SUBSTANCES DERIVED FROM 4-DE-*N*-METHYLFORTIMICIN B

PAUL KURATH, JACK TADANIER, PAULETTE JOHNSON, DAVE GRAMPOVNIK,
RICHARD S. EGAN, RUTH S. STANASZEK, MOMIR CIROVIC,
WILLIAM H. WASHBURN and JAMES E. LEONARD

Department of Chemical Research and Department of Analytical Research
Abbott Laboratories, North Chicago, Illinois 60064, U.S.A.

(Received for publication December 29, 1980)

The preparation of 4-de-*N*-methylfortimicin A analogs as well as the preparation of 4-de-*N*-methyl-4-*N*-(β-aminoethyl)-4-*N*-ethylfortimicin B is reported. It was shown that the 4-*N*-methyl group in fortimicin analogs is essential for antibacterial activity since neither the 4-de-*N*-methylfortimicin A nor the 4-de-*N*-methyl-4-*N*-(β-aminoethyl)-4-*N*-ethylfortimicin B exhibited useful biological activity.

The chemical structure[1] and the antimicrobial properties[2] of the aminoglycoside antibiotic fortimicin A were reported in 1977. As a part of a continuing effort to correlate the effect of chemical modifications of fortimicin A and the biological properties of the derived substances, it was decided to prepare a series of 4-de-*N*-methylfortimicin A analogs. The desired 4-de-*N*-methyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**2**), an intermediate needed in the synthesis of such analogs, was obtained by Ruschig degradation of the previously described 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**1**).[3] Reaction of **2** with the *N*-hydroxysuccinimide active ester of *N*-benzyloxycarbonylglycine gave rise to 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-4-de-*N*-methylfortimicin A (**3a**) which upon hydrogenolysis in methanolic hydrochloric acid over palladium-on-carbon[3] afforded the tetrahydrochloride salt of **4a**. The lack of antimicrobial activity of the tetrahydrochloride salt of **4a** was observed.

Detailed studies of the NMR spectra of the fortimicin A and fortimicin B free bases in aqueous solutions revealed that the aminocyclitol portion of fortimicin A adopts that chair conformation in which the 4-N(CH_3)$COCH_2NH_2$ group is equatorial while the aminocyclitol portion of fortimicin B prefers that conformation in which the 4-$NHCH_3$ group is axial.[1]

Spin decoupling experiments carried out at 100 MHz on **4a** (free base) in aqueous solution reveal that the coupling constants $J_{1,2}$, $J_{5,6}$ and $J_{1,6}$ are 9.3 Hz each. This shows that the aminocyclitol of **4a** has four axial protons and in aqueous solution, assumes the fortimicin B conformation.

In Table 1 the ^{13}C-NMR spectra of **4a**, fortimicin A, and fortimicin B are compared at basic pD-values (~10). The spectrum of **4a** shows the absence of the 4-*N*-CH_3 signal which appears at 36.0 ppm and 32.3 ppm in fortimicin B and fortimicin A, respectively. The comparison of the purpurosamine chemical shifts in Table 1 shows that they are almost identical for the three compounds. When one takes into consideration that the 4-*N*-CH_3 group is missing in **4a** it is quite reasonable that the signal of C-4 is shifted upfield from 60.8 ppm in fortimicin B to 50.1 ppm in **4a**. When the effect of the 4-*N*-CH_3 group is considered, the aminocyclitol resonances recorded in Table 1 show that the values of **4a** are more similar to those of fortimicin B than to those of fortimicin A.

This finding is in agreement with the above conclusion, based on the study of the ^{1}H-NMR spectra of the three compounds. that the aminocyclitol conformation of **4a** resembles that of fortimicin B.

It was postulated that the introduction of more space-filling substituents at the 4-NH_2 group would result in 4-de-*N*-methylfortimicin A analogs in which the 4-NH-aminoacyl group would force the diaminocyclitol portion of the molecule into that chair conformation in which the 4-NH-aminoacyl group is equatorial. The intermediates **3b** and **3c** were prepared from **2** by coupling with the *N*-hydroxy-5-norbornene-2,3-dicarboximide active esters of L-4-benzyloxycarbonylamino-2-hydroxybutyric acid and L-benzyloxycarbonylisoleucine, respectively. Deprotection of the intermediates **3b** and **3c** in the usual manner[3] led to the isolation of the tetrahydrochloride salts of **4b** and **4c**, respectively.

Table 1. ^{13}C Chemical shifts of **4a**, fortimicin B and fortimicin A at pD~10.

Carbon	**4a**	Fortimicin B	Fortimicin A
C=O(Gly)	167.8	—	168.8
1′	102.4	102.4	100.1
6	83.3	84.1	78.4
3	80.6	79.9	73.0
5′	75.3	75.1	75.1
5	71.1	71.1	71.1
2	69.9	71.1	73.6
4	50.1	60.8	55.0
3-OCH_3	59.0	59.3	56.4
1	53.6	53.8	52.5
6′	50.5	50.6	50.5
2′	50.3	50.4	50.2
CH_2(Gly)	44.6	—	43.3
NCH_3	—	36.0	32.3
4′	27.3	27.4	27.3
3′	27.0	27.1	27.1
7′-CH_3	18.6	18.7	18.7

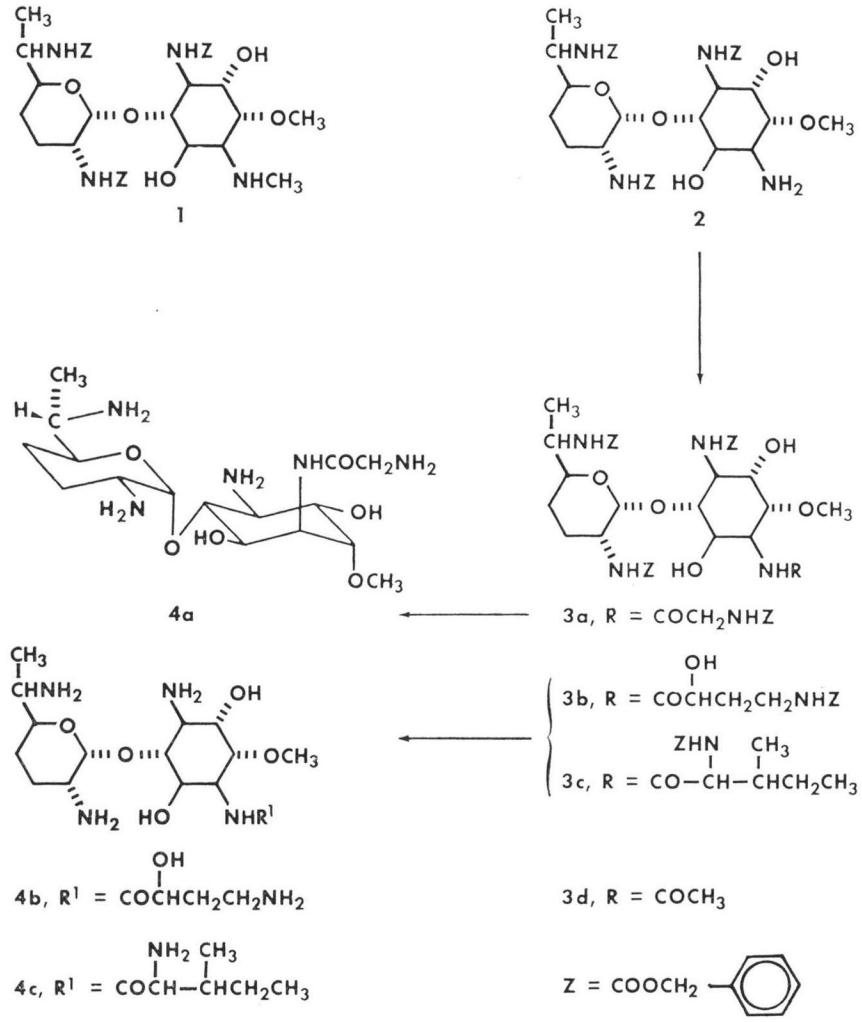
The tetrahydrochloride salts of both, **4b** and **4c**, were found to be biologically inactive. NMR studies of the free bases **4b** and **4c** did not lead to a definite conclusion regarding their conformation. The new substituents caused the spectra to be more complex and thus less amenable to interpretation.

It appeared of interest to make 4-de-*N*-methyl-4-*N*-ethylfortimicin A analogs in order to obtain substances in which the fortamine ring of the molecule would be forced into the fortimicin A conformation[1] and examine the biological effect of the replacement of the 4-*N*-methyl group in fortimicin A by an ethyl group.

A reaction of **2** with *N*-acetoxy-5-norbornene-2,3-dicarboximide afforded 4-de-*N*-methyl-4-*N*-acetyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**3d**), and the reduction of **3d** with 1 M borane tetrahydrofuran complex in a tetrahydrofuran solution[4] yielded 4-de-*N*-methyl-4-*N*-ethyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**5**). The reaction of **2** with acetaldehyde followed by treatment of the reaction mixture with sodium cyanoborohydride in an aqueous methanolic buffer solution[5] likewise afforded **5**.

Reaction of **5** with the *N*-hydroxysuccinimide active *N*-benzyloxycarbonylglycyl ester gave rise to the desired 4-de-*N*-methyl-4-*N*-ethyl-4-*N*-(*N*-benzyloxycarbonylglycyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**6**). This substance was found to decompose to **5** during silica gel chromatography in solvent systems containing alcohols. The intermediate **6** could be successfully purified by silica gel chromatography in ethyl acetate. When **5** was allowed to react with the *N*-hydroxy-5-norbornene-2,3-dicarboximide active *N*-benzyloxycarbonyl-β-alanyl ester, the product isolated from the reaction was not the expected 4-*N*-substituted fortimicin derivative since the tertiary amide band in the IR of the substance was missing. The compound of the reaction was formulated as 4-de-*N*-methyl-4-*N*-ethyl-5-*O*-(*N*-benzyloxycarbonyl-β-alanyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**7**) by analogy with a similar 5-*O*-acylation which occurred as a consequence of intramolecular base catalysis by the 4-*N*-amino group.[6]

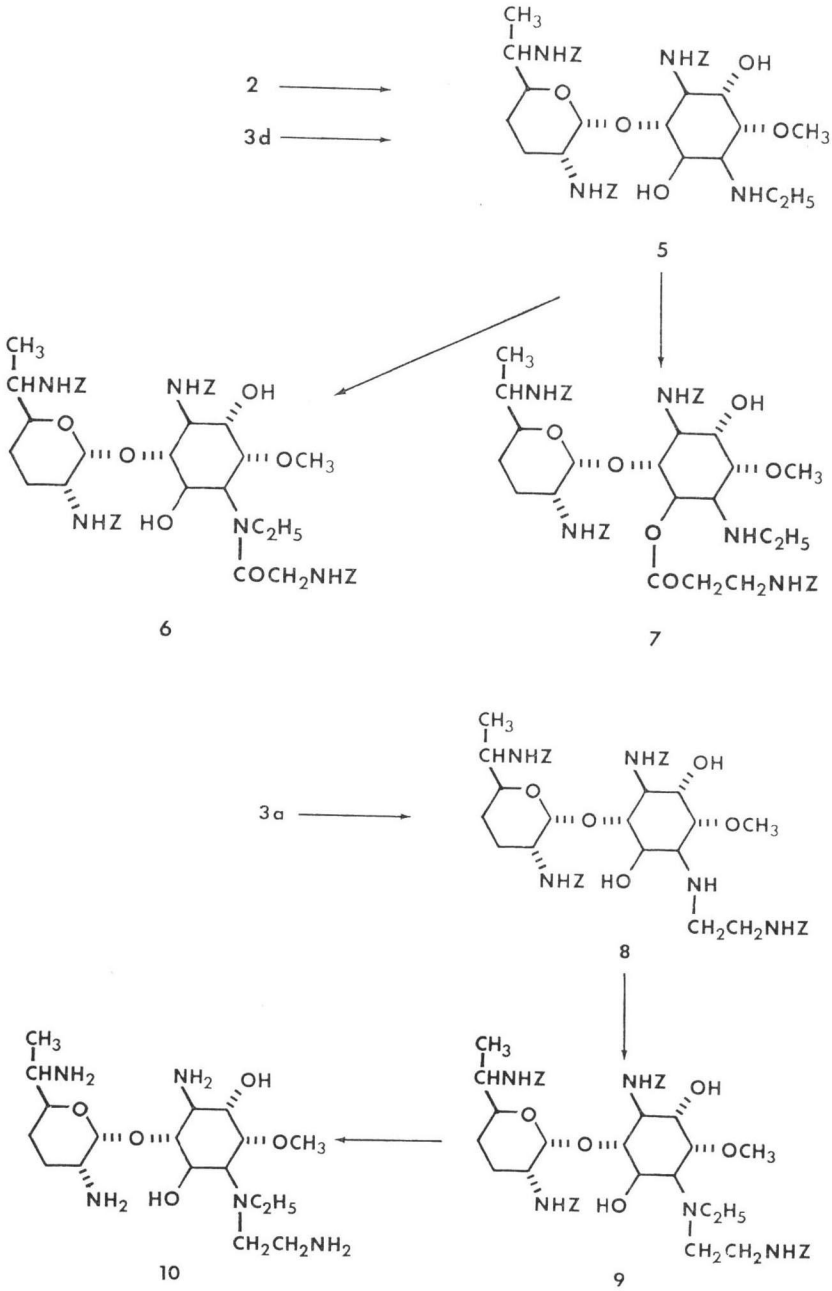
Both compounds, **6** and **7**, on deprotection under the usual conditions[3] gave rise to decomposition



mixtures which were not further characterized. In order to examine the biological effect of replacement of the 4-*N*-methyl group by a 4-*N*-ethyl group in a fortimicin derivative which would be stable, we decided to prepare 4-de-*N*-methyl-4-*N*-(β-aminoethyl)-4-*N*-ethylfortimicin B (**10**). In the latter substance (**10**) the 4-*N*-methyl group of 4-*N*-(β-aminoethyl)fortimicin B, which was previously shown to exhibit good antimicrobial activity,[4] is replaced by a 4-*N*-ethyl group.

The above prepared intermediate **3a** was treated with a 1 M borane tetrahydrofuran complex[4] to afford 4-de-*N*-methyl-4-*N*-(*N*-benzyloxycarbonyl-β-aminoethyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**8**). This compound, **8**, was allowed to react with acetaldehyde and the resulting reaction mixture was treated with sodium cyanoborohydride[5] to yield 4-de-*N*-methyl-4-*N*-(*N*-benzyloxycarbonyl-β-aminoethyl)-4-*N*-ethyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**9**). Deprotection[3] of **9** afforded the desired pentahydrochloride salt of 4-de-*N*-methyl-4-*N*-(β-aminoethyl)-4-*N*-ethylfortimicin B (**10**). The latter was found to be biologically inactive.

The work outlined in this paper leads to the conclusion that the 4-*N*-methyl group in fortimicin analogs is essential for biological activity. Neither the 4-de-*N*-methylfortimicin A analogs (**3a** ~ **3c**) nor



the 4-de-*N*-methyl-4-*N*-ethyl derivative **10** exhibited useful antimicrobial activity.

Experimental

General Methods

All evaporations were conducted with a rotary evaporator under reduced pressure. Silica gel chromatography was performed on Silica Woelm 32-63 (particle size 32~63 μm, weight per ml about 0.4 g). Optical rotations were obtained on a Hilger and Watts polarimeter. IR spectra were recorded with a

Perkin-Elmer Model 521 grating spectrometer. ^{1}H-NMR spectra were determined at 100 MHz with a Varian Associates HA-100 spectrometer. Chemical shifts are reported in ppm from internal tetramethylsilane ($\delta=0$) for the spectra recorded of compounds in deuteriochloroform ($CDCl_3$) solutions, and in ppm from external tetramethylsilane ($\delta=0$) for the spectra recorded of compounds in deuterium oxide (D_2O) solutions. ^{13}C-NMR spectra were determined at 25.2 MHz with a Varian Associates XL-100-15/NTC TT-100 spectrometer system. Chemical shifts are reported downfield from TMS and were measured from internal dioxane (67.4 ppm). Mass spectra were recorded with an A.E.I. MS-902 mass spectrometer with an ionization energy of 70 eV.

4-De-*N*-methyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (2)

A solution of 6.76 g of 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**1**)[3] and 1.42 g of *N*-chlorosuccinimide in 220 ml of dichloromethane was stirred at room temperature for 40 minutes. The reaction mixture was diluted with 350 ml of dichloromethane and the solution was washed with 400 ml of water, then with two 300-ml portions of water and finally with 300 ml of a saturated sodium chloride solution. The aqueous layers were extracted in series with two 300-ml portions of dichloromethane, the dichloromethane extracts were dried over anhydrous magnesium sulfate, filtered, combined, and evaporated to leave a residue of 6.75 g of 4-*N*-chloro-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B.

A solution of the above prepared 4-*N*-chloro derivative (6.75 g) and 9.25 g of 1,8-diazabicyclo-[5.4.0]undec-7-ene in 185 ml of benzene was stirred at room temperature for one hour and then at reflux temperature for an additional hour. After cooling, 185 ml of water was added and the mixture was stirred at room temperature for one hour. After the addition of 800 ml of benzene to the mixture, the aqueous phase was separated and extracted with two 500-ml portions of benzene. The benzene layers were washed with three 300-ml portions of a 5% aqueous sodium bicarbonate solution and then with five 100-ml portions of a saturated sodium chloride solution. The organic extracts were dried over anhydrous magnesium sulfate, filtered, combined, and evaporated to leave a residue of 6.28 g. This residue was purified by chromatography on 280 g of silica gel using benzene - methanol (85: 15, v/v) as the eluent. From the early fractions of the chromatogram 3.48 g of by-products not containing **2** were obtained after evaporation of the solvent.

The residues of a second group of fractions containing the desired substance **2** and the same nonpolar compounds as the preceding fractions amounted to 1.70 g. After rechromatography of this mixture 0.26 g of the desired product **2** was isolated.

Further elution of the original column, combination and evaporation of the appropriate fractions afforded a residue of 0.82 g of 4-de-*N*-methyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**2**).

Table 2. Physical constants of 4-de-*N*-methyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**2**) and its 4-*N*-acylated derivatives (**3a**~**3d**).

Measurement	**2**	**3a** R=$COCH_2NHZ$	**3b** R=$COCH(OH)\cdot CH_2CH_2NHZ$	**3c** R=$COCH(NHZ)\cdot CH(CH_3)CH_2CH_3$	**3d** R=$COCH_3$
Sum formula	$C_{38}H_{48}N_4O_{11}\cdot\frac{1}{2}H_2O$	$C_{48}H_{57}N_5O_{14}$	$C_{50}H_{61}N_5O_{15}$	$C_{52}H_{65}N_5O_{14}$	$C_{40}H_{50}N_4O_{12}$
Anal. Calcd.	C 61.19 H 6.62 N 7.51%	C 62.12 H 6.19 N 7.55%	C 61.78 H 6.33 N 7.20%	C 63.46 H 6.66 N 7.12%	C 61.68 H 6.47 N 7.19%
Found	C 61.30 H 6.52 N 7.32%	C 61.97 H 6.31 N 7.52%	C 61.93 H 6.61 N 7.25%	C 63.72 H 6.75 N 7.04%	C 61.32 H 6.56 N 7.07%
Optical rotation (c, $CHCl_3$)	$[\alpha]_D^{24}+29°$ (c 1.07)	$[\alpha]_D^{26}+23°$ (c 0.98)	$[\alpha]_D^{22}+18°$ (c 1.02)	$[\alpha]_D^{22}+22°$ (c 1.08)	$[\alpha]_D^{24}+35°$ (c 1.02)
IR $\nu_{max}^{CDCl_3}$ cm^{-1}	1699, 1498	1702, 1503	1698, 1505	1702, 1500	1700, 1503
^{1}H NMR ($CDCl_3$) δ ppm	7.3 (Ar-Z) 5.07 (CH_2-Z) 3.40 (OCH_3) 1.0 (7′-CH_3)	7.28 (Ar-Z) 5.04 (CH_2-Z) 3.39 (OCH_3) 0.95 (7′-CH_3)	7.27 (Ar-Z) 5.02 (CH_2-Z) 3.37 (OCH_3) 0.97 (7′-CH_3)	7.28 (Ar-Z) 5.04 (CH_2-Z) 3.39 (OCH_3) 0.87 (7′-CH_3)	7.3 (Ar-Z) 5.07 (CH_2-Z) 3.47 (OCH_3) 1.93 ($COCH_3$) 1.00 (7′-CH_3)

After rechromatography of the above obtained 4-de-*N*-methyl derivative **2** on silica gel in benzene - methanol (85: 15, v/v), an analytical sample of **2** was obtained, the physical constants of which are recorded in Table 2.

4-De-*N*-methyl-4-*N*-(*N*-benzyloxycarbonylglycyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**3a**)

A solution of 0.92 g of the above prepared **2** and 0.78 g of the *N*-hydroxysuccinimide active *N*-benzyloxycarbonylglycyl ester in 5 ml of tetrahydrofuran containing 5 drops of triethylamine was stirred at room temperature for 22 hours. Evaporation of the solvent left a residue of 1.78 g which was purified by chromatography on 180 g of silica gel using a mixture of 1,2-dichloroethane - methanol - ethanol - concentrated ammonium hydroxide (1170: 35: 135: 10, v/v) as the eluting solvent. The early fractions of the chromatogram contained **3a** which was contaminated by less polar material. The residue from these fractions amounted to 0.24 g. Further elution of the column followed by combination and evaporation of the appropriate fractions led to the isolation of 0.86 g of the desired substance **3a**. The physical constants of this compound are listed in Table 2.

4-De-*N*-methyl-4-*N*-(L-4-*N*-benzyloxycarbonylamino-2-hydroxybutyryl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**3b**)

The *N*-hydroxy-5-norbornene-2,3-dicarboximide active ester of L-4-*N*-benzyloxycarbonylamino-2-hydroxybutyric acid was prepared according to a previously published procedure[7] by reacting 0.58 g of L-4-*N*-benzyloxycarbonylamino-2-hydroxybutyric acid and 0.4 g of *N*-hydroxy-5-norbornene-2,3-dicarboximide in 3 ml of tetrahydrofuran - dioxane (1: 1, v/v) with 0.47 g of dicyclohexylcarbodiimide in the cold. The dicyclohexylurea was collected on a filter and washed with three 1-ml portions of tetrahydrofuran - dioxane (1: 1, v/v).

The filtrate containing the active ester was allowed to react with 0.72 g of 4-de-*N*-methyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**2**) for 24 hours. Evaporation of the solvent left a residue of 1.83g of crude coupling product which was purified by chromatography on 130 g of silica gel in a benzene - methanol - ethanol - concentrated ammonium hydroxide (1170: 34: 136: 10, v/v) solvent mixture.

The residues from the fractions containing the desired compound (**3b**) amounted to 0.66 g. The substance was further purified by chromatography on 120 g of silica gel using benzene - methanol (85: 15, v/v) as the eluent to afford 0.58 g of compound. After Sephadex LH-20 filtration in 95% ethanol a yield of 0.56 g of analytically pure **3b** was obtained. The analytical data and physical constants of **3b** are listed in Table 2.

4-De-*N*-methyl-4-*N*-(L-*N*-benzyloxycarbonylisoleucyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**3c**)

A solution containing 0.53 g of 4-de-*N*-methyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**2**) and 0.62 g of the *N*-hydroxy-5-norbornene-2,3-dicarboximide active ester of L-*N*-benzyloxycarbonylisoleucine[7], mp 100~102°C, in 1 ml of *N*,*N*-dimethylformamide was stirred overnight at room temperature. Evaporation of the solvent left a residue of 1.36 g of crude coupling product which was purified by chromatography on 120 g of silica gel using a benzene - methanol (85: 15, v/v) mixture as the eluent.

The fractions containing the desired product **3c** were still contaminated by the *N*-benzyloxycarbonyl protected active ester which was employed in the coupling reaction. Combination and evaporation of the solvent from these fractions led to the isolation of 0.83 g of contaminated coupling product. Further chromatography of this residue on silica gel in benzene - methanol - ethanol - concentrated ammonium hydroxide (1170: 34: 136: 10, v/v) mixture followed by Sephadex LH-20 chromatography in 95% ethanol afforded 0.66 g of pure **3c**. The analytical data and physical constants of **3c** are listed in Table 2.

4-De-*N*-methyl-4-*N*-acetyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**3d**)

A chloroform solution (4 ml) of 0.83 g of **2** and 0.40 g of *N*-acetoxy-5-norbornene-2,3-dicarboximide of mp 114~115°C prepared from equimolecular amounts of *N*-hydroxy-5-norbornene-2,3-dicarboximide and acetic anhydride in methanol, was stirred at room temperature for 24 hours. Evaporation of the solvent left a residue of 1.35 g from which, after chromatography on 100 g of silica gel in benzene - methanol (85: 15, v/v), 0.68 g of **3d** was obtained. Rechromatography of the substance on a Sephadex LH-20 column yielded 0.40 g of pure **3d**; the physical constants of **3d** are recorded in Table 2.

Table 3. Physical constants of 4-de-*N*-methylfortimicin B (deprotected **2**), 4-de-*N*-methyl-4-*N*-ethylfortimicin B (deprotected **5**), and 4-de-*N*-methyl-4-*N*-aminoacylfortimicin B (**4a**~**4c**) tetrahydrochlorides.

Measurement	Deprotected **2**	Deprotected **5**	**4a** $R^1=COCH_2NH_2$	**4b** $R^1=COCH(OH)\cdot CH_2CH_2NH_2$	**4c** $R^1=COCH\cdot(NH_2)CH(CH_3)\cdot CH_2CH_3$
^1H NMR (D_2O) δ ppm	5.94 (anom. H) 3.99 (OCH_3) 1.83 (7′-CH_3)	5.88 (anom. H) 3.96 (OCH_3) 1.80 (7′-CH_3)	6.2 (anom. H) 4.01 (OCH_3) 1.82 (7′-CH_3)	6.16 (anom. H) 4.0 (OCH_3) 1.81 (7′-CH_3)	6.16 (anom. H) 4.0 (OCH_3) 1.81 (7′-CH_3)
IR $\tilde{\nu}_{max}^{KBr}$ cm^{-1}	3420, 2920, 1585, 1485	3400, 2930, 1583, 1490	1682, 1483	1650, 1487	1674, 1482
Optical rotation (*c*, CH_3OH)	$[\alpha]_D^{25}+76°$ (0.55)	$[\alpha]_D^{25}+87°$ (0.98)	$[\alpha]_D^{24}+36°$ (1.01)	$[\alpha]_D^{25}+32°$ (1.01)	$[\alpha]_D^{25}+48°$ (1.04)
MS calcd. formula	Calcd. for $C_{14}H_{31}N_4O_5$: 335.2294	Calcd. for $C_{16}H_{35}N_4O_5$: 363.2607	Calcd. for $C_{16}H_{34}N_5O_6$: 392.2509	Calcd. for $C_{18}H_{38}N_5O_7$: 436.2771	Calcd. for $C_{20}H_{42}N_5O_6$: 448.3135
Type ion observed	$[M+H]^+$ found *m/z*: 335.2292	$[M+H]^+$ found *m/z*: 363.2565	$[M+H]^+$ found *m/z*: 392.2528	$[M+H]^+$ found *m/z*: 436.2747	$[M+H]^+$ found *m/z*: 448.3128

4-De-*N*-methyl-4-*N*-aminoacylfortimicins B (**4a**~**4c**) tetrahydrochloride salts and deprotection of **2** and **5**

The compounds **3a**, **3b** and **3c** were hydrogenolyzed in 0.2 N methanolic hydrochloric acid in the same manner as the corresponding fortimicin A analogs[3] to afford, after filtration and evaporation of the solvent, the desired tetrahydrochloride salts of **4a**, **4b** and **4c**, respectively. The physical constants of these tetrahydrochloride salts are listed in Table 3.

The physical constants of the deprotected tetrahydrochloride salts obtained from **2** and **5** in the same manner[3] are likewise recorded in Table 3.

4-De-*N*-methyl-4-*N*-ethyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**5**)

A. From 4-de-*N*-methyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**2**)

A solution of 2.36 g of **2** in 175 ml of methanol and 52.5 ml of Sørensen's pH 8 phosphate buffer solution,[8] and 6 ml of acetaldehyde was stirred at room temperature for 15 minutes. The addition of 0.75 g of sodium cyanoborohydride was followed by the addition of 20 ml of methanol 10 minutes later and stirring at room temperature was continued for 3 hours. The methanol was removed from the reaction mixture under reduced pressure, the resulting slurry was diluted with 120 ml of water and extracted with 200 ml of benzene. The aqueous layer was separated and extracted with three 150-ml portions of benzene. The benzene extracts were washed with two 120-ml portions of water, dried over anhydrous magnesium sulfate, filtered, combined and evaporated under reduced pressure to leave 2.46 g of crude reaction product.

The above residue, together with 0.82 g of hydroxylamine hydrochloride was dissolved in 160 ml of methanol containing 2.25 ml of acetic acid and the solution was refluxed and stirred for one hour. Evaporation of the solvent afforded a residue of 3.88 g which was chromatographed in a benzene - methanol - ethanol - concentrated ammonium hydroxide (1170: 34: 136: 10, v/v) mixture on 270 g of silica gel to yield 1.45 g of the desired substance **5**. An analytical sample was obtained after rechromatography of the above product on a Sephadex LH-20 column in 95% ethanol. The physical constants of this sample were as follows: $[\alpha]_D^{24}+26°$ (*c* 1.00, $CHCl_3$); $\tilde{\nu}_{max}^{CDCl_3}$ 1700, 1497 cm^{-1}; ^1H NMR ($CDCl_3$) 7.30 (Ar-Z), 5.07 (CH_2-Z), 3.41 (OCH_3) ppm.

Anal. Calcd. for $C_{40}H_{52}N_4O_{11}\cdot H_2O$: C, 61.36; H, 6.95; N, 7.16.
Found: C, 61.48; H, 6.56; N, 7.09.

B. From 4-de-*N*-methyl-4-*N*-acetyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**3d**)

To an ice cold stirred solution of 0.68 g of **3d** in 10 ml of tetrahydrofuran there was added 2 ml of 1 M borane tetrahydrofuran complex (Aldrich Chemical Company, Inc) in a nitrogen atmosphere. Stir-

ring of the mixture under nitrogen was continued for 3 hours when an additional 2 ml of the borane tetrahydrofuran complex was added to the reaction mixture. After 1.5 hours another 3 ml of the complex was added and the reaction was allowed to proceed for one more hour. The excess borane complex was decomposed by the careful addition of 3 ml of water and the solution was evaporated under reduced pressure. The residue was repeatedly dissolved in methanol followed by evaporation of the solvent to yield a residue of 0.73 g which was subjected to chromatography on 80 g of silica gel in a benzene - methanol (85: 15, v/v) mixture to yield 0.49 g which contained the desired substance **5**. Further purification of this residue was achieved by repeated rechromatography on silica gel in a benzene - methanol - concentrated ammonium hydroxide (80: 20: 1, v/v) mixture. The final purification of the compound was accomplished by chromatography on a Sephadex LH-20 column in 95% ethanol to afford 0.38 g of pure 4-de-*N*-methyl-4-*N*-ethyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**5**) with the following physical constants: $[\alpha]_D^{24}+25°$ (*c* 0.98, $CHCl_3$); $\tilde{\nu}_{max}^{CDCl_3}$ 1702, 1498 cm^{-1}; ^1H NMR ($CDCl_3$) 7.32 (Ar-Z), 5.07 (CH_2-Z), 3.42 (OCH_3) ppm.

Anal. Calcd. for $C_{40}H_{52}N_4O_{11}\cdot H_2O$: C, 61.36; H, 6.95; N, 7.16.
Found: C, 61.19; H, 6.75; N, 6.85.

The substances prepared under A and B above were shown to be identical by tlc-chromatography in several solvent systems.

4-De-*N*-methyl-4-*N*-ethyl-4-*N*-(*N*-benzyloxycarbonylglycyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**6**)

A solution of 0.54 g of **5** and 0.46 g of *N*-hydroxysuccinimide active *N*-benzyloxycarbonylglycyl ester in 4 ml of tetrahydrofuran was stirred at room temperature for 2 days. Evaporation of the solvent left a residue of 1.02 g which was chromatographed on 110 g of silica gel in ethyl acetate as the eluent to afford 0.55 g of **6** which was rechromatographed on 60 g of silica gel in the same solvent to give an analytical sample of the desired product **6**: $[\alpha]_D^{26}+24°$ (*c* 1.04, $CHCl_3$); $\tilde{\nu}_{max}^{CDCl_3}$ 1715, 1635 (tertiary amide), 1505 cm^{-1}; ^1H NMR ($CDCl_3$) 7.3 (Ar-Z), 5.1 (CH_2-Z), 3.3 (OCH_3) ppm.

Anal. Calcd. for $C_{50}H_{61}N_5O_{14}$: C, 62.81; H, 6.43; N, 7.33.
Found C, 62.69; H, 6.64; N, 7.11.

4-De-*N*-methyl-4-*N*-ethyl-5-*O*-(*N*-benzyloxycarbonyl-β-alanyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**7**)

A solution of 0.52 g of 4-de-*N*-methyl-4-*N*-ethyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**5**) and 0.58 g of *N*-hydroxy-5-norbornene-2,3-dicarboximide active *N*-benzyloxycarbonyl-β-alanyl ester[7] in 5 ml of tetrahydrofuran was stirred at room temperature for 3 days. Evaporation of the solvent afforded a residue of 1.11 g which was subjected to chromatography on 70 g of silica gel in ethyl acetate to afford 0.55 g of partially purified **7**. After repeated chromatography on silica gel in ethyl acetate, an analytical sample of **7** was obtained: $[\alpha]_D^{23}+18°$ (*c* 0.90, $CHCl_3$); $\tilde{\nu}_{max}^{CDCl_3}$ 1702, 1497 cm^{-1}; ^1H NMR ($CDCl_3$) 7.3 (Ar-Z), 5.07 (CH_2-Z), 3.36 (OCH_3) ppm.

Anal. Calcd. for $C_{51}H_{63}N_5O_{14}$: C, 63.14; H, 6.55; N, 7.22.
Found C, 63.02; H, 6.69; N, 7.03.

4-De-*N*-methyl-4-*N*-(*N*-benzyloxycarbonyl-β-aminoethyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**8**)

To an ice-cooled stirred solution of 1.01 g of **3a** in 20 ml of tetrahydrofuran there was added, in an atmosphere of nitrogen, 13 ml of a 1 M borane tetrahydrofuran complex solution and the mixture was stirred under nitrogen for 1.5 hours. The excess reagent was decomposed by the careful addition of 4 ml of water. The solvent was evaporated under reduced pressure and the residue was repeatedly dissolved in methanol followed by evaporation of the solvent. The residue obtained amounted to 1.05 g which was chromatographed on 100 g of silica gel in a benzene - ethanol (980: 20, v/v) mixture to give 0.72 g of a mixture containing the starting material **3a** and the desired product **8**.

After chromatography of this mixture on silica gel in 1,2-dichloroethane - methanol - ethanol - acetic acid (1170: 35: 135: 10, v/v), 0.36 g of the starting material **3a** was separated from 0.35 g of the desired product **8**. The latter (**8**) was dissolved in ethyl acetate and the solution was washed with a 5% aqueous solution of sodium bicarbonate and two small portions of water. The aqueous washes were

extracted with two portions of ethyl acetate. The ethyl acetate extracts were dried over anhydrous magnesium sulfate, filtered, combined and evaporated to leave a residue of 0.24 g of the desired substance **8**. The compound was chromatographed on 25 g of silica gel in ethyl acetate - ethanol (980: 20, v/v) to afford 0.22 g of an analytical sample of 4-de-*N*-methyl-4-*N*-(*N*-benzyloxycarbonyl-β-aminoethyl)-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**8**) with the following physical constants: $[\alpha]_D^{25}+19°$ (*c* 1.00, $CHCl_3$); $\nu_{max}^{CDCl_3}$ 1705, 1503 cm^{-1}; ^1H NMR ($CDCl_3$) 7.3 (Ar-Z), 5.06 (CH_2-Z), 3.39 (OCH_3), 1.03 (7′-CH_3) ppm.

Anal. Calcd. for $C_{48}H_{59}N_5O_{13}$: C, 63.07; H, 6.51; N, 7.66.
Found C, 63.10; H, 6.66; N, 7.59.

4-De-*N*-methyl-4-*N*-(*N*-benzyloxycarbonyl-β-aminoethyl)-4-*N*-ethyl-1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin B (**9**)

A solution of 0.50 g of the above prepared intermediate **8** and 0.8 ml of acetaldehyde in 27 ml of methanol was stirred for ten minutes, 9.6 ml of Sørenson's pH 6 buffer solution[8] was added and stirring was continued for 40 minutes when 0.93 g of sodium cyanoborohydride was added to the solution. Stirring at room temperature was continued for 24 hours. The solvents were evaporated and the remaining residue was partitioned between 100 ml of chloroform and 100 ml of water. The aqueous layer was separated and extracted with two 50-ml portions of chloroform. The combined chloroform extracts were washed with two 100-ml portions of water and 100 ml of a 5% aqueous sodium bicarbonate solution. The chloroform extract was dried over anhydrous magnesium sulfate, filtered and evaporated to leave a residue of 0.49 g. The substance was purified by chromatography on 55 g of silica gel in the lower phase of dichloromethane - methanol - 37% aqueous formaldehyde solution (700: 35: 10, v/v). Combination of the appropriate fractions and evaporation afforded a residue of 0.27 g of **9**.

An analytical sample, obtained after dissolving a part of the above substance in a small amount of ethyl acetate, filtration of the solution and evaporation of the solvent, had the following physical constants: $[\alpha]_D^{25}+19°$ (*c* 0.47, $CHCl_3$); $\nu_{max}^{CDCl_3}$ 1712, 1508 cm^{-1}; ^1H NMR ($CDCl_3$) 7.3 (Ar-Z), 5.06 (CH_2-Z), 3.35 (OCH_3), 1.1 (7′-CH_3), 0.96 (Et-CH_3) ppm.

Anal. Calcd. for $C_{50}H_{63}N_5O_{13}\cdot H_2O$: C, 62.55; H, 6.82; N, 7.30.
Found C, 62.33; H, 7.02; N, 7.50.

4-De-*N*-methyl-4-*N*-(β-aminoethyl)-4-*N*-ethylfortimicin B (**10**)

A solution of 0.24 g of **9** in 0.2 N methanolic hydrochloric acid was deprotected over 5% palladium-on-carbon in the usual manner[3] to afford 0.15 g of the pentahydrochloride salt of **10**: $[\alpha]_D^{22}+79°$ (*c* 1.02, CH_3OH); ν_{max}^{KBr} 3380, 2930, 1575, 1455 cm^{-1}; ^1H NMR (D_2O) 5.87 (C-1′ H), 4.01 (OCH_3), 1.9 (Et-CH_3), 1.82 (7′-CH_3) ppm.

Anal. Calcd. for $C_{18}H_{40}N_5O_5$ $(M+1)^+$ 406.3029; Found *m/z* 406.3021.

The above pentahydrochloride salt was converted to the free base by passing it over a small AG1-X2 (OH^- form) ion exchange column: ^1H NMR (D_2O, pD ~ 10.95) 5.44 (C-1′ H, $J_{1',2'}$ 3.2 Hz), 3.91 (OCH_3), 1.52 (7′-CH_3, $J_{6',7'}$ 7 Hz) ppm. No conclusion could be reached regarding the conformation of the aminocyclitol part of the substance.

Acknowledgments

The authors wish to thank Ms. S. MUELLER and Mr. P. HILL for mass spectra, Ms. J. HOOD for microanalyses, Mr. D. A. DUNNIGAN for catalytic hydrogenations and Dr. R. L. GIROLAMI for determining antibacterial activities. The authors are indebted to Profs. PETER BEAK of the University of Illinois, STEPHEN HANESSIAN of the University of Montreal and LESTER MITSCHER of the University of Kansas for stimulating discussions.

References

1) EGAN, R. S.; R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, J. TADANIER, J. R. MARTIN, P. COLLUM, A. W. GOLDSTEIN, R. L. DEVAULT, 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
2) 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 fortimicins. J. Antibiotics 30: 533~540, 1977

3) TADANIER, J.; J. R. MARTIN, P. KURATH, A. W. GOLDSTEIN & P. JOHNSON: 4-*N*-Acylfortimicins B and the preparation of fortimicin A from fortimicin B. Carbohydr. Res 79: 91~102, 1980
4) SATO, M. & Y. MORI: Chemical modification of fortimicins: Preparation of 4-*N*-substituted fortimicin B. J. Antibiotics 32: 371~378, 1979
5) FREIBERG, L. A.; R. R. RASMUSSEN & A. ALTER: The synthesis of ^{14}C-methyl labelled fortimicin A. in preparation.
6) TADANIER, J.; J. R. MARTIN, A. M. NADZAN, P. JOHNSON, J. HOLMS, R. HALLAS, R. STANASZEK, M. CIROVIC, D. GRAMPOVNIK & A. W. GOLDSTEIN: 2-Substituted fortimicins by ring opening of 1,2-epiimino-2-deoxy-2-*epi*-fortimicin B and by nucleophilic displacements of 2-*O*-methanesulfonyl derivatives. Carbohydr. Res. in press.
7) 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
8) "Handbook of Biochemistry", H. A. SOBER, *Ed.*, pp. J-234~J-237, The Chemical Rubber Company, Cleveland, Ohio, 1970