CHEMICAL MODIFICATION OF FORTIMICINS

II. SELECTIVE PROTECTION OF FORTIMICINS A AND B*

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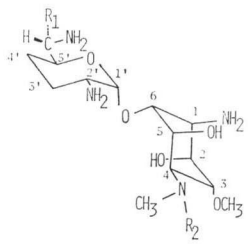
The four amino groups of fortimicin B(**4**) could be differentiated from each other and 2′-N-benzyloxycarbonyl-, 2′,6′-di-N-benzyloxycarbonyl-, 2′-N-*tert*-butoxycarbonyl-, 6′-N-*tert*-butoxycarbonyl- and 2′,6′-di-N-*tert*-butoxycarbonyl-fortimicin B (**7**, **8**, **16**, **18**, **17**) were prepared from **4**. From these fortimicin B derivatives, selectively protected fortimicin A derivatives **13**, **15**, **21**, **22** and **25** were prepared by combination of procedures of benzyloxycarbonylation or *tert*-butoxycarbonylation, 4-N-glycylation.

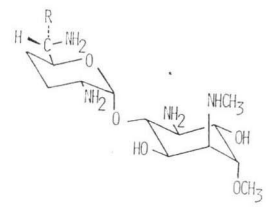
Fortimicins (FM) are aminoglycoside antibiotics produced by *Micromonospora* sp.[1~6] FM-A (**1**), C (**2**) and D (**3**) have potent antibacterial activities, while their deacylated components, *i.e.*, FM-B (**4**) and KE (**5**) are only weakly active compounds. The first attempt of chemical modification to prepare antibiotics with stronger antibacterial activity was aimed toward the preparation of 4-N-substituted FM-B.[7,8]

In the next step, we undertook a program of modification at other positions of FM-A. This paper describes the preparation of selectively protected FM-A derivatives.

Fig. 1. Structures of fortimicins.



	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

Selective Protection of Fortimicin B

To determine the order of the reactivity of the primary amino groups, the reaction of FM-B and N-benzyloxycarbonyloxysuccinimide (Cbz-NOS) was examined under various conditions. Treatment of FM-B with 1 equivalent of Cbz-NOS in aqueous tetrahydrofuran or methanol gave 2′-N-benzyl-

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oxycarbonyl-FM-B(2′-N-Cbz-FM-B, **7**) as well as a smaller amount of 2′,6′-di-N-Cbz-FM-B (**8**) which were easily isolated by solvent extraction, followed by chromatography. When a larger amount of Cbz-NOS was applied, formation of **7** was decreased while **8** and 1,2′,6′-tri-N-Cbz-FM-B[8] (**6**) were increased in the reaction mixture (Table 1).

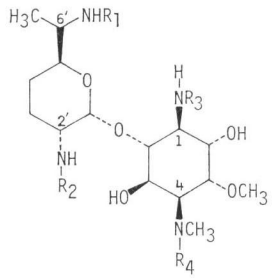
Table 1. Benzyloxycarbonylation of fortimicin B.

Experimental conditions			Yield (%)			
Cbz-NOS*	Et_3N*	Solvent	**4**	**7**	**8**	**6**
1.3	—	50% aq. THF	14	34	28	
3.0	3.1	〃			25	49
1.0	—	methanol	24	36	19	
1.5	—	〃	9	42	27	10
2.0	2.9	〃		19	48	9
3.1	4.0	〃			15	59

4: FM-B, **7**: 2′-N-Cbz-FM-B, **8**: 2′,6′-Di-N-Cbz-FM-B, **6**: 1,2′,6′-Tri-N-Cbz-FM-B.
* Equivalents to FM-B.

Treatment of FM-B with 1.5~2 equivalents of Cbz-NOS in methanol gave **7** and **8** in satisfactory yield. The yield of the positional isomers of the above products was almost negligible, although they were detected by tlc. Addition of triethylamine decreased the formation of the positional isomers of **7** and/or **8**, when 2 or more equivalents of Cbz-NOS were used. The order of the reactivity of the amino groups of FM-B to Cbz-NOS was 2′>6′>1>4 in basic conditions (Personal Communication by Dr. L. FREIBERG, Abbott Laboratories, *i.e.*, treatment of FM-B with 2.5 equivalents of Cbz-NOS in aqueous methanol at the pH 6 gave 1,2′-di-N-Cbz-FM-B as a main product, and no 2′,6′-isomer was detected by tlc. Presumably the reactivity of the 6′-amino group was decreased by protonation under these conditions).

Fig. 2. Protected fortimicin A and B derivatives.



Cbz: Benzyloxycarbonyl
Boc: *tert*-Butoxycarbonyl
Gly: Glycyl

	R_1	R_2	R_3	R_4		R_1	R_2	R_3	R_4
6	Cbz	Cbz	Cbz	H	**17**	Boc	Boc	H	H
7	H	Cbz	H	H	**18**	Boc	H	H	H
8	Cbz	Cbz	H	H	**19**	Cbz	Boc	Cbz	H
9	Boc	Cbz	H	H	**20**	Cbz	Boc	Cbz	Cbz-Gly
10	Boc	Cbz	Boc	H	**21**	Cbz	H	Cbz	Cbz-Gly
11	Cbz	Cbz	Boc	H	**22**	Boc	Boc	H	Boc-Gly
12	Boc	Cbz	Boc	Boc-Gly	**23**	Boc	Boc	Cbz	H
13	Boc	H	Boc	Boc-Gly	**24**	Boc	Boc	Cbz	Boc-Gly
14	Cbz	Cbz	Boc	Cbz-Gly	**25**	H	Cbz	Cbz	Cbz-Gly
15	Cbz	Cbz	H	Cbz-Gly	**26**	Boc	Cbz	Cbz	H
16	H	Boc	H	H	**27**	Boc	Cbz	Cbz	Cbz-Gly

The Structures of N-Benzyloxycarbonyl-fortimicin B Derivatives

Pmr parameters of 2′-N-Cbz-FM-B (**7**) and 2′,6′-di-N-Cbz-FM-B (**8**) are listed in Table 2. In the pmr spectrum of **7**, the five aromatic protons appeared at 7.27 ppm, indicating **7** is mono-N-Cbz-FM-B. The facts that the C-CH_3 and the N-CH_3 appeared at 1.08 and 2.36 ppm respectively, and they were observed at 1.36 and 2.56 ppm, respectively, under acidic conditions, revealed that 4- and 6′-amino were not benzyloxycarbonylated. Irradiation of the C-CH_3 caused collapse of the signal of H-6′ at 2.80 ppm. Irradiation at 4.9 ppm (the anomeric proton) did not affect the signals between 2.6 to 3.1 ppm, showing that the signal of H-2′ was absent in this region. Two proton signals at 2.96 ppm, therefore, were attributed to H-1 and H-4. These finding shows that the amino groups at 1, 4 and 6′ positions are not acylated. The position of the Cbz group must be 2′-N.

Table 2. Pmr parameters of 2′-N-Cbz-FM-B (**7**) and 2′,6′-di-N-Cbz-FM-B (**8**).[a]

Proton	Chemical shifts[b] (J in Hz)	
	2′-N-Cbz-FM-B (**7**)	2′,6′-Di-N-Cbz-FM-B (**8**)
H-1′	~4.9	4.90 (d, 3)
2′	3.4~3.8	3.4~4.0
3′, 4′	1.3~2.0	1.3~2.0
5′	3.4~3.8	3.4~4.0
6′	2.80 (q, 7)	~3.7
1	2.96 (t, 9.5)	3.02 (t, 9.5)
2	3.4~3.8	3.4~4.0
3	3.4~3.8	3.4~4.0
4	2.96 (dd, 4.5, 3)	3.02 (dd, 4.5, 3)
5	3.92 (dd, 10, 4.5)	3.92 (dd, 10, 4.5)
6	3.4~3.8	3.4~4.0
CH_3-6′	1.08 (d, 7) [1.36][c]	1.12 (d, 7) [1.14][c]
NCH_3	2.36 (s) [2.56][c]	2.36 (s) [2.53][c]
OCH_3	3.41 (s)	3.42 (s)
PhCH_2	5.01 (s)	5.03 (s)
Ph	7.27 (s)	7.26 (s)

[a] Measured in CD_3OD.
[b] ppm downfield from internal TMS.
[c] Chemical shifts of their hydrochlorides.

In the pmr spectrum of **8**, the presence of 10 aromatic protons at 7.26 ppm indicated **8** is di-N-Cbz-FM-B. The C-CH_3 doublet at 1.12 ppm and the anomeric proton doublet at 4.90 ppm were collapsed by irradiation at 3.7 ppm. These downfield shifts of the H-2′ and H-6′ revealed both amino groups at 2′ and 6′ positions were benzyloxycarbonylated, thus, **8** is 2′,6′-di-N-Cbz-FM-B.

The structures of **7** and **8** were supported by their cmr and the assignments are presented in Table 3.

Lithium aluminum hydride reduction of **7** and **8** gave 2′-N-methyl-FM-B and 2′,6′-di-N-methyl-FM-B, respectively. The mass spectrum of 2′-N-methyl-FM-B showed a distinct molecular ion at m/z 362, and an ion at m/z 157 which was attributable to the methylated 6-*epi*-purpurosamine B. The mass spectrum of 2′,6′-N,N-dimethyl-FM-B indicated a molecular ion at m/z 376, and an ion at m/z 171 attributable to N,N-dimethylated 6-*epi*-purpurosamine B. In the mass spectra of the both compounds, fragment ions at m/z 235 and 207 were observed, which revealed that aminocyclitol parts were not methylated in both cases.[3,5]

Selective Protection of Fortimicin A

tert-Butoxycarbonyl (Boc) groups can be removed without affecting benzyloxycarbonyl (Cbz) groups and *vice versa*. By combination of these protection methods selective protection of FM-A could be accomplished.

Treatment of 2′-N-Cbz-FM-B (**7**) with 1 equivalent of *tert*-butyl 4,6-dimethylpyrimid-2-ylthiocarbonate (Boc-S)[9] in methanol gave 2′-N-benzyloxycarbonyl-6′-N-*tert*-butoxycarbonyl-FM-B(2′-N-Cbz-6′-N-Boc-FM-B, **9**) as a main product, together with a smaller amount of 2′-N-Cbz-1,6′-di-N-Boc-FM-B (**10**). When 2 or more equivalents of Boc-S was used, **10** was obtained in good yield. Treatment

Table 3. Cmr parameters for 2′-N-Cbz-FM-B (7) and 2′,6′-di-N-Cbz-FM-B (8).[a,d]

	2′-N-Cbz-FM-B (7)[b]		2′,6′-Di-N-Cbz-FM-B (8)[c]	
	Free base	Hydrochloride	Free base	Hydrochloride
C-1′	99.6	98.4	101.4	100.2
2′	51.8	52.0	51.2	51.1
3′	24.5	22.9	25.3	24.4
4′	27.1	27.4	27.3	27.1
5′	74.7 (4.1)	70.6	72.9	72.1
6′	50.8	50.2	52.6	51.1
6′-CH_3	18.5	15.3	17.5	17.2
C-1	54.3	53.6	55.5	54.5
2	70.9 (5.6)	65.3	71.9 (5.3)	66.6
3	79.7 (6.1)	73.6	80.6 (6.0)	74.6
4	61.3	57.6	62.3	58.1
5	70.9 (5.6)	65.3	71.2 (5.1)	66.1
6	83.3 (9.7)	73.6	86.0 (10.3)	75.1
OCH_3	59.3	57.3	59.2	57.6
NCH_3	35.4	31.2	35.5	31.7
$PhCH_2CO$	157.9	158.2	158.0, 158.2	158.0, 158.5
$PhCH_2CO$	67.4	67.9	67.3	67.5
Ph C-1	137.3	136.9	138.2	138.0, 138.7
2, 6	128.5	128.9	128.7 ~ 129.3	128.7 ~ 129.5
3, 5	129.4	129.7		
4	129.0	129.4		

[a] Protonation shifts were given in parenthesis.
[b] In D_2O.
[c] In CD_3OD.

of 2′,6′-di-N-Cbz-FM-B (**8**) with Boc-S gave 2′,6′-di-N-Cbz-1-N-Boc-FM-B (**11**). Isolation and purification of **9**, **10** and **11** could be achieved by the same procedures as those used for di- and tri-Cbz-FM-B derivatives. The structures of **9**, **10** and **11** were easily deduced from their pmr spectra (see experimental section).

N-*tert*-Butoxycarbonylglycine (Boc-Gly) was condensed with the free amino group at 4-position of **10** by N,N′-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HBT)[8] to give 2′-N-Cbz-1,6′,2″-tri-N-Boc-FM-A* (**12**). Hydrogenolysis of **12** gave 1,6′,2″-tri-N-Boc-FM-A (**13**) which was a starting material for 2′-modified FM-A derivatives. When **10** was condensed with N-benzyloxycarbonylglycine (Cbz-Gly), the product could be used for 1,6′- or 2′,2″-modification of FM-A. **11** was condensed with Cbz-Gly to give 2′,6′,2″-tri-N-Cbz-1-N-Boc-FM-A (**14**), and which when treated with trifluoroacetic acid (TFA) gave 2′,6′,2″-tri-N-Cbz-FM-A (**15**). **15** was used as a starting material for the preparation of 1-N-modified-FM-A derivatives.

The order of reactivity of the amino groups of FM-B to *tert*-butoxycarbonylation with Boc-S was 2′>6′>1>4, similar to those of benzyloxycarbonylation with Cbz-NOS. 2′-N-Boc-FM-B (**16**) and 2′,6′-di-N-Boc-FM-B (**17**) were derived from FM-B by treatment with Boc-S in satisfactory yield, together with a small amount of 6′-N-Boc-FM-B (**18**). Benzyloxycarbonylation of 1- and 6′-amino

* The 2″-N refers to the amino group of the 4-N-glycyl moiety.

groups of **16** gave 1,6′-di-N-Cbz-2′-Boc-FM-B (**19**), which was condensed with Cbz-Gly to give 1,6′,2″-tri-N-Cbz-2′-Boc-FM-A (**20**). Treatment of **20** with TFA gave 1,6′,2″-tri-N-Cbz-FM-A (**21**). In the same sequence, 2′,6′,2″-tri-N-Boc-FM-A (**22**) was prepared from **17**, *via* intermediated **23** and **24**, and 1,2′,2″-tri-N-Cbz-FM-A (**25**) was prepared from **18**, *via* intermediates **26** and **27**. Reactions of Boc-S with FM-B were relatively slow compared to Cbz-NOS, especially to 1 and 4-amino groups. Therefore, **21** was prepared more easily than **13** from FM-B and both compounds are useful as starting materials for 1-N-modified FM-A. Similarly, **22** was prepared more easily than **15**.

Modification of FM-A at the 1,2′ and 6′-positions are reported in the subsequent paper.[10]

Experimental

Mass spectra were obtained on a JEOL JMS-01SG-2 spectrometer equipped with a field desorption-field ionization - electron impact combination ion-source, model MS-FD-01. The EI mass were measured at 30 eV. Pmr and cmr spectra were measured on a JEOL PS-100, a JEOL PFT-100 or a Varian T-60 spectrometer in the CW or FT mode. Chemical shifts of pmr were reported in ppm downfield from internal TMS. Chemical shifts of cmr were measured from internal dioxane (67.4 ppm) and reported in ppm downfield from TMS. Elemental analyses were performed on a Yanagimoto CHN Corder MT-1. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter.

2′-N-Benzyloxycarbonyl- and 2′,6′-di-N-benzyloxycarbonyl-fortimicin B (**7** and **8**)

To a stirred solution of FM-B (**4**, 348 mg, 1.0 mmole) in methanol (20 ml), Cbz-NOS (374 mg, 1.6 mmole) in tetrahydrofuran (5 ml) was added dropwise over a period of 1 hour in an ice-bath. The reaction was continued for another 1 hour under ice-cooling, followed by 16 hours at room temperature. The reaction mixture was evaporated to dryness, and the residue was partitioned between water (20 ml) and chloroform (20 ml). Organic layer was washed with water (10 ml×2), and combined aqueous layer was extracted with chloroform (10 ml). The aqueous layer was applied to a column of Amberlite CG-50 (NH_4^+, 20 ml). Elution was performed with 0.1 N NH_4OH to give **7** (201 mg, 42%), m.p. 95~98°C, $[\alpha]_D^{23}$ +35° (*c* 1.0, methanol). *Anal.* Calcd. for $C_{23}H_{38}N_4O_7$: C, 57.24; H, 7.94; N, 11.61. Found: C, 57.23; H, 7.94; N, 11.51.

Unreacted FM-B (32 mg, 9.2%) was eluted with 0.2 N NH_4OH. The organic layer was dried over sodium sulfate, the solvent was evaporated. The residue was applied to a column of silica gel (15 g). 1,2′,6′-Tri-N-Cbz-FM-B (**6**, 72 mg, 9.6%) was eluted with chloroform - methanol (96: 4). Elution with chloroform - methanol (9: 1) gave **8** (169 mg, 27%), m.p. 80~82.5°C, $[\alpha]_D^{23}$ +4.1° (*c* 1.0, methanol). *Anal.* Calcd. for $C_{31}H_{44}N_4O_9$: C, 60.37; H, 7.19; N, 9.09. Found: C, 60.36; H, 7.08; N, 9.18.

When FM-B was treated with 2 equivalents of Cbz-NOS, **8** was the main product (48%).

2′-N-Methyl- and 2′,6′-di-N-methyl-fortimicin B

To a solution of 2′-N-Cbz-FM-B (**7**, 600 mg, 1.24 mmole) in tetrahydrofuran (30 ml), excess lithium aluminum hydride was added and the reaction mixture was refluxed for 3 hours. After cooling, the mixture was poured into ice-water. Insoluble material was removed by filtration. After the pH of the filtrate was adjusted to 7, the solution was applied to a column of Amberlite CG-50 (NH_4^+, 30 ml), which was eluted with 0.2 N NH_4OH to give crude sample.

Further purification by silica gel (25 g) column chromatography eluted with lower phase of chloroform - methanol - 14% NH_4OH (2: 1: 1) gave pure 2′-N-methyl-FM-B (120 mg, 27%).

2′,6′-Di-N-Cbz-FM-B (**8**, 940 mg, 1.52 mmole) was treated with lithium aluminum hydride in the same procedure as above. Purification by Amberlite CG-50 (NH_4^+) chromatography gave 2′,6′-di-N-methyl-FM-B (350 mg, 61%).

2′-N-Benzyloxycarbonyl-6′-N-*tert*-butoxycarbonylfortimicin B (**9**) and 2′-N-benzyloxycarbonyl-1,6′-di-N-*tert*-butoxycarbonylfortimicin B (**10**)

A solution of 2′-N-Cbz-FM-B (**7**, 241 mg, 0.50 mmole) and Boc-S (115 mg, 0.48 mmole) in tetrahydrofuran (5 ml) was stirred at room temperature for 4 days. After evaporation of the solvent,

the residue was dissolved in ethyl acetate, washed with water, dried over sodium sulfate, filtered and then evaporated. The residue was chromatographed on a column of silica gel (10 g). The column, when eluted with chloroform - methanol (95: 5), gave **10** (74 mg, 22%). *Anal.* Calcd. for $C_{33}H_{54}N_4O_{11}$: C, 58.04; H, 7.97; N, 8.21. Found: C, 57.84; H, 7.99; N, 8.08. Pmr (CD_3OD): δ 1.15 (3H, d, J=6.5 Hz, CH_3-6'), 1.43 (18H, s, $C(CH_3)_3$), 2.37 (3H, s, NCH_3), 2.92 (1H, t, J=5 Hz, H-4), 3.47 (3H, s, OCH_3), 5.07 (2H, s, $PhCH_2$), 5.27 (1H, d, J=3 Hz, H-1'), 7.34 (5H, s, Ph).

Further elution with chloroform - methanol (9: 1) gave **9** (110 mg, 38%). *Anal.* Calcd. for $C_{28}H_{46}N_4O_9$: C, 57.71; H, 7.97; N, 9.62. Found: C, 57.62; H, 7.99; N, 9.53. Pmr (CD_3OD): δ 1.10 (3H, d, J=7 Hz, CH_3-6'), 1.43 (9H, s, $C(CH_3)_3$), 2.37 (3H, s, NCH_3), 2.97 (1H, t, J=9 Hz, H-1), 3.04 (1H, dd, J=3.5, 5 Hz, H-4), 3.47 (3H, s, OCH_3), 4.93 (1H, d, J=3 Hz, H-1'), 5.10 (2H, s, $PhCH_2$), 7.30 (5H, s, Ph). When **7** was treated with 2.5 equivalents of Boc-S, **10** was the main product (50%).

2',6'-Di-N-benzyloxycarbonyl-1-N-*tert*-butoxycarbonylfortimicin B (**11**)

2',6'-Di-N-Cbz-FM-B (**8**, 123 mg, 0.20 mmole) and Boc-S (240 mg, 1.0 mmole) in tetrahydrofuran (5 ml) were stirred for 2 days. After working up and chromatography in the similar manner to the preparation of **9** and **10**, **11** (77 mg, 54%) was obtained. $[\alpha]_D^{23}$ +42.4° (c 1.0, methanol). MS (m/z); 716 (M^+), 369, 277, 91. Pmr (CD_3OD): δ 1.12 (3H, d, J=6.5 Hz, CH_3-6'), 1.47 (9H, s, $C(CH_3)_3$), 2.33 (3H, s, NCH_3), 2.90 (1H, t, J=4 Hz, H-4), 3.42 (3H, s, OCH_3), 5.07 (4H, s, $PhCH_2$), 5.45 (1H, d, J=3 Hz, H-1'), 7.31 (10H, s, Ph).

2'-N-Benzyloxycarbonyl-1,6',2''-tri-N-*tert*-butoxycarbonylfortimicin A (**12**) and 1,6',2''-tri-N-*tert*-butoxycarbonylfortimicin A (**13**)

To an ice-cooled, stirred solution of Boc-Gly (0.77 g, 4.40 mmole) and HBT (0.67 g, 5.62 mmole) in tetrahydrofuran (60 ml) was added DCC (0.90 g, 4.4 mmole). After 1 hour 2'-N-Cbz-1,6'-di-N-Boc-FM-B (**10**, 2.50 g, 3.66 mmole) was added to the reaction mixture and the solution was stirred at room temperature for 18 hours. After removal of insoluble material, the solvent was evaporated. The residue was chromatographed on a column of silica gel (100 g). Elution with chloroform - methanol (97: 3) gave **12** (2.55 g, 83%). $[\alpha]_D^{23}$ +45.2° (c 1.0, methanol). *Anal.* Calcd. for $C_{40}H_{65}N_5O_{14}\cdot\frac{1}{2}H_2O$: C, 56.59; H, 7.83; N, 8.25. Found: C, 56.41; H, 7.82; N, 8.39.

12 (2.2 g, 2.62 mmole) was dissolved in methanol (50 ml), acetic acid (2 ml) and 10% Pd-C were added to the solution, then hydrogen was added. The catalyst was removed by filtration and the solvent was evaporated. The residue was dissolved in ethyl acetate, washed with 5% sodium bicarbonate solution followed by water and dried over sodium sulfate, then filtered. Evaporation of the solvent gave **13** (1.80 g, 97%). Pmr (CD_3OD): δ 1.17 (3H, d, J=6.5 Hz, CH_3-6'), 1.45 (27H, s, $C(CH_3)_3$), 3.08 (3H, s, NCH_3), 3.38 (3H, s, OCH_3).

2',6',2''-Tri-N-benzyloxycarbonyl-1-N-*tert*-butoxycarbonylfortimicin A (**14**) and 2',6',2''-tri-N-benzyloxycarbonylfortimicin A (**15**)

To a solution of Cbz-Gly (335 mg, 1.6 mmole) and HBT (216 mg, 1.81 mmole) in tetrahydrofuran (20 ml) was added DCC (331 mg, 1.60 mmole) and the solution was stirred for 1 hour under ice-cooling. 2',6'-Di-N-Cbz-1-N-Boc-FM-B (**11**, 885 mg, 1.24 mmole) was added to the solution, then the solution was stirred at room temperature overnight. After removal of insoluble material, the solvent was evaporated. The residue was chromatographed on a column of silica gel (50 g). Elution with chloroform - methanol (98: 2) gave **14** (258 mg, 23%). Pmr (CD_3OD): δ 1.16 (3H, d, J=6 Hz, CH_3-6'), 1.40 (27H, s, $C(CH_3)_3$), 2.94 and 3.03 (3H, each s, NCH_3), 3.33 (3H, s, OCH_3), 5.00 and 5.03 (2H, each s, $PhCH_2$), 7.20 and 7.24 (5H, each s, Ph).

14 (258 mg, 0.28 mmole) was dissolved in a mixture of methylene chloride (2 ml) and TFA (1 ml), and the solution was stirred at room temperature for 2 hours. The solvent was evaporated and the residue was dissolved in ethyl acetate, washed with 5% sodium bicarbonate solution, then water, and dried over sodium sulfate. The solvent was evaporated and the residue was chromatographed on a column of silica gel (5 g). Elution with chloroform - methanol (97: 3) gave **15** (168 mg, 73%). $[\alpha]_D^{23}$ +50.6° (c 1.0, methanol). $MH^+ = m/z$ 808 (FD-MS).

2'-N-*tert*-Butoxycarbonyl-, 6'-N-*tert*-butoxycarbonyl- and 2',6'-di-N-*tert*-butoxycarbonylfortimicin

B (**16**, **18** and **17**)

FM-B (2.3 g, 6.60 mmole) and Boc-S (1.9 g, 7.9 mmole) were dissolved in 50% aqueous tetrahydrofuran (120 ml) and allowed to stand at room temperature for 8 days. After evaporation of the solvent, water (40 ml) was added and the solution was washed with ethyl acetate. The pH of the aqueous layer was adjusted to 5.5 and applied to a column of Amberlite CG-50 (NH_4^+, 140 ml). The column was eluted with 0.05 N NH_4OH to give **18** (0.32 g, 11%). $[\alpha]_D^{23}$ −3.6° (*c* 1.0, methanol). MS (*m/z*): 448 (M^+), 375, 261, 235, 207. Pmr (CD_3OD): δ 1.10 (3H, d, *J*=6.5 Hz, CH_3-6′), 1.40 (9H, s, $C(CH_3)_3$), 2.39 (3H, s, NCH_3), 3.43 (3H, s, OCH_3), 4.90 (1H, d, *J*=3 Hz, H-1′). Irradiation at 3.4 ppm collapsed CH_3-6′ doublet.

Further elution of the column with 0.1 N NH_4OH gave **16** (0.84 g, 28%). $[\alpha]_D^{23}$ +37.7° (*c* 1.0, methanol). MS (*m/z*): 448 (M^+), 360, 260, 235, 207, 187. Pmr (CD_3OD): δ 1.07 (3H, d, *J*=6.5 Hz, CH_3-6′), 1.43 (9H, s, $C(CH_3)_3$), 2.41 (3H, s, NCH_3), 3.47 (3H, s, OCH_3), 4.98 (1H, d, *J*=3 Hz, H-1′). Irradiation at 3.6 ppm collapsed H-1′ doublet. Elution with 0.2 N NH_4OH gave unreacted FM-B (0.73 g, 32%).

The same reactions were repeated (total FM-B used were 8.4 g, 24.1 mmole), and the combined ethyl acetate layer was concentrated. The residue was chromatographed on a column of silica gel (60 g). Elution with chloroform - methanol (9: 1) gave **17** (1.6 g, 12%). $[\alpha]_D^{23}$ −0.2° (*c* 1.0, methanol). MS (*m/z*): 548 (M^+), 475, 287, 235, 231, 187. Pmr (CD_3OD): δ 1.10 (3H, d, *J*=6 Hz, CH_3-6′), 1.43 (18H, s, $C(CH_3)_3$), 2.41 (3H, s, NCH_3), 3.45 (3H, s, OCH_3), 4.85 (1H, d, *J*=3 Hz, H-1′).

1,6′-Di-N-benzyloxycarbonyl-2′-N-*tert*-butoxycarbonylfortimicin B (**19**)

2′-N-Boc-FM-B (**16**, 2.00 g, 4.46 mmole), triethylamine (1.37 ml) and Cbz-NOS (2.44 g, 10.5 mmole) in chloroform (120 ml) were stirred at room temperature for 3 hours. The solution was washed with water, dried and evaporated. The residue was chromatographed on a column of silica gel (70 g), which was eluted with chloroform - methanol (96: 4) to give **19** (2.42 g, 76%). $[\alpha]_D^{23}$ +19.6° (*c* 0.5, methanol). MS (*m/z*): 716 (M^+), 369, 321, 277, 91. Pmr (CD_3OD): δ 1.02 (3H, d, *J*=6 Hz, CH_3-6′), 1.40 (9H, s, $C(CH_3)_3$), 2.37 (3H, s, NCH_3), 3.47 (3H, s, OCH_3), 5.03 (4H, s, $PhCH_2$), 5.31 (1H, br, H-1′), 7.30 (10H, s, Ph).

1,6′,2″-Tri-N-benzyloxycarbonyl-2′-N-*tert*-butoxycarbonylfortimicin A (**20**)

Cbz-Gly (175 mg, 0.85 mmole), HBT (113 mg, 0.95 mmole) and DCC (200 mg, 0.97 mmole) in tetrahydrofuran (20 ml) were stirred under ice-cooling for 1.5 hours. To the solution 1,6′-di-N-Cbz-2′-N-Boc-FM-B (**19**, 500 mg, 0.70 mmole) in tetrahydrofuran (10 ml) was added dropwise. After the solution was stirred for 2 days, the solvent was evaporated. Chloroform was added to the residue and insoluble material was removed by filtration. The filtrate was washed with water, dried and the solvent was evaporated. The residue was chromatographed on a column of silica gel (35 g). The column was eluted with chloroform - methanol (98: 2) to give **20** (540 mg, 85%). $[\alpha]_D^{23}$ +49.0° (*c* 1.0, methanol). *Anal.* Calcd. for $C_{46}H_{61}N_5O_{14}$: C, 60.84; H, 6.77; N, 7.71. Found: C, 60.60; H, 6.81; N, 7.76. Pmr (CD_3OD): δ 1.13 (3H, d, *J*=6 Hz, CH_3-6′), 1.35 (9H, s, $C(CH_3)_3$), 3.07 (3H, s, NCH_3), 3.35 (3H, s, OCH_3), 5.03 and 5.07 (6H, each s, $PhCH_2$), 7.29 (15H, s, Ph).

1,6′,2″-Tri-N-benzyloxycarbonylfortimicin A (**21**)

To a solution of 1,6′,2″-tri-N-Cbz-2′-N-Boc-FM-A (**20**, 500 mg, 0.55 mmole) in chloroform (5 ml), TFA (2 ml) was added and the solution was stirred at room temperature for 1 hour. After the solvent was evaporated, the residue was dissolved in ethyl acetate and the solution was washed with saturated sodium bicarbonate, water and saturated sodium chloride. The solution was dried over sodium sulfate, filtered and the solvent was evaporated to give **21** (450 mg, quant.). $[\alpha]_D^{23}$ +45.4° (*c* 0.5, methanol). $MH^+ = m/z$ 808 (FD-MS). Pmr (CD_3OD): δ 1.13 (3H, d, *J*=6 Hz, CH_3-6′), 3.07 (3H, s, NCH_3), 3.37 (3H, s, OCH_3), 5.06 and 5.10 (6H, each s, $PhCH_2$), 5.48 (1H, br, H-1′), 7.30 (15H, s, Ph).

1-N-Benzyloxycarbonyl-2′,6′-di-N-*tert*-butoxycarbonylfortimicin B (**23**)

2′,6′-Di-N-Boc-FM-B (**17**, 3.0 g, 5.47 mmole) and Cbz-NOS (1.63 g, 6.99 mmole) in chloroform (60 ml) was stirred at room temperature for 18 hours. The solvent was evaporated and the residue was chromatographed on a column of silica gel (100 g). The column was eluted with chloroform - methanol (95: 5) to give **23** (2.2 g, 59%). $[\alpha]_D^{23}$ +16.4° (*c* 0.5, methanol). MS (*m/z*): 682 (M^+), 651, 369, 287,

231, 91. Pmr (CD_3OD): 1.02 (3H, d, J=6 Hz, CH_3-6′), 1.43 (18H, s, $C(CH_3)_3$), 2.42 (3H, s, NCH_3), 2.97 (1H, t, J=6 Hz, H-4), 3.50 (3H, s, OCH_3), 5.10 (2H, br, $PhCH_2$), 5.30 (1H, br, H-1′), 7.37 (5H, s, Ph).

1-N-Benzyloxycarbonyl-2′,6′,2″-tri-N-*tert*-butoxycarbonylfortimicin A (**24**) and 2′,6′,2″-tri-N-*tert*-butoxycarbonylfortimicin A (**22**)

To an ice-cooled solution of Boc-Gly (620 mg, 3.54 mmole) and HBT (470 mg, 3.95 mmole) in tetrahydrofuran (80 ml) was added DCC (730 mg, 3.54 mmole), and stirred for 1 hour under ice-cooling. To the reaction mixture was added 1-N-Cbz-2′,6′-di-N-Boc-FM-B (**23**, 2.00 g, 2.93 mmole), and the solution was stirred for 2 days. After evaporation of the solvent, ethyl acetate was added to the residue and insoluble material was removed by filtration. The filtrate was washed with water, dried over sodium sulfate and the solvent was evaporated. The residue was chromatographed on a column of silica gel (100 g), the column was eluted with chloroform - methanol (98: 2) to give **24** (2.04 g, 83%). $[\alpha]_D^{23}$ +51.4° (c 1.0, methanol). *Anal.* Calcd. for $C_{40}H_{65}N_5O_{14}\cdot\frac{1}{2}H_2O$: C, 56.59; H, 7.83; N, 8.25. Found: C, 56.65; H, 7.92; N, 8.46.

24 (2.00 g, 2.38 mmole) was dissolved in a mixture of methanol (50 ml) and acetic acid (0.4 ml). To the solution was added 10% Pd-C and hydrogen was bubbled through. The catalyst was removed and the solvent was evaporated. The residue was dissolved in ethyl acetate and the solution was washed with 5% sodium bicarbonate solution, water, dried over sodium sulfate, filtered and the solvent was evaporated to give **22** (1.4 g, 83%). $[\alpha]_D^{23}$ +52.8° (c 0.5, methanol). $MH^+=m/z$ 706 (FD-MS). Pmr (CD_3OD): δ 1.13 (3H, d, J=6 Hz, CH_3-6′), 1.47 (27H, s, $C(CH_3)_3$), 3.10 (3H, s, NCH_3), 3.50 (3H, s, OCH_3).

1,2′-Di-N-benzyloxycarbonyl-6′-N-*tert*-butoxycarbonylfortimicin B (**26**)

6′-N-Boc-FM-B (**18**, 1.7 g, 3.79 mmole) and Cbz-NOS (2.0 g, 8.58 mmole) in chloroform (100 ml) were stirred at room temperature for 3 hours. The solution was washed with water, dried over sodium sulfate, filtered and the solvent was evaporated. The residue was chromatographed on a column of silica gel (60 g), the column was eluted with chloroform - methanol (98: 2) to give **26** (2.0 g, 74%). Pmr (CD_3OD): δ 1.03 (3H, d, J=6.5 Hz, CH_3-6′), 1.43 (9H, s, $C(CH_3)_3$), 2.37 (3H, s, NCH_3), 3.47 (3H, s, OCH_3), 5.02 (4H, s, $PhCH_2$), 5.33 (1H, br, H-1′), 7.27 (10H, s, Ph).

1,2′,2″-Tri-N-benzyloxycarbonyl-6′-N-*tert*-butoxycarbonylfortimicin A (**27**) and 1,2′,2″-tri-N-benzyloxycarbonylfortimicin A (**25**)

Cbz-Gly (610 mg, 2.92 mmole), HBT (390 mg, 3.27 mmole) and DCC (660 mg, 3.20 mmole) in tetrahydrofuran (50 ml) were stirred under ice-cooling for 1 hour. 1,2′-Di-N-Cbz-6′-N-Boc-FM-B (**26**, 1.90 g, 2.65 mmole) was added to the solution, and the solution was stirred for 18 hours. After insoluble material was removed by filtration, the solvent was evaporated. The residue was chromatographed on a column of silica gel (60 g). The column was eluted with chloroform - methanol (98: 2) to give **27** (1.8 g, 75%).

27 (1.6 g, 1.76 mmole) was dissolved in chloroform (10 ml). After addition of TFA (5 ml), the solution was stirred at room temperature for 1 hour. The solvent was evaporated and the residue was dissolved in ethyl acetate. The solution was washed with saturated sodium bicarbonate, water and dried over sodium sulfate. Evaporation of the solvent gave **25** (1.1 g, 77%). $[\alpha]_D^{23}$ +57.8° (c 1.0, methanol). $MH^+=m/z$ 808 (FD-MS). Pmr (CD_3OD): δ 1.13 (3H, d, J=6 Hz, CH_3-6′), 3.03 (3H, s, NCH_3), 3.33 (3H, s, OCH_3), 5.00 and 5.66 (6H, each s, $PhCH_2$), 7.23 and 7.30 (15H, each s, Ph).

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