SUBSTANCES DERIVED FROM 4-DE-\(N\)-METHYLFORTIMICIN B

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The preparation of 4-de-\(N\)-methylfortimicin A analogs as well as the preparation of 4-de-\(N\)-methyl-4-\(N\)-(\(\beta\)-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-\(N\)-(\(\beta\)-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\(_2\)NH\(_2\) 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 I the \(^{13}\)C-NMR spectra of \(4a\), fortimicin A, and fortimicin B are compared at basic pD-values (\(\sim 10\)). 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 I 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 I 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-NH₂ 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-benzyloxy-carbonylamino-2-hydroxybutyric acid and L-benzyloxy-carbonylisoleucine, respectively. De-protection of the intermediates 3b and 3c in the usual manner led to the isolation of the tetrahydrochloride salts of 4b and 4c, respectively.

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 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 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 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.

Both compounds, 6 and 7, on deprotection under the usual conditions 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 complex⁴) 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 cyanoborohydride⁵) to yield 4-de-N-methyl-4-N-(N-benzyloxycarbonyl-β-aminoethyl)-4-N-ethyl-1,2',6'-tri-N-benzyloxycarbonylfortimicin B (9). Deprotection³) 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$_2$O) 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 non-polar 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>
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<th>3b</th>
<th>3c</th>
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<td>C$<em>{48}$H$</em>{57}$N$<em>{5}$O$</em>{14}$</td>
<td>C$<em>{48}$H$</em>{57}$N$<em>{5}$O$</em>{14}$</td>
<td>C$<em>{52}$H$</em>{65}$N$<em>{5}$O$</em>{14}$</td>
<td>C$<em>{40}$H$</em>{50}$N$<em>{4}$O$</em>{12}$</td>
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<td>C 61.78</td>
<td>C 63.46</td>
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<td>N 7.55 %</td>
<td>N 7.20 %</td>
<td>N 7.12 %</td>
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<td>H 6.75</td>
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<tr>
<td></td>
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<td>N 7.52 %</td>
<td>N 7.25 %</td>
<td>N 7.04 %</td>
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<td>[a]$_D^{24} +23^\circ$ (c 0.98)</td>
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<td>1702, 1503</td>
<td>1698, 1505</td>
<td>1702, 1500</td>
<td>1700, 1503</td>
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<td>7.28 (Ar-Z)</td>
<td>7.27 (Ar-Z)</td>
<td>7.28 (Ar-Z)</td>
<td>7.3 (Ar-Z)</td>
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<td></td>
<td>5.07 (CH$_2$-Z)</td>
<td>5.04 (CH$_2$-Z)</td>
<td>5.02 (CH$_2$-Z)</td>
<td>5.04 (CH$_2$-Z)</td>
<td>5.07 (CH$_2$-Z)</td>
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<td>3.39 (OCH$_3$)</td>
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<td>3.39 (OCH$_3$)</td>
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<td>1.0 (7'-CH$_3$)</td>
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</tr>
</tbody>
</table>
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-benzyloxy carbonylglycyl)-1,2',6'-tri-N-benzyloxy carbonylfortimicin B (3a)
A solution of 0.92 g of the above prepared 2 and 0.78 g of the N-hydroxysuccinimide active N-benzyloxy carbonylglycyl 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-benzyloxy carbonylamino-2-hydroxybutyryl)-1,2',6'-tri-N-benzyloxy carbonylfortimicin B (3b)
The N-hydroxy-5-norbornene-2,3-dicarboximide active ester of L-4-N-benzyloxy carbonylamino-2-hydroxybutyric acid was prepared according to a previously published procedure7) by reacting 0.58 g of 1,4-N-benzyloxy carbonylamino-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-benzyloxy carbonylfortimicin B (2) for 24 hours. Evaporation of the solvent left a residue of 1.83 g 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 0.56 g of analytically pure 3b. The physical constants of this compound are listed in Table 2.

4-De-N-methyl-4-N-(l-N-benzyloxy carbonylisoleucyl)-1,2',6'-tri-N-benzyloxy carbonylfortimicin B (3c)
A solution containing 0.53 g of 4-de-N-methyl-1,2',6'-tri-N-benzyloxy carbonylfortimicin B (2) and 0.62 g of the N-hydroxy-5-norbornene-2,3-dicarboximide active ester of L-N-benzyloxy carbonylisoleucine7), 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 in a benzene - methanol - ethanol - concentrated ammonium hydroxide (1170: 34: 136: 10, v/v) mixture as the eluent.

The fractions containing the desired product 3c were still contaminated by the N-benzyloxy carbonyl 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-benzyloxy carbonylfortimicin 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.

<table>
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<th>Measurement</th>
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<th>4b</th>
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<tr>
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<td>4.00 (OCH3)</td>
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<tr>
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</table>

4-De-N-methyl-4-N-ethylfortimicins 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 analogs3) 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 manner3) 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: [α]D²⁰ +26° (c 1.00, CHCl₃); c¹DCI₁₃ 1700, 1497 cm⁻¹; 1H NMR (CDCl₃) 7.30 (Ar-Z), 5.07 (CH₃-Z), 3.41 (OCH₃) ppm.

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: [α]D24 +24° (c 0.98, CHCl3); δCDCl3max 1702, 1498 cm-1; 1H NMR (CDCl3) 7.32 (Ar–Z), 5.07 (CH2–Z), 3.42 (OCH3) ppm.


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: [α]D24 +24° (c 1.04, CHCl3); δCDCl3max 1715, 1635 (tertiary amide), 1505 cm-1; 1H NMR (CDCl3) 7.3 (Ar–Z), 5.1 (CH2–Z), 3.3 (OCH3) ppm.


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 ester7) 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: [α]D23 +18° (c 0.90, CHCl3); δCDCl3max 1702, 1497 cm-1; 1H NMR (CDCl3) 7.3 (Ar–Z), 5.07 (CH2–Z), 3.36 (OCH3) ppm.


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: \([a]_{D}^{26} +19° (c 1.00, \text{CHCl}_3)\); \(\varepsilon_{\text{max}}^\text{DCI} 1705, 1503 \text{ cm}^{-1}\); \(^{1}H\) 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$_{5}$O$_{13}$: 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 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: \([a]_{D}^{25} +19° (c 0.47, \text{CHCl}_3)\); \(\varepsilon_{\text{max}}^\text{DCI} 1712, 1508 \text{ cm}^{-1}\); \(^{1}H\) NMR (CDCl$_3$) 7.3 (Ar-Z), 5.06 (CH$_2$-Z), 3.35 (OCH$_3$), 1.11 (7'-CH$_3$), 0.96 (Et-CH$_3$) ppm.

**Anal.** Calcd. for C$_{50}$H$_{83}$N$_{5}$O$_{13}$·H$_2$O: 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 to afford 0.15 g of the pentahydrochloride salt of 10: \([a]_{D}^{22} +79° (c 1.02, \text{CH}_3\text{OH})\); \(\varepsilon_{\text{max}}^\text{KBr} 3380, 2930, 1575, 1455 \text{ cm}^{-1}\); \(^{1}H\) NMR (D$_2$O) 5.87 (C-1' H), 4.01 (OCH$_3$), 1.9 (Et-CH$_3$), 1.82 (7'-CH$_3$) ppm.

**Anal.** Calcd. for C$_{28}$H$_{46}$N$_{5}$O$_{5}$(M+1)$^+$ 406.3029; **Found** m/z 406.3021.

The above pentahydrochloride was converted to the free base by passing it over a small AG1-X2 (OH$^-$ form) ion exchange column: \(^{1}H\) NMR (D$_2$O, 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.

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