CHEMICAL MODIFICATION OF FORTIMICINS
IV. PREPARATION OF 4,2′-DI-N-SUBSTITUTED FORTIMICIN B DERIVATIVES*

MORIYUKI SATO, KENICHI MOCHIDA, SHIGEO YOSHIIE, YASUKI MORI and KUNIKATSU SHIRAHATA**

Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.
Asahimachi, Machida, Tokyo, Japan

KOZO KITAURA

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.
Nagaizumi, Shizuoka, Japan

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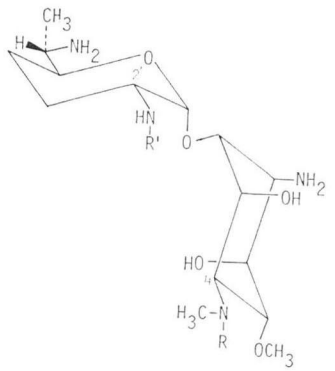
2-Aminoethyl, glycyl, (*S*)-4-amino-2-hydroxybutyl and (*S*)-4-amino-2-hydroxybutyryl groups were introduced to the 2′-amino group of 4-N-[(*S*)-4-amino-2-hydroxybutyl]fortimicin B (**2**). All of the derivatives of this type prepared showed antibacterial activity almost as strong as fortimicin A (**1**) and were also active against fortimicin A resistant bacteria which can inactivate fortimicin A by producing an inactivating enzyme AAC(3)-I.

In the study of chemical modification of fortimicin (FM), we have prepared several 4-N-substituted FM-B analogs[1]. Among them, 4-N-[(*S*)-4-amino-2-hydroxybutyl]-FM-B (**2**) has the most potent antibacterial activity, equal to or slightly better than that of FM-A (**1**). Its chemical stability is also better than that of FM-A. On the other hand, 2′-N-(2-aminoethyl)-FM-A (**3**)[2] and 2′-N-[(*S*)-4-amino-2-hydroxybutyl]-FM-A (**4**)[3] (Fig. 1) have potent activity against a resistant strain producing an enzyme AAC(3)-I which inactivates FM-A[4]. Thus our interest was directed toward introduction of 2-aminoethyl or 4-amino-2-hydroxybutyl group to the 2′-amino group of **2**. In this paper, preparation of 2′-N-(2-aminoethyl)-4-N-[(*S*)-4-amino-2-hydroxybutyl]-FM-B (**9**), 4,2′-di-N-[(*S*)-4-amino-2-hydroxybutyl]-FM-B (**12**) and the related compounds together with their antibacterial activities are reported.

Fig. 1. 4-N- or 4,2′-di-N-substituted fortimicin B.



1 R=$COCH_2NH_2$, R′=H
2 R=$CH_2CH(OH)(CH_2)_2NH_2$, R′=H
3 R=$COCH_2NH_2$, R′=$(CH_2)_2NH_2$
4 R=$COCH_2NH_2$, R′=$CH_2CH(OH)(CH_2)_2NH_2$

Preparation of 4,2′-Di-N-substituted Fortimicin B Derivatives

4,2′-Di-N-substituted FM-B derivatives were synthesised from 2′-N-substituted FM-B by similar

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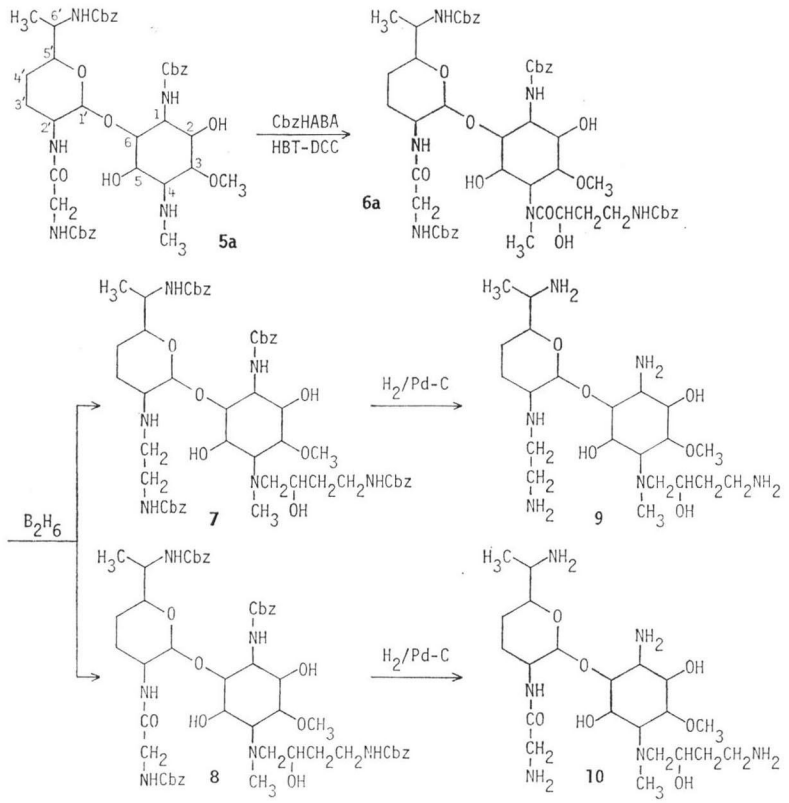
** To whom inquiries should be addressed.

Scheme 1. Synthesis of 4,2′-di-N-substituted fortimicin B.



sequences to those for preparation of 2′-N-substituted FM-A[3] (Schemes 1 and 2). (*S*)-4-Benzyloxycarbonylamino-2-hydroxybutyric acid (Cbz-HABA) was condensed with the 4-amino group of 1,6′-di-N-benzyloxycarbonyl-2′-N-(N-benzyloxycarbonylglycyl)-FM-B (**5a**) and 1,6′-di-N-benzyloxycarbonyl-2′-N-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-FM-B (**5b**)[3] by treatment with N,N-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HBT)[5] to give tetra-N-benzyloxycarbonyl-4-N-[(*S*)-4-amino-2-hydroxybutyryl]-2′-N-glycyl-FM-B (**6a**) and tetra-N-benzyloxycarbonyl-4,2′-di-N-[(*S*)-4-amino-2-hydroxybutyryl]-FM-B (**6b**), respectively. Amide carbonyl reduction of **6a** with diborane[1,6] gave a mixture of 1,6′-di-N-benzyloxycarbonyl-4-N-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyl]-2′-N-(2-benzyloxycarbonylaminoethyl)-FM-B (**7**) and partially reduced compound 1,6′-di-N-benzyloxycarbonyl-4-N-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyl]-2′-N-(N-benzyloxycarbonylglycyl)-FM-B (**8**), which were separated by silica gel chromatography. Hydrogenolysis of **7** and **8** gave 4-N-[(*S*)-4-amino-2-hydroxybutyl]-2′-N-(2-aminoethyl)-FM-B (**9**) and 4-N-[(*S*)-4-amino-2-hydroxybutyl]-2′-N-glycyl-FM-B (**10**), respectively. Hydrogenolysis of **6b** gave 4,2′-di-N-[(*S*)-4-amino-2-hydroxybutyryl]-FM-B (**11**) which was isolated as its trifluoroacetate salt because the 4-amino-2-hydroxybutyryl group at 4-N-position was easily hydrolysed under basic conditions[1,5]. Diborane reduction of the trifluoroacetate salt[1,7] of **11** gave 4,2′-di-N-[(*S*)-4-amino-2-hydroxybutyl]-FM-B (**12**) and 4-N-[(*S*)-4-amino-2-hydroxybutyl]-2′-N-[(*S*)-4-amino-2-hydroxybutyryl]-FM-B (**13**).

Scheme 2. Synthesis of 4,2′-di-N-substituted fortimicin B.

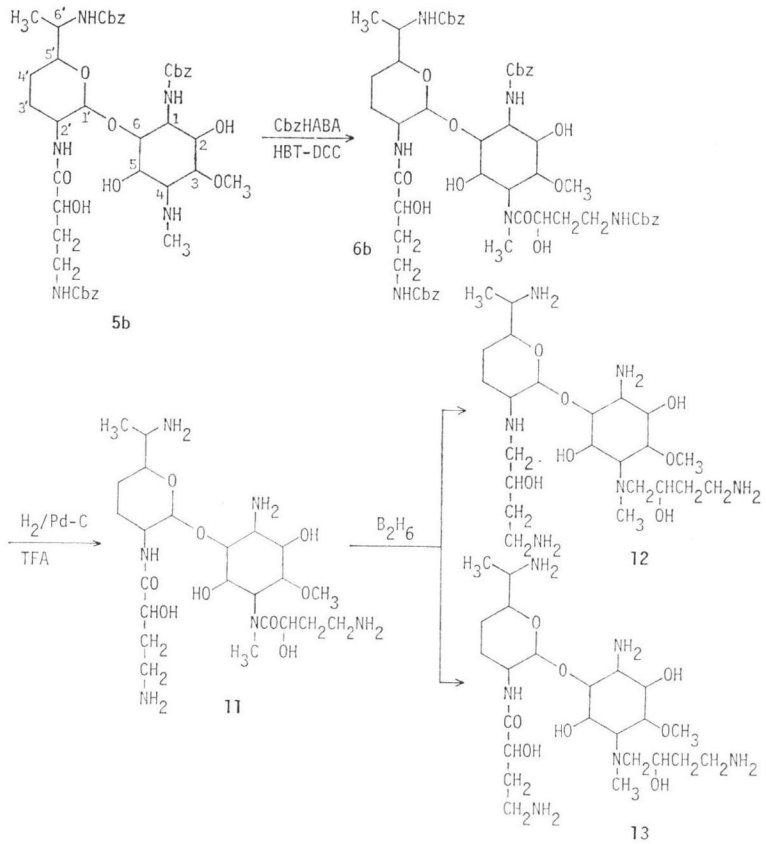


Table 1. Antibacterial spectra (MIC in mcg/ml at pH 7.2).

Organisms	Marker	1	9	12	10	13
S. aureus FDA 209P	G(+)	0.78	0.78	0.78	1.56	0.78
〃 Smith	〃	1.56	1.56	0.78	0.78	1.56
S. epidermidis	〃	1.56	0.78	0.78	1.56	1.56
M. lutea ATCC 9341	〃	1.56	1.56	0.78	0.78	3.12
B. subtilis ATCC 6633	〃	0.78	0.4	0.4	0.78	0.78
E. coli NIHJ-2	G(−)	1.56	3.12	1.56	3.12	1.56
K. pneumoniae 8045	〃	1.56	0.78	1.56	1.56	1.56
P. aeruginosa KY 4276	〃	6.25	6.25	6.25	25	12.5
S. marcescens T-55	〃	1.56	1.56	1.56	1.56	3.12
P. vulgalis ATCC 6897	〃	25	25	50	25	25
E. coli KY 8349	APH(3′)-I	1.56	1.56	1.56	1.56	1.56
P. aeruginosa KY 8571	AAC(6′)-III	12.5	12.5	12.5	25	25
〃 KY 8510	AAC(6′)-IV	12.5	12.5	12.5	25	50
S. marcescens KY 4248	〃	3.12	6.25	3.12	12.5	6.25
Providencia sp. KY 8464	AAC(2′)	3.12	12.5	3.12	6.25	6.25
E. coli KY 8348	AAC(3)-I	>100	0.78	6.25	3.12	3.12
P. aeruginosa KY 8563	AAC(3)-II	12.5	50	50	100	100
E. coli KY 8356	AAD(2″)	6.25	3.12	3.12	6.25	3.12

Antibacterial Activities of 4,2′-Di-N-substituted Fortimicin B Derivatives

Minimum inhibitory concentrations (MIC) were measured by agar dilution method using a medium of pH 7.2. MIC's of the 4-N-[(*S*)-4-amino-2-hydroxybutyl]-FM-B derivatives (**9**, **10**, **12** and **13**) were shown in Table 1. Although all compounds are active against the FM-A resistant strain, no remarkable enhancement of antibacterial activity compared to that of fortimicin A was observed against sensitive strains.

Experimental

Mass spectra were obtained on a JEOL JMS-01SG-2 spectrometer at 30 eV. PMR spectra were measured on a JEOL PS-100, a JEOL PFT-100A or a Varian T-60 spectrometer in the CW or FT mode. Chemical shifts of PMR are reported in ppm downfield from internal DSS or TMS. Elemental analyses were performed on a Yanagimoto CHN Corder MT-1. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. Reported pD values are uncorrected pH meter readings of deuterated solutions.

Tetra-N-benzyloxycarbonyl-4-N-[(*S*)-4-amino-2-hydroxybutyryl]-2′-N-glycyl-fortimicin B (**6a**)

To an ice-cooled solution of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid (Cbz-HABA, 205 mg) and 1-hydroxybenzotriazole (HBT, 110 mg) in tetrahydrofuran (THF, 5 ml) was added dicyclohexylcarbodiimide (DCC, 167 mg), and stirred for 1 hour. To the reaction mixture was added 1,6′-di-N-benzyloxycarbonyl-2′-N-(N-benzyloxycarbonylglycyl)-FM-B[2,3] (**5a**, 435 mg), and the mixture was stirred for 18 hours at room temperature. Insoluble material was removed by filtration and the solvent was evaporated to dryness. The resulting residue was applied to a column of silica gel (25 g) which was eluted with chloroform - methanol (97: 3) to give **6a** (344 mg, 61%). PMR (CD_3OD): δ 1.13 (3H, d, J=6.5 Hz, CH_3–6′), 1.2~2.0 (6H, m, CH_2–3′, 4′, 3″), 3.07 (3H, s, NCH_3), 3.33 (3H, s, OCH_3), 7.30 (20H, br s, aromatic protons).

Tetra-N-benzyloxycarbonyl-4,2′-di-N-[(*S*)-4-amino-2-hydroxybutyryl]fortimicin B (**6b**)

1,6′-Di-N-benzyloxycarbonyl-2′-N-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-FM-B[3] (**5b**) was condensed with Cbz-HABA in the similar way described above to give **6b** (28%). PMR (CD_3OD): δ 1.13 (3H, d, J=6.5 Hz, CH_3–6′), 1.2~1.9 (8H, m, CH_2), 3.10 (3H, s, NCH_3), 3.35 (3H, s, OCH_3), 7.33 (20H, br s, aromatic protons).

1,6′-Di-N-benzyloxycarbonyl-4-N-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyl]-2′-N-(2-benzyloxycarbonylaminoethyl)fortimicin B (**7**) and 1,6′-Di-N-benzyloxycarbonyl-4-N-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyl]-2′-N-(N-benzyloxycarbonylglycyl)fortimicin B (**8**)

To a solution of **6a** (324 mg) in THF (10 ml) was added 1 M diborane solution in THF (5 ml), and the solution was stirred for 5 minutes at room temperature. After excess diborane was decomposed by adding a small amount of water, the reaction mixture was evaporated to dryness. The residue was dissolved in 0.2 M methanolic hydrogen chloride (20 ml), stirred for 46 hours at room temperature, and then the solvent was evaporated. The residue was dissolved in the mixture of chloroform (20 ml) and 5% aqueous sodium hydrogen carbonate (20 ml). The organic layer was washed with water, dried over sodium sulfate and the solvent was evaporated. The residue was chromatographed on a column of silica gel (15 g), which was eluted with chloroform - methanol (96: 4) to give **8** (51 mg, 16%). PMR (CD_3OD): δ 1.03 (3H, d, J=6.5 Hz, CH_3–6′), 1.2~1.9 (6H, m, CH_2–3′, 4′, 3″), 2.46 (3H, s, NCH_3), 3.40 (3H, s, OCH_3), 7.30 (20H, br s, aromatic protons). Further elution of the column with the same solvent system gave **7** (121 mg, 38%). PMR (CD_3OD): δ 1.02 (3H, d, J= 6.5 Hz, CH_3–6′), 1.2~1.9 (6H, m, CH_2–3′, 4′, 3″), 2.40 (3H, s, NCH_3), 3.99 (3H, s, OCH_3), 7.33 (20H, br s, aromatic protons).

4-N-[(*S*)-4-Amino-2-hyroxybutyl]-2′-N-(2-aminoethyl)fortimicin B (**9**)

A solution of **7** (121 mg) in 0.2 M methanolic hydrogen chloride (15 ml) was bubbled with hydrogen in the presence of 10% palladium on carbon (*ca.* 20 mg) for 16 hours. The catalyst was removed by

filtration and the methanol was evaporated. The resulting residue was dissolved in water (5 ml). The solution, after its pH was adjusted to 7 with 1 N sodium hydroxide, was chromatographed on a column of Amberlite CG-50 (NH_4^+, 5 ml) with 0.6 N ammonium hydroxide to give **7** (27 mg, 47%). MS: *m/z* 479 (MH^+), 322, 294, 186, 169. PMR (D_2O): δ 1.04 (3H, d, J=6.6 Hz, CH_3–6′), 1.2~1.9 (6H, m, CH_2–3′, 4′, 3″), 2.43 (3H, s, NCH_3), 3.43 (3H, s, OCH_3), 5.13 (1H, d, J=3.4 Hz, H–1′). An aqueous solution of **9** obtained above, after adjusting the pH to 2 with 5 N sulfuric acid, was added to 10-fold volume of ethanol. The resulting precipitate was collected, washed with ethanol and dried to give the sulfate of **9**. $[\alpha]_D^{23}$ +67.0° (*c* 0.2, H_2O).

Anal. Calcd. for $C_{21}H_{46}N_6O_6 \cdot 3H_2SO_4 \cdot C_2H_5OH \cdot H_2O$: C, 33.00; H, 7.23; N, 9.98.
Found: C, 32.76; H, 7.18; N, 9.70.

4-N-[(*S*)-4-Amino-2-hydroxybutyl]-2′-N-glycylfortimicin B (**10**)

Hydrogenation of **8** followed by purification in the same manner as the preparation of **9** gave **10** (40%). MS: *m/z* 493 (MH^+), 492 (M^+), 322, 294, 200. PMR (D_2O, pD10.9): δ 1.06 (3H, d, J=6.4 Hz, CH_3–6′), 1.2~1.9 (6H, m, CH_2–3′, 4′, 3″), 2.40 (3H, s, NCH_3), 3.29 (2H, s, CH_2–gly), 3.43 (3H, s, OCH_3), 5.10 (1H, d, J=2.6 Hz, H–1′). Sulfate: $[\alpha]_D^{23}$ +70.0° (*c* 0.2, H_2O).

Anal. Calcd. for $C_{21}H_{44}N_6O_7 \cdot 2.5H_2SO_4 \cdot C_2H_5OH \cdot H_2O$: C, 34.45; H, 7.17; N, 10.48.
Found: C, 34.29; H, 7.45; N, 10.61.

4,2′-Di-N-[(*S*)-4-amino-2-hydroxybutyl]fortimicin B (**12**) and 4-N-[(*S*)-4-amino-2-hydroxybutyl]-2′-N-[(*S*)-4-amino-2-hydroxybutyryl]fortimicin B (**13**)

A solution of **6b** (1.07 g) in a mixture of methanol (60 ml) and trifluoroacetic acid (0.6 ml) was bubbled with hydrogen in the presence of 10% palladium on carbon for 16 hours. The catalyst was removed by filtration and the solvents were evaporated to dryness to give the trifluoroacetate of 4,2′-di-N-[(*S*)-4-amino-2-hydroxybutyryl]-FM-B (**11**, 1.06 g). To a solution of the trifluoroacetate in THF (30 ml) was added 1 M diborane solution in THF (15 ml) and the reaction mixture was stirred for 30 minutes at room temperature. After excess diborane was decomposed by adding a small amount of water, the reaction mixture was evaporated to dryness. The resulting residue was dissolved in 0.2 M methanolic hydrogen chloride (50 ml), and heated at 50°C for 4 hours. The solution was evaporated to dryness, the residue was redissolved in water and the pH of the solution was adjusted to 7 with 1 N sodium hydroxide. The resulting solution was chromatographed on a column of Amberlite CG-50 (NH_4^+, 25 ml) with 0.5 N ammonium hydroxide to give **13** (86 mg, 16%). MS: *m/z* 537 (MH^+), 322, 294, 244, 226. PMR (D_2O, pD 10.8): δ 1.05 (3H, d, J=6.6 Hz, CH_3–6′), 1.2~1.9 (8H, m, CH_2), 2.43 (3H, s, NCH_3), 3.43 (3H, s, OCH_3), 5.07 (1H, d, J=3 Hz, H–1′). Sulfate: $[\alpha]_D^{25}$ +87.5° (*c* 0.2, H_2O).

Anal. Calcd. for $C_{23}H_{48}N_6O_5 \cdot 2.5H_2SO_4 \cdot 1.5H_2O$: C, 34.76; H, 7.35; N, 9.73.
Found: C, 34.84; H, 7.61; N, 9.78.

Further elution of the column with 0.6 N ammonium hydroxide gave **12** (150 mg, 29%). MS: *m/z* 523 (MH^+), 322, 294, 230, 213. PMR (D_2O, pD 11.0): δ 1.05 (3H, d, J=6.6 Hz, CH_3–6′), 1.2~1.9 (8H, m, CH_2), 2.44 (3H, s, NCH_3), 3.43 (3H, s, OCH_3), 5.20 (1H, d, J=2.9 Hz, H–1′), $[\alpha]_D^{25}$ +88.0° (*c* 0.2, H_2O).

Anal. Calcd. for $C_{23}H_{50}N_6O_7 \cdot 3H_2SO_4 \cdot 1.5H_2O$: C, 33.74; H, 7.36; N, 9.44.
Found: C, 33.77; H, 7.61; N, 9.20.

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