CHEMICAL MODIFICATION OF FORTIMICINS

III. PREPARATION OF N-SUBSTITUTED FORTIMICIN A DERIVATIVES*

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

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

KOZO KITAURA

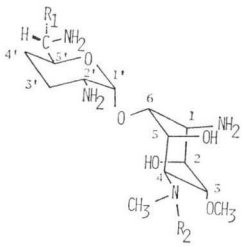
Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.
Nagaizumi, Shizuoka, Japan

(Received for publication November 25, 1980)

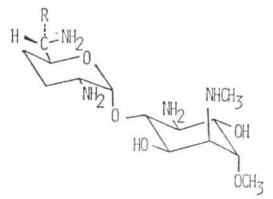
The 1, 2′ or 6′-amino group of fortimicin A was alkylated or acylated and the antimicrobial activities of the derivatives were compared with each other. 2′-N-Substituted fortimicins A were active against the fortimicin A resistant strain which produced AAC(3)-I. AAC(3)-I is the only enzyme which can inactivate fortimicin A. Among the derivatives prepared in the present study, 2′-N-[(*S*)-4-amino-2-hydroxybutyl]fortimicin A showed stronger activity than fortimicin A.

Fortimicins (FM) are pseudodisaccharide aminocyclitol antibiotics produced by *Micromonospora* sp. FM-A (**1**), C (**2**), and D (**3**) have strong antimicrobial activity but their 4-N-deacyl analogs, FM-B (**4**) and KE (**5**), have only weak activity.[1~4] On the basis of these findings, a variety of 4-N-substituted FM-B analogs has been prepared by the Abbott group[5] and in our laboratory.[6] At the next step of fortimicin chemical modification, the reactivity of the four amino groups of FM-B to acylating agents was examined and the procedure for regiospecific protection of three of them has been established[7] which was useful for chemical modification of the amino groups other than at position-4 of FM-B.

Fig. 1.



	R_1	R_2
FM-A (**1**)	CH_3	$COCH_2NH_2$
FM-C (**2**)	CH_3	$COCH_2NHCONH_2$
FM-D (**3**)	H	$COCH_2NH_2$



	R
FM-B (**4**)	CH_3
FM-KE (**5**)	H

* A part of this work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 2~4 (Abstr. Paper, p. 276).

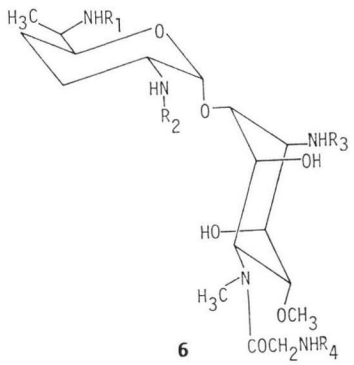
** To whom inquiries should be addressed.

In this paper we will describe the reactivity of the four amino groups, derivation of 1-N, 2′-N, and 6′-N-monosubstituted FM-A from tri-N-acylated FM-A, and their antimicrobial activity.

Preparation of 1-N-, 2′-N-, and 6′-N-Substituted FM-A and their Antibacterial Activity

Initially, the same kind of substituent was attached to each of the 1, 2′, and 6′-amino groups to determine which amino groups should be modified for the best antibacterial activity. Methyl, ethyl, and (*S*)-4-amino-2-hydroxybutyryl (AHBA) were selected for the purpose because it is known that the AHBA group can add desired activity to aminocyclitol antibiotics against bacteria resistant to them.[8] AHBA derivatives were prepared from tri-N-benzyloxycarbonyl (Cbz) FM-A (**6a**, **6b**, and **6c**), which have only one free amino group, by reaction with N-Cbz-AHBA-succinimide,[8] followed by removal of the Cbz groups. 1-N-, 2′-N- and 6′-N-methyl and ethyl derivatives were obtained from **6a**, **6b** and **6c** by reductive alkylation with formaldehyde and acetaldehyde, respectively, and sodium borohydride, followed by the same deprotection. In the case of 6′ and 2′-free amino derivatives (**6a** and **6b**, respectively), their methylation with formaldehyde and sodium borohydride gave 6′-N-dimethyl and 2′-N-dimethyl derivatives as well as monomethyl derivatives (**6d**) and (**6g**), respectively. On the other hand the similar

Fig. 2.



6

	R_1	R_2	R_3	R_4
6a*	H	Cbz	Cbz	Cbz
6b*	Cbz	H	Cbz	Cbz
6c	Cbz	Cbz	H	Cbz

* The conformation of the fortamine moieties of these compounds are not certain.

Table 1. N-Substituted fortimicin A derivatives (**6**).

Compound	R_1	R_2	R_3	R_4	Rf[b]	$[\alpha]_D$[c]	Mass fragments (*m/z*)
6d	CH_3	H	H	H	0.69	+84.0[d]	419 (M^+), 376, 292, 246, 235, 207, 157.
6e	CH_2CH_3	H	H	H	0.77	+98.0[d]	433 (M^+), 345, 305, 274, 246, 207, 196, 171.
6f	AHBA[a]	H	H	H	0.40	+44.5[d]	[g]
6g	H	CH_3	H	H	0.69	+78.5[e]	419 (M^+), 362, 292, 274, 246, 207, 157.
6h	H	CH_2CH_3	H	H	0.75	+70.0[e]	433 (M^+), 376, 292, 274, 246, 207, 189, 171.
6i	H	AHBA	H	H	0.42	+78.0[f]	506 (M^+), 431, 400, 301, 244, 235, 226, 207, 143.
6j	H	H	CH_3	H	0.67	+87.0[d]	419 (M^+), 359, 306, 260, 221, 174, 154, 143.
6k	H	H	CH_2CH_3	H	0.74	+86.0[d]	433 (M^+), 320, 274, 235, 200, 185, 143.
6l	H	H	AHBA	H	0.42	+62.0[d]	[g]

[a] AHBA = $COCH(OH)CH_2CH_2NH_2$ (*S*).
[b] TLC on silica gel (solvent; $CHCl_3$ - CH_3OH - 28 % NH_4OH, 1 : 1 : 1, lower layer).
[c] Measured as sulfates in water (*c* 0.2).
[d] Measured at 20°C.
[e] Measured at 23°C.
[f] Measured at 21°C.
[g] Satisfactory mass spectra of these compounds could not been obtained because of decomposition.

Table 2. Antibacterial spectra of N-substituted fortimicin A (**6**). MIC (mcg/ml).[a]

Organisms	Inactivating enzyme	**1**	**6d**	**6e**	**6g**	**6h**	**6i**	**6j**
S. aureus 209-P		1.56	1.56	1.56	1.56	1.56	0.78	50
S. aureus Smith		0.78	0.78	1.56	0.78	1.56	0.78	25
B. subtilis ATCC 6633		0.78	0.78	1.56	0.78	0.78	0.78	6.25
E. coli NIH JC-2		1.56	1.56	3.12	1.56	6.25	3.12	50
E. coli Juhl		3.12	3.12	6.25	1.56	1.56	1.56	100
K. pneumoniae # 8045		1.56	1.56	3.12	1.56	1.56	1.56	100
S. sonnei ATCC 9290		3.12	6.25	3.12	1.56	3.12	3.12	100
P. vulgaris ATCC 6897		50	25	50	50	50	50	>100
P. aeruginosa KY-4276		12.5	12.5	25	25	50	50	>100
E. coli 57R/W677	ANT (2″)	3.12	3.12	3.12	6.25	3.12	3.12	25
E. coli KY-8332	AAC (6′)	1.56	1.56	1.56	3.12	3.12	3.12	25
E. coli KY-8348	ACC (3)-I	>100	>100	>100	100	50	6.25	100
P. aeruginosa KY-8516	AAC (6′)	25	50	50	100	100	50	>100
Providencia sp. KY-8464	AAC (2′)	3.12	3.12	6.25	3.12	1.56	3.12	>100
K. pneumoniae KY-3020	ANT (2″)	6.25	12.5	25	6.25	6.25	6.25	>100

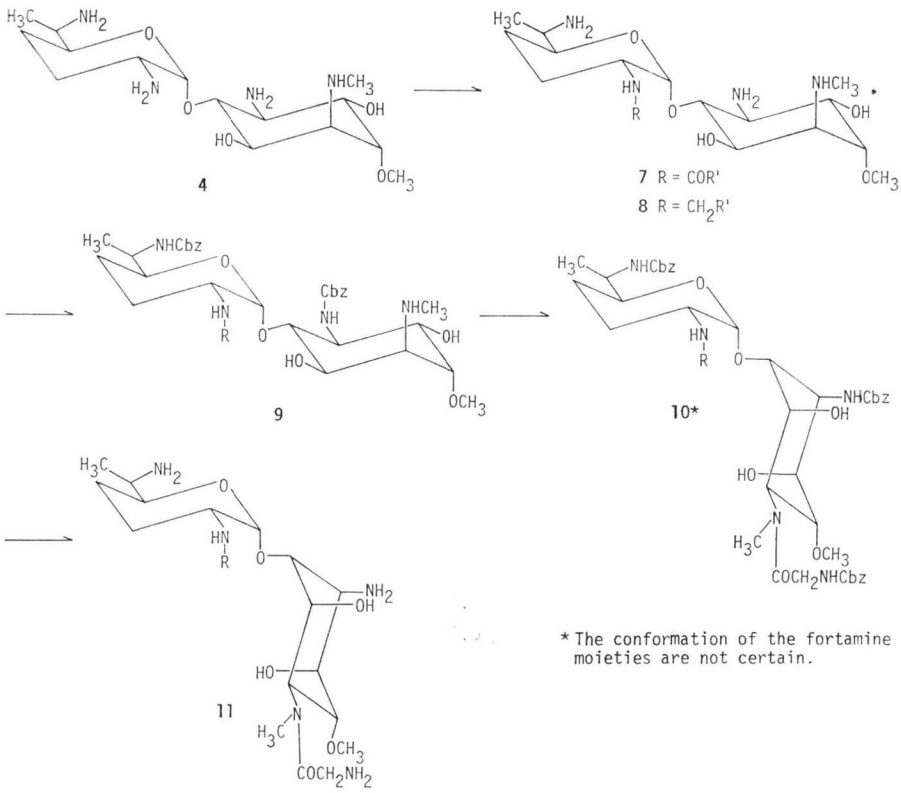
[a] The minimum inhibitory concentration was measured by agar dilution method at pH 7.2. Compounds **6f**, **6k**, and **6l** have no antimicrobial activity below the concentration of 100 mcg/ml.

methylation of 1-N-free amino derivative (**6c**) gave only monomethyl derivative (**6j**). The structures of the products were identified by their pmr and mass spectra. The compounds synthesized and their antimicrobial activity were listed in Tables 1 and 2, respectively. The strength of their activity depended on the position modified. 6′-N-Methyl and 2′-N-methyl FM-A (**6d**[9], **6g**) showed activity similar to that of FM-A, whereas 1-N-methyl FM-A (**6j**) showed only weak activity and ethylation of the 1-N-amino group caused complete loss of activity. The 2′-N-AHBA derivative (**6i**) showed strong activity comparable to that of FM-A. It is also noteworthy that **6i** is active against the FM-A resistant strain producing ACC(3)-I. AAC(3)-I is known as the only enzyme which can inactivate FM-A by acetylation of the amino group at position-1.[10] On the other hand, 6′ and 1-AHBA derivatives (**6f** and **6l**) showed no antibacterial activity. The following conclusion may be drawn from the present study on fortimicin: (1) Introduction of an alkyl or an acyl group to the 6′-amino group results in a compound with possibly decreased activity relative to the parent compound: (2) Activity is also reduced markedly by introduction of a substituent to the 1-amino group. Because more than a dozen derivatives of 4-N-acyl[5,6] and 4-N-alkyl FM-B[6] have been prepared for antibacterial assay and none of them were sufficiently more active than FM-A, our next interest was directed towards the preparation of 2′-N-acyl and alkyl FM-A analogs.

Preparation of 2′-N-Substituted FM-A and their Antibacterial Activity

2′-N-Alkyl derivatives were prepared according to the route shown in Scheme 1. Reaction of FM-B (**4**) with N-hydroxysuccinimide ester of N-Cbz-amino acids gave 2′-N-acyl derivatives (**7**), the amide carbonyl groups of which were reduced with diborane to afford 2′-N-alkyl FM-B (**8**).[6] After the amino groups at C-1 and C-6′ were protected with Cbz, the remaining free amino group at C-4 of **9** was acylated with N-Cbz-glycine by the active ester method using 1-hydroxybenzotriazole. Finally, all the four Cbz groups of **10** were removed by hydrogenolysis to give the desired compounds (**11**)

Scheme 1.



(method A). Other 2′-N-alkyl derivatives were derived from 1,6′-di-N-Cbz-4-N-(N-Cbz-glycyl) FM-B (**6b**) by the reductive alkylation, followed by removal of the Cbz groups in the similar procedure to preparation of 2′-N-methyl FM-A (**6g**) already mentioned (method B). 2′-N-Acyl FM-A were also prepared from **6b** by acylation and deprotection (method C). The structures of the 2′-N-substituted FM-A were determined from their mass and pmr spectra. A number of the FM-A derivatives thus obtained and their antibacterial activity are listed in Tables 3 and 4, respectively. Although they are limited test results, these data reveal the following structure-activity relationships; (1) increase of carbon atoms in a simple alkyl group introduced to 2′-amino group results in reduced activity. The tendency was remarkable especially to Gram-negative bacteria; (2) when the substituents, whether they are alkyls or acyls, contain a hydroxyl and an amino group (**11g**～**11k**), the activities are strong to both Gram-positive and Gram-negative bacteria including the resistant strain which produce the aminoglycoside inactivating enzyme AAC(3)-I; (3) when the substituents have the same number of carbon atoms, alkyl derivatives are slightly more active than the corresponding acyl derivatives (**11e** to **11f**, **11g** to **11h**, and **6i** to **11k**); (4) no significant difference of activity is found between α-amino-ω-hydroxyalkyl derivatives (**11i** and **11g**) and α-hydroxy-ω-aminoalkyl derivatives (**11h** and **11f**); (5) di-aminoalkylation and dihydroxyalkylation result in compounds with rather decreased activity relative to FM-A. The di-hydroxyalkyl derivatives (**11m** and **11n**) are inactive against the resistant strain. Among the N-substituted FM-A prepared in the present study 2′-N-[(*S*)-4-amino-2-hydroxybutyl] FM-A (**11k**) showed the strongest activity.

Table 3. 2′-N-Substituted fortimicin A derivatives (**11**).

Compound	R	Method	Rf[a]	$[\alpha]_D$[b]	Mass fragments (*m/z*)
11a	$CH_2CH_2CH_3$	B	0.75	+74.5[e]	447 (M^+), 390, 292, 246, 207, 185.
11b	$CH(CH_3)_2$	B	0.78	+67.0[e]	447 (M^+), 390, 292, 246, 207, 185.
11c	$CH_2CH_2CH_2CH_3$	B	0.80	+73.5[e]	461 (M^+), 404, 324, 274, 246, 207, 198.
11d	$CH_2C_6H_5$	B	0.81	+75.0[e]	495 (M^+), 382, 354, 336, 207, 143.
11e	$COCH_2OH$	C	0.66	+110.0[c]	464 (MH^+), 407, 235, 207, 187.
11f	CH_2CH_2OH	B	0.64	+75.5[e]	449 (M^+), 392, 235, 207, 187.
11g	$COCH(OH)CH_2NH_2$ (*S*)	C	0.51	+90.5[c]	492 (M^+), 349, 246, 235, 230, 207.
11h	$CH_2CH(OH)CH_2NH_2$ (*S*)	A	0.46	+67.5[c]	478 (M^+), 460, 361, 235, 216, 207.
11i	$CH_2CH(NH_2)CH_2OH$ (*S*)	A	0.50	+57.5[c]	460 (M^+-H_2O), 235, 216, 207, 143.
11j	$CH_2CH(NH_2)CH_2CH_2OH$ (*S*)	A	0.54	+84.5[c]	492 (M^+), 400, 361, 270, 230, 207.
11k	$CH_2CH(OH)CH_2CH_2NH_2$ (*S*)	A	0.38	+63.0[c]	475 (M^+-NH_3), 474 (M^+-H_2O), 400, 361, 230, 207.
11l	$CH_2CH(NH_2)CH_2CH_2NH_2$ (*S*)	A	0.35	+55.5[c]	474 (M^+-NH_3), 473 (M^+-H_2O), 361, 229, 207, 155.
11m	$CH_2CH(OH)CH_2OH$ (*RS*)	B	0.25	+70.0[d]	479 (M^+), 404, 361, 292, 235, 217, 207.
11n	$CH(CH_2OH)_2$	B	0.48	+67.5[d]	[f]
11o	$CONH_2$	C	0.56	+116.0[d]	448 (M^+), 292, 274, 264, 235, 207, 189.
11p	$COCH_2NHCONH_2$	C	0.41	+103.0[e]	[f]
11q	$C(=NH)NH_2$	C	0.13	+65.0[d]	[f]

[a] TLC on silica gel (solvent; $CHCl_3$ - CH_3OH - 28 % NH_4OH, 1 : 1 : 1, lower layer).
[b] Measured as sulfates in water (*c* 0.2).
[c] Measured at 21°C.
[d] Measured at 22°C.
[e] Measured at 23°C.
[f] Satisfactory mass spectra of these compounds could not been obtained because of decomposition.

Table 4. Antibacterial spectra of 2′-N-substituted fortimicin A (**11**). MIC (mcg/ml).[a]

Organisms	Inactivating enzyme	**1**	**11b**	**11g**	**11h**	**11i**	**11j**	**11k**	**11l**	**11m**	**11q**
S. aureus 209-P		1.56	0.78	3.12	3.12	3.12	1.56	0.78	6.25	6.25	1.56
S. aureus Smith		0.78	1.56	1.56	0.78	1.56	0.78	0.78	3.12	1.56	0.78
B. subtilis ATCC 6633		0.78	0.78	1.56	1.56	1.56	0.78	0.78	3.12	3.12	0.78
E. coli NIH JC-2		1.56	1.56	3.12	1.56	3.12	1.56	1.56	6.25	12.5	6.25
E. coli Juhl		3.12	6.25	3.12	6.25	12.5	0.78	6.25	12.5	25	6.25
K. pneumoniae #8045		1.56	3.12	3.12	1.56	3.12	1.56	1.56	6.25	6.25	1.56
S. sonnei ATCC 9290		3.12	3.12	6.25	6.25	12.5	3.12	3.12	50	12.5	3.12
P. vulgaris ATCC 6897		50	50	25	50	50	6.25	50	50	50	50
P. aeruginosa KY-4276		12.5	100	50	25	25	25	12.5	12.5	50	25
E. coli 57R/W677	ANT (2″)	3.12	3.12	6.25	6.25	6.25	0.78	3.12	12.5	12.5	3.12
E. coli KY-8332	AAC (6′)	1.56	3.12	3.12	1.56	1.56	0.78	1.56	6.25	12.5	0.78
E. coli KY-8348	AAC (3)-I	>100	25	6.25	6.25	12.5	3.12	6.25	12.5	>100	3.12
P. aeruginosa KY-8516	AAC (6′)	25	>100	25	12.5	25	50	25	25	50	12.5
Providencia sp. KY-8464	AAC (2′)	3.12	6.25	6.25	6.25	6.25	3.12	3.12	25	3.12	3.12
K. pneumoniae KY-3020	ANT (2″)	6.25	12.5	12.5	6.25	6.25	3.12	6.25	12.5	25	6.25

[a] The minimum inhibitory concentration was measured by agar dilution method at pH 7.2.

Experimental

Mass spectra were obtained on a JEOL JMS-01SG-2 spectrometer at 30 eV. Pmr spectra were measured on a JEOL PS-100 or a JEOL PFT-100A spectrometer in D_2O solution and chemical shifts are reported in ppm downfield from internal DSS or TMS. Optical rotation were measured with a Perkin-Elmer model 141 polarimeter. Reported pD values are uncorrected pH meter readings of deuterated solution.

General Procedure for Preparation of N-Alkyl FM-A from Tri-N-protected FM-A by Reductive Alkylation Followed by Hydrogenolysis

To an ice-cooled solution of 1 mmole of tri-N-Cbz-FM-A **6a**, **6b**, or **6c** and 2~4 mmole of aldehyde or ketone in 20 ml of methanol was added 1~4 mmole of sodium borohydride. To the reaction mixture, after stirring for 4~8 hours at room temperature, were added 2 ml of 1 N hydrochloric acid and 100 mg of 10% palladium on carbon and hydrogen gas was bubbled through the reaction mixture for several hours at room temperature. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The crude material thus obtained was chromatographed on a column of 50 ml of Amberlite CG-50 (NH_4^+) resin with 0.1~0.2 N ammonium hydroxide. Fractions containing the product were combined and evaporated under reduced pressure to give pure material.

Typical Procedure for Method A; Preparation of 2′-N-[(*S*)-4-Amino-2-hydroxybutyl] FM-A (**11k**)

(1) 2′-N-[(*S*)-4-Cbz-amino-2-hydroxybutyryl] FM-B (**7**, R=$COCH(OH)CH_2CH_2NHCbz$)

To a solution of 5.0 g (14.3 mmole) of FM-B in 200 ml of methanol was added dropwise a solution of 6.0 g (17.1 mmole) of N-[(*S*)-4-Cbz-amino-2-hydroxybutyryloxy]succinimide for about 1 hour, and the solution was stirred for 3 hours at room temperature. The reaction mixture was evaporated under reduced pressure. The residue was then dissolved in 100 ml of water and charged to a column of 150 ml of Amberlite CG-50 (NH_4^+) after adjusting the pH to 6 with 1 N hydrochloric acid. The column was washed with 150 ml of water and eluted with 0.1 N ammonium hydroxide. The fractions containing the product were combined and evaporated under reduced pressure to give 3.4 g of white power **7** (40.6%). TLC on silica gel (lower layer of $CHCl_3$ - MeOH - 14% NH_4OH=2: 1: 1); Rf 0.37. Pmr (CD_3OD); 1.04 (3H, d, J=6.5, CH_3-6′), 1.2~1.9 (6H, m), 2.37 (3H, s, NCH_3), 3.43 (3H, s, OCH_3), 5.03 (2H, s, CH_2Ph), 5.06 (1H, d, J=3, H-1′), 7.26 (5H, s, Ph).

(2) 2′-N-[(*S*)-4-Cbz-amino-2-hydroxybutyl]FM-B (**8**, R=$CH_2CH(OH)CH_2CH_2NHCbz$)

To a solution of 2.5 g of **7** in 50 ml of tetrahydrofuran was added 1 M solution of diborane in 50 ml of tetrahydrofuran and the solution was stirred for 2 hours 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 a mixture of 5 ml of 2 N hydrochloric acid and 45 ml of methanol, and stirred for 18 hours at room temperature. The solution was evaporated under reduced pressure. The residue was chromatographed on a column of Amberlite CG-50 (NH_4^+) to give **8** (1.8 g, 71%). TLC (lower layer of $CHCl_3$ - MeOH - 14% NH_4OH=2: 1: 1); Rf 0.30. Pmr (CD_3OD); 1.05 (3H, d, J=6.5, 6′-CH_3), 1.2~1.9 (6H, m), 2.37 (3H, s, NCH_3), 3.44 (3H, s, OCH_3), 5.05 (2H, s, CH_2Ph), 7.26 (5H, s, Ph).

(3) 2′-N-[(*S*)-4-Cbz-amino-2-hydroxybutyl]-1, 6′-di-N-Cbz-FM-B (**9**, R=$CH_2CH(OH)CH_2CH_2NHCbz$)

To a solution of 1.2 g of **8** in 40 ml of tetrahydrofuran was added 1.6 g of N-(benzyloxycarbonyloxy) succinimide in portions over a period of 1 hour and the solution was stirred for 3 hours at room temperature. The reaction mixture was evaporated under reduced pressure. The residue was dissolved in 50 ml of ethyl acetate, washed with aqueous 5% sodium hydrogen carbonate, and then with water, dried over sodium sulfate and the solvent was removed. The resulting crude material was chromatographed on a column of 100 g of silica gel with chloroform - methanol (9: 1) to give **9*** (1.13 g, 64%). TLC ($CHCl_3$ - MeOH, 9: 1); Rf 0.16.

* The pmr of the product showed complex signals and could not be analyzed exactly but it indicated presence of three Cbz groups.

(4) 2′-N-[(*S*)-4-Amino-2-hydroxybutyl]FM-A (**11k**)

To an ice-cooled solution of 180 mg of N-Cbz-glycine and 120 mg of 1-hydroxybenzotriazole in 16 ml of tetrahydrofuran was added 185 mg of dicyclohexylcarbodiimide, and stirred 1 hour under ice-cooling. To the reaction mixture was added 600 mg of **9** obtained above, and the solution was stirred for 18 hours at room temperature. After removal of the precipitate formed by filtration, the solution was concentrated *in vacuo* to give a residue, which was re-dissolved in a mixture of 2 ml of 1 N hydrochloric acid and 20 ml of methanol and 100 mg of 10% palladium on carbon. Hydrogen gas was passed through the reaction mixture with bubbling for 4 hours at room temperature. The catalyst was removed and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on Amberlite CG-50 (NH_4^+) as mentioned above, to give 220 mg (61%) of **11k**. Mass (*m/z*); 429 (M^+), 418, 400, 361, 332, 247, 207, 155. Pmr (D_2O, pD=10.8); 1.04 (3H, d, J=6.6, 6′-CH_3), 1.2~2.0 (6H, m), 2.6~2.9 (7H, m), 3.05 (3H, s, NCH_3), 3.44 (3H, s, OCH_3), 3.52 (2H, s, CH_2-Gly), 4.4 (1H, m, H-2), 4.90 (1H, dd, J=11.4, 3.2, H-4), 5.04 (1H, d, J=2.0, H-1′).

Typical Procedure for Method C; 2′-N-[(*S*)-3-Amino-2-hydroxypropionyl]FM-A (**11g**)

N-[(*S*)-3-Cbz-amino-2-hydroxypropionyloxy]succinimide (280 mg) was added to a solution of 500 mg of 1,6′-di-N-Cbz-4-N-(N-Cbz-glycyl)FM-B (**6b**) in 12 ml of tetrahydrofuran and was stirred for 3 hours at room temperature. The reaction mixture was evaporated under reduced pressure. The residue was dissolved in 20 ml of ethyl acetate washed with aqueous 5% sodium hydrogen carbonate and water, dried over sodium sulfate and then the solvent was removed. Catalytic hydrogenolysis of the residue, followed by chromatography on Amberlite CG-50 as above mentioned, gave 260 mg (86%) of **11g**. Mass (*m/z*); 492 (M^+), 444, 349, 246, 235, 207, 154. Pmr (D_2O, pD=10.7); 1.01 (3H, d, J=6.5, 6′-CH_3), 1.5~1.9 (4H, m), 2.6~2.9 (4H, m), 3.06 (3H, s, NCH_3), 3.44 (3H, s, OCH_3), 3.51 (2H, s, CH_2-Gly), 4.87 (1H, d, J=2.5, H-1′).

Typical Procedure for Method B; 2′-N-Isopropyl-FM-A (**11b**)

To an ice-cooled, stirred solution of 800 mg of **6b** and 230 mg of acetone in 20 ml of methanol was added 38 mg of sodium borohydride, and stirring was continued 4 hours under ice-cooling. Hydrogenolysis of the reaction mixture after addition of 2 ml of 1 N hydrochloric acid, followed by column chromatography as described above gave 290 mg (65%) of **11b**. Mass (*m/z*); 447 (M^+), 390, 292, 246, 207, 185. Pmr (D_2O, pD=10.9); 1.03 (3H, d, J=6.4, 6′-CH_3), 1.04 (6H, d, J=4.9, $CH(CH_3)_2$), 3.06 (3H, s, NCH_3), 3.44 (3H, s, OCH_3), 3.52 (2H, s, CH_2-Gly), 4.92 (1H, dd, J=11.4, 3.1, H-4), 5.07 (1H, d, J=2.7, H-1′).

References

1) Nara, T.; M. Yamamoto, I. Kawamoto, K. Takayama, R. Okachi, S. Takasawa, T. Sato & S. Sato: Fortimicins A and B, new aminoglycoside antibiotics. I. Producing organism, fermentation and biological properties of fortimicians. J. Antibiotics 30: 533~540, 1977
2) Okachi, R.; S. Takasawa, T. Sato, S. Sato, M. Yamamoto, I. Kawamoto & T. Nara: Fortimicins A and B, new aminoglycoside antibiotics. II. Isolation, physico-chemical and chromatographic properties. J. Antibiotics 30: 541~551, 1977
3) Egan, R. S.; R. S. Stanaszek, M. Cirovic, S. L. Mueller, J. Tadanier, J. R. Martin, P. Collum, A. W. Goldstein, R. L. De Vault, A. C. Sinclair, E. E. Fager & L. A. Mitscher: Fortimicins A and B, new aminoglycoside antibiotics. III. Structural identification. J. Antibiotics 30: 552~563, 1977
4) Sugimoto, M.; S. Ishii, R. Okachi & T. Nara: Fortimicins C, D and KE, new aminoglycoside antibiotics. J. Antibiotics 32: 868~873, 1979
5) Tadanier, J.; J. R. Martin, P. Kurath, A. W. Goldstein & P. Johnson: 4-N-Acylfortimicin B and the preparation of fortimicin A from fortimicin B. Carbohyd. Res. 79: 91~102, 1980
6) Sato, M. & Y. Mori: Chemical modification of fortimicins. Preparation of 4-N-substituted fortimicin B. J. Antibiotics 32: 371~378, 1979
7) Sato, M.; K. Mochida, Y. Mori & K. Shirahata: Chemical modification of fortimicins. II. Selective protection of fortimicins A and B. J. Antibiotics 34: 513~521, 1981
8) Richardson, K.; S. Jevons, J. W. Moore, B. C. Ross & J. R. Wright: Synthesis and antibacterial activities of 1-N-[(*S*)-amino-2-hydroxyalkyl]kanamycin A derivatives. J. Antibiotics 30: 843~846, 1977

9) TADANIER, J.; D. A. DUNNIGAN, J. R. MARTIN, L. FREIBERG & M. CIROVIC: 6′-N-Methylfortimicins A and B and 6′,6′-di-N-methylfortimicins A and B. J. Antibiotics 34: 193~201, 1981
10) SATO, S.; T. IIDA, R. OKACHI, K. SHIRAHATA & T. NARA: Enzymatic acetylation of fortimicin A and seldomycin factor 5 by aminoglycoside 3-acetyltransferase I: [AAC(3)-I] of *E. coli* KY 8348. J. Antibiotics 30: 1025~1027, 1977