), and stirred for 2 hours at room temperature. Removal of dioxane by evaporation gave the precipitate, which was washed with 0.01 N HCl to yield **4a** (127.9 mg, 93% yield) as a colorless powder. MP 166~168°C (dec); $[\alpha]_D^{27}$ +9.2° (*c* 1.0, dioxane); IR cm^{-1} 3350, 2970, 1740 (sh), 1710, 1660, 1560, 1310, 1290, 1230, 720; ^{1}H NMR (dioxane-d_8) δ 1.51 (4H, br, 4-H_2 and 5-H_2), 2.34 (1H, dd, J=7.7, 15.4 Hz, 2-H), 2.41 (1H, dd, J=6, 15.4 Hz, 2-H), 3.08 (2H, m, 6-H_2), 3.84 (1H, br, 3H), 3.87

(2H, d, J=6.4 Hz, 1'-H_2), 5.01 (4H, s, benzyl CH_2), 6.09 (1H, br, NH), 6.29 (1H, br d, J=9 Hz, NH), 7.08 (1H, br t, NH), 7.27 (2H, m, aromatic H), 7.29 (4H, s, aromatic H), 7.31 (4H, s, aromatic H); ^{13}C NMR (dioxane-d_8) δ 171.7 (s), 171.2 (s), 156.8 (s), 156.5 (s), 138.4 (s), 138.3 (s), 128.9 (d), 128.6 (d), 128.5 (d), 128.3 (d), 66.7 (t), 66.4 (t), 49.4 (d), 41.2 (t), 41.2 (t), 41.0 (t), 32.1 (t), 27.4 (t); EI-MS m/z 471 (M^+), SI-MS m/z 472 $(M+H)^+$.

Bis(*N*-benzyloxycarbonyl)-D-β-lysylglycyl Azide (**5a**)

Following the method of OVERMAN *et al*,[6] *N*,*N*-diisopropylethylamine (0.21 ml, 1.18 mmol) and ethyl chloroformate (0.085 ml, 0.89 mmol) in THF (1.5 ml) were added to a solution of **4a** (279.2 mg, 0.59 mmol) in THF (3.5 ml) at 0°C in argon atmosphere, and the mixture was stirred for 4 hours. After addition of sodium azide (77 mg, 1.18 mmol) in H_2O (1 ml), the mixture was stirred for an additional 2 hours at 0°C and then poured into ice water (50 ml) to yield a precipitate. The precipitate was dissolved in methylene chloride (50 ml) and the solution was filtered to remove the insoluble matter. Evaporation of the filtrate gave **5a** (192.2 mg, 65% yield), IR ν_{azide} 2130 cm^{-1}.

Tris(*N*-benzyloxycarbonyl)bellenamine (**6a**)

From **5a**: A solution of **5a** (189.0 mg, 0.38 mmol) in dioxane (2 ml) was added over 30 minutes to a refluxing mixture of benzyl alcohol (0.13 ml, 1.26 mmol), 4-*tert*-butylcatechol (4 mg), dry toluene (5 ml) and pyridine (2 drops).[6] Reflux was continued overnight and the reaction mixture was concentrated to afford **6a** (47.5 mg, 22% yield from the azide) which was washed with MeOH (10 ml). It was identical with the **6a** derived from the natural **1a** in all respects.

From natural **1a**: Benzyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate (71 mg, 0.26 mmol) in MeOH (1 ml) and triethylamine (0.039 ml, 0.28 mmol) were added to a solution of **1a** (10.6 mg, 0.061 mmol) in 50% aqueous MeOH (1 ml). After being stirred for 4 hours at room temperature, the precipitate was filtered, and washed with H_2O (2 ml) and MeOH (2 ml) to give **6a** (27.7 mg, 79% yield) as a colorless powder, MP 216~219°C (dec); $[\alpha]_D^{27}$ +4° (c 0.1, dioxane); IR cm^{-1} 3320, 1700, 1660, 1550, 1540, 1280, 1260, 710; ^{1}H NMR (DMSO-d_6) δ 1.31 (2H, m, 5-H_2), 1.39 (2H, m, 4-H_2), 2.17 (1H, dd, J=14, 8 Hz, 2-H), 2.23 (1H, dd, J=14, 6.4 Hz, 2-H), 2.93 (2H, br, 6-H_2), 3.77 (1H, br, 3-H), 4.33 (2H, br t, 1'-H_2), 4.98 (4H, s, benzyl CH_2), 5.01 (2H, s, benzyl CH_2), 7.07 (1H, d, J=10 Hz, 3-NH), 7.19 (1H, t, J=5.5 Hz, 6-NH), 7.32 (15H, aromatic H), 7.81 (1H, t, J=6 Hz, NH), 8.39 (1H, t, J=6 Hz, NH); ^{13}C NMR (DMSO-d_6) δ 172.2 (s, C-1), 156.2 (s, CO), 156.0 (s, CO), 155.5 (s, CO), 137.2 (s, aromatic C×2), 137.0 (s, aromatic C), 128.3 (d, aromatic CH×9), 127.7 (d, aromatic CH×6), 65.3 (t, benzyl CH_2), 65.1 (t, benzyl CH_2×2), 48.0 (d, C-3), 45.3 (t, C-1'), 40.9 (t, C-6), 40.3 (t, C-2), 31.5 (t, C-4), 26.1 (t, C-5); EI-MS m/z 576 (M^+).

Anal Calcd for $C_{31}H_{36}N_4O_7$: C 64.57, H 6.29, N 9.72.
Found: C 64.30, H 6.17, N 9.91.

Bellenamine (**1a**)

A solution of **5a** (6.8 mg, 0.012 mmol) in 50% aqueous acetic acid (1 ml) was hydrogenated with 10% Pd-carbon (11.2 mg) overnight at room temperature in a Parr apparatus (3.2 kg/cm^2). The reaction mixture was filtered and concentrated to give the crude **1a** (2.7 mg). Purification by column chromatography on Amberlite CG-50 (NH_4^+, 0.5 ml) eluted with 1.5% aqueous ammonia afforded pure **1a** (1.4 mg, 68% yield) as a colorless hygroscopic syrup. It was identical with the natural **1a** in all respects including antibacterial activity.[1]

L-β-Lysylmethanediamine (**1b**)

The enantiomer **1b** was similarly prepared from **2b** which was synthesized from L-ornithine by the method of VAN TAMELEN and SMISSMAN.[4]

3b[7]: 68% yield from **2b**. **4b**: 93% yield from **3b**.

6b: colorless powder, 73% yield from **4b**. MP 217~221°C (dec); $[\alpha]_D^{24}$ −5° (c 0.1, dioxane).

Anal Calcd for $C_{31}H_{36}N_4O_7$: C 64.57, H 6.29, N 9.72.
Found: C 64.34, H 6.27, N 9.98.

1b: colorless syrup, 55% yield from **6b**. $[\alpha]_D^{22}$ +19° (c 0.1, 0.08 N HCl); ^{1}H NMR (D_2O, pD 5.8) δ 1.78 (4H, m, 4-H_2 and 5-H_2), 2.70 (1H, dd, J=8.3, 16.8 Hz, 2-H), 2.84 (1H, dd, J=4.6, 16.8 Hz, 2-H), 3.05

(2H, br, 6-H_2), 3.73 (1H, br, 3-H), 4.50 (2H, s, 1'-H_2); ^{13}C NMR δ 173.9 (s, C-1), 48.8 (d, C-3), 46.0 (t, C-1'), 39.7 (t, C-6), 36.9 (t, C-2), 29.8 (t, C-4), 23.7 (t, C-5); SI-MS m/z 175 $(M+H)^+$.

D-Lysylmethanediamine (**7a**)

D-Lysyl congener **7a** was synthesized from **8a** monohydrochloride (Peptide Institute Inc.) by the similar procedure.

9a: 55% yield from **8a**. **10a**: 98% yield from **9a**.

11a: 29% yield from **10a**. MP 157~165°C (dec).

Anal Calcd for $C_{31}H_{36}N_4O_7$: C 64.57, H 6.29, N 9.72.
Found: C 64.40, H 6.21, N 10.44.

7a: colorless syrup, 44% yield from **11a**. $[\alpha]_D^{20}$ −39° (*c* 0.37, 0.06 N HCl); SI-MS m/z 175 $(M+H)^+$. The spectral data except optical rotation were identical with those of **7b**.

L-Lysylmethanediamine (**7b**)

L-Lysyl congener **7b** was prepared from **9b** (Peptide Institute, Inc.).

10b: colorless powder, 98% yield from **9b**. MP 144~152°C (dec); $[\alpha]_D^{27}$ −10° (*c* 1.0, dioxane); IR cm^{-1} 3320, 2940, 1720, 1700, 1680, 1660, 1550, 1360, 1280, 700.

11b: colorless powder, 9% yield from **10b**. MP 191~194°C (dec); $[\alpha]_D^{27}$ −5.5° (*c* 0.2, dioxane); IR cm^{-1} 3310, 1690, 1660, 1550 (sh), 1540, 1270, 1240, 700; ^{1}H NMR (DMSO-d_6) δ 1.18 (2H, m, 4-H_2), 1.34 (2H, m, 5-H_2), 1.50 (2H, m, 3-H_2), 2.94 (2H, dt, J=6, 6.5 Hz, 6-H_2) 3.93 (1H, br, 2-H), 4.35 (2H, m, 1'-H_2), 4.98 (2H, s, benzyl CH_2), 4.99 (2H, s, benzyl CH_2), 5.03 (2H, s, benzyl CH_2), 7.21 (1H, t, J=6 Hz, 6-NH), 7.29 (1H, NH), 7.33 (15H, aromatic H), 7.86 (1H, t, J=6.2 Hz, NH), 8.40 (1H, br t, NH); ^{13}C NMR (DMSO-d_6) δ 172.2 (s, C-1), 156.2 (s, CO), 156.0 (s, CO), 155.9 (s, CO), 137.2 (s, aromatic C), 137.0 (s, aromatic C × 2), 128.3 (d, aromatic CH × 9), 127.7 (d, aromatic CH × 6), 65.3 (t, benzyl CH_2 × 2), 65.1 (t, benzyl CH_2), 54.4 (d, C-2), 45.5 (t, C-1'), 40.1 (t, C-6), 31.6 (t, C-5), 29.0 (t, C-3), 22.7 (t, C-4): EI-MS m/z 576 (M^+).

Anal Calcd for $C_{31}H_{36}N_4O_7$: C 64.57, H 6.29, N 9.73.
Found: C 64.60, H 6.30, N 10.01.

7b: colorless syrup, 77% yield from **11b**. $[\alpha]_D^{21}$ +39° (*c* 0.37, 0.06 N HCl); ^{1}H NMR (D_2O, pD 4.4) δ 1.51 (2H, m, 4-H_2), 1.74 (2H, m, 5-H_2), 1.98 (2H, m, 3-H_2), 3.03 (2H, t, J=12 Hz, 6-H_2), 4.13 (1H, t, J=10 Hz, 2-H), 4.59 (2H, ABq, J=13 Hz, 1'-H_2); ^{13}C NMR (D_2O, pD 4.4) δ 172.0 (s, C-1), 53.9 (d, C-2), 46.3 (t, C-1'), 40.0 (t, C-6), 31.0 (t, C-5), 27.3 (t, C-3), 22.2 (t, C-4); SI-MS m/z 175 $(M+H)^+$.

References

1) IKEDA, Y.; S. KONDO, D. IKEDA, K. YOKOSE, H. FURUTANI, T. IKEDA, M. HAMADA, M. ISHIZUKA, T. TAKEUCHI & H. UMEZAWA: D-β-Lysylmethanediamine, a new biogenetic amine produced by a *Streptomyces*. J. Antibiotics 39: 476~478, 1986
2) IKEDA, R.; H. HOSHINO, Y. IKEDA, S. KONDO & T. TAKEUCHI: Activity and mechanism of bellenamine, a new anti-HIV agent. Abstracts of 40th Annual Meeting of the Society of Japanese Virologists, p. 178, Kobe, Oct. 30, 1992
3) VAN TAMELEN, E. E. & E. E. SMISSMAN: Streptolin. The structure and synthesis of isolysine. J. Am. Chem. Soc. 74: 3713~3714, 1952
4) VAN TAMELEN, E. E. & E. E. SMISSMAN: Streptolin. The structure and synthesis of isolysine. J. Am. Chem. Soc. 75: 2031~2035, 1953
5) FUJINO, M.; S. KOBAYASHI, M. OBAYASHI, T. FUKUDA, S. SHINAGAWA & O. NISHIMURA: The use of *N*-hydroxy-5-norbornene-2,3-dicarboximide active esters in peptide synthesis. Chem. Pharm. Bull. 22: 1857~1863, 1974
6) OVERMAN, L. E.; G. F. TAYLOR, C. B. PETTY & P. J. JESSUP: *trans*-1-*N*-Acylamino-1,3-dienes: Preparation from dienoic acids. J. Org. Chem. 43: 2164~2167, 1978
7) WAKAMIYA, T.; H. URATANI, T. TESHIMA & T. SHIBA: Synthesis of acyl derivatives of β-lysine for peptide synthesis. Bull. Chem. Soc. Jpn. 48: 2401~2402, 1975