4-*N*-AMINOACYLFORTIMICINS E

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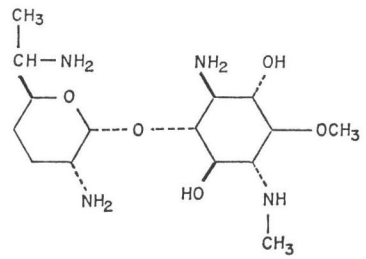
The conversion of fortimicin E, a minor metabolite from the *Micromonospora olivoasterospora* fermentation which also produces fortimicin A and fortimicin B, to four 4-*N*-aminoacylfortimicins E was accomplished. The new 4-*N*-aminoacylfortimicins E showed only weak antimicrobial activity against several Gram-negative and Gram-positive microorganisms.

The aminoglycoside fortimicin E (**1**) was isolated as a by-product in the *Micromonospora olivoasterospora* ATCC 21819 fermentation which produces fortimicin A (**2**) and fortimicin B (**3**).[1] The structure of fortimicin E was shown to be represented by **1** on the basis of ^{1}H-NMR, ^{13}C-NMR and mass spectral studies.[2] In contrast to fortimicin A (**2**)[3] and fortimicin B (**3**)[3] all the substituents in the aminocyclitol ring of fortimicin E (**1**) are equatorially oriented.[2] Fortimicin A (**2**)[1] and some of its analogous 4-*N*-aminoacylfortimicins B[4,4a] were found to be better antimicrobial agents than fortimicin B (**3**).[1] Since fortimicin E (**1**) was shown to exhibit only weak antibiotic properties[2] it appeared possible that 4-*N*-aminoacylation of fortimicin E (**1**) might lead to substances with improved antibiotic properties. For this reason the synthesis of some 4-*N*-aminoacylfortimicins E was attempted.



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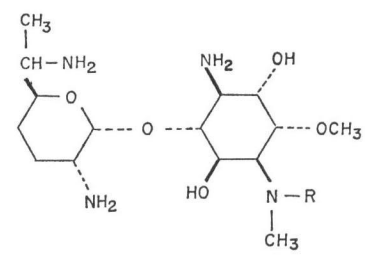


2: R = $COCH_2NH_2$
3: R = H

In preliminary experiments it was shown that the reaction of fortimicin E (**1**) with three equivalents of *N*-benzyloxycarbonyloxysuccinimide led to the formation of 4,2′,6′-tri-*N*-benzyloxycarbonylfortimicin E rather than the desired 1,2′,6′-tri-*N*-benzyloxycarbonylfortimicin E which could have been used as an intermediate in the proposed 4-*N*-aminoacylation reactions. It was therefore necessary to employ a blocking group which would allow the selective protection of the primary amino functions at C-1, C-2′ and C-6′ while leaving the 4-methylamino group available for acylation. It appeared attractive to attempt the preparation of 1,2′,6′-tri-*N*-salicylidenefortimicin E (**4**) by reacting fortimicin E (**1**) with three equivalents of salicylaldehyde.[5] Since the equatorial 4-methylamino group and the

equatorial 5-hydroxyl group of fortimicin E (**1**) are *trans*-oriented it was not expected that a 4,5-oxazolidine would form.[6,7] It was shown earlier that neighboring *N*-methylamino groups and hydroxyl groups in six-membered rings do not form oxazolidines with aldehydes when the two groups are *trans*-oriented[6,7] while in the case of *cis* orientation oxazolidine formation was observed.[6~10]

The 1,2′,6′-tri-*N*-salicylidenefortimicin E (**4**), obtained by reacting fortimicin E (**1**) with three equivalents of salicylaldehyde, was coupled with *N*-benzyloxycarbonylglycine-*N*-hydroxysuccinimide active ester,[11,12] *N*-benzyloxycarbonyl-*β*-alanine *N*-hydroxy-5-norbornene-2,3-dicarboximide active ester,[13] *N*-benzyloxycarbonylsarcosine *N*-hydroxy-5-norbornene-2,3-dicarboximide active ester, and L-2-hydroxy-4-*N*-benzyloxycarbonylaminobutyric acid *N*-hydroxy-5-norbornene-2,3-dicarboximide active ester and afforded the 4-*N*-(*N*-benzyloxycarbonylaminoacyl)-1,2′,6′-tri-*N*-salicylidenefortimicins E, **5a~5d**, respectively. The intermediates **5a~5d** were treated with 0.2 N aqueous hydrochloric

Table 1. Physical constants of tetra-*N*-benzyloxycarbonyl-4-*N*-aminoacylfortimicins E (**7a~7d**).

Measurement	**7a**, R=$COCH_2NHZ$	**7b**, R=$COCH_2CH_2NHZ$	**7c**, R=$COCH_2N(CH_3)Z$	**7d**, R=L-$COCH(OH)CH_2CH_2NHZ$
Optical rotation (*c* in $CHCl_3$)	$[\alpha]_D^{24}$ +32° (*c* 0.99)	$[\alpha]_D^{24}$ +29° (*c* 1.02)	$[\alpha]_D^{24}$ +30° (*c* 0.96)	$[\alpha]_D^{25}$ +25° (*c* 0.99)
IR $\nu_{max}^{CDCl_3}$ cm^{-1}	1690, 1642, 1500	1697, 1627, 1502	1690, 1655(sh), 1500	1692, 1624, 1497
^{1}H-NMR ($CDCl_3$) *δ*ppm	7.28 (Ar-Z), 5.02 (CH_2-Z), 3.43 (OCH_3), 2.82 (4-*N*-CH_3), 0.92 (7′-CH_3)	7.28 (Ar-Z), 5.02 (CH_2-Z), 3.4, 3.37 (OCH_3)[a], 2.82, 2.75 (4-*N*-CH_3)[a], 0.9 (7′-CH_3)	7.3 (Ar-Z), 5.02 (CH_2-Z), 3.48 (OCH_3), 2.94 and 2.8 (Sar-*N*-CH_3 and 4-*N*-CH_3), 0.93 (7′-CH_3)	7.28 (Ar-Z), 5.03 (CH_2-Z), 3.45 (OCH_3), 2.81 (4-*N*-CH_3), 0.92 (7′-CH_3)[b]
Sum formula	$C_{49}H_{59}N_5O_{14}$	$C_{50}H_{61}N_5O_{14}$	$C_{50}H_{61}N_5O_{14}$	$C_{51}H_{63}N_5O_{15}$
Anal. Calcd.	C, 62.47; H, 6.31; N, 7.44%	C, 62.81; H, 6.43; N, 7.33%	C, 62.81; H, 6.43; N, 7.33%	C, 62.12; H, 6.44; N, 7.10%
Found	C, 62.64; H, 6.43; N, 7.41%	C, 62.67; H, 6.58; N, 7.28%	C, 62.52; H, 6.41; N, 7.25%	C, 61.92; H, 6.53; N, 6.90%

[a] Doublets due to hindered rotation
[b] Recorded at 55°

Table 2. Physical constants of 4-*N*-aminoacylfortimicins E tetrahydrochlorides (**8a~8d**).

Measurement	**8a**, R′=$COCH_2NH_2$	**8b**, R′=$COCH_2CH_2NH_2$	**8c**, R′=$COCH_2NHCH_3$	**8d**, R′=L-$COCH(OH)CH_2CH_2NH_2$
Optical rotation (*c* in CH_3OH)	$[\alpha]_D^{24}$ +54° (*c* 1.00)	$[\alpha]_D^{26}$ +48° (*c* 1.04)	$[\alpha]_D^{22}$ +52° (*c* 1.06)	$[\alpha]_D^{22}$ +46° (*c* 1.06)
MS calcd. formula	Calcd. for $C_{17}H_{35}N_5O_6$: 405.2587	Calcd. for $C_{18}H_{37}N_5O_6$: 419.2744	Calcd. for $C_{18}H_{37}N_5O_6$: 419.2744	Calcd. for $C_{19}H_{37}N_5O_6$: 431.2744
Type ion observed	M^+ found *m/e*: 405.2605	M^+ found *m/e*: 419.2747	M^+ found *m/e*: 419.2738	M^+-H_2O found *m/e*: 431.2675
IR ν_{max}^{KBr} cm^{-1}	1640	1618	1640	1618
^{1}H-NMR (DMSO) ppm	6.3 (anom. H), 3.42 (OCH_3), 2.88 (4-*N*-CH_3), 1.26 (7′-CH_3)	6.3 (anom. H), 3.43 (OCH_3), 2.88 (*N*-CH_3), 1.3 (7′-CH_3)	6.29(anom. H), 3.43 (OCH_3), 2.78 and 2.56 (4-*N*-CH_3 and Sar-*N*-CH_3), 1.26 (7′-CH_3)	6.17 (anom. H), 3.39 (OCH_3), several signals in subst. 4-*N*-CH_3, area, not assigned, 1.25 (7′-CH_3)
δ(Temp.)	(ambient temp)[a]	(150°)[a]	(ambient temp)[a]	(140°)[b]

[a] Measured from internal tetramethylsilane
[b] Measured from internal hexamethyldisiloxane

Table 3. ^{1}H-NMR data of the 4-*N*-aminoacylfortimicins E (**9a**~**9d**) and fortimicin E (**1**) free bases.

Substance	4-*N*-Side chain	^{1}H-NMR-data δppm
9a	R′=$COCH_2NH_2$	4.86 (anom. H), 3.37 (OCH_3), 2.76 (4-*N*-CH_3), 0.92 (7′-CH_3) (DMSO, ambient temp)[a]
9b	R′=$COCH_2CH_2NH_2$	4.88 (anom. H), 3.37 (OCH_3), 2.72 (4-*N*-CH_3), 0.92 (7′-CH_3) (DMSO, ambient temp)[a]
9c	R′=$COCH_2$-NH-CH_3	4.95 (anom. H), 3.38 (OCH_3), 2.82 (4-*N*-CH_3), 2.29 (Sar-*N*-CH_3), 0.95 (7′-CH_3) (DMSO, 130°)[a]
9d	R′=CO-CH(OH)$CH_2CH_2NH_2$	decomposed to fortimicin E (**1**), 4.78 (anom. H), 3.39 (OCH_3), 2.32 (4-*N*-CH_3), 0.85 (7′-CH_3) (DMSO, ambient temp)[b]
1	R′=H	4.78 (anom. H), 3.39 (OCH_3), 2.32 (4-*N*-CH_3), 0.85 (7′-CH_3) (DMSO, ambient temp)[b]

[a] Measured from internal tetramethylsilane
[b] Measured from internal hexamethyldisiloxane

acid to cleave the SCHIFF base protecting groups, and the crude 4-*N*-(*N*-benzyloxycarbonylaminoacyl)-fortimicin E trihydrochlorides were subjected to silica gel chromatography in solvent systems containing ammonium hydroxide to afford the partially purified substances **6a**~**6d**, respectively.

The intermediates **6a**~**6d** still contained impurities and were therefore not suitable for the direct conversion to the desired 4-*N*-acylaminofortimicins E (**8a**~**8d**). The partially purified intermediates **6a**~**6d** were allowed to react with *N*-benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide[14] to afford the tetra-*N*-benzyloxycarbonyl-4-*N*-aminoacylfortimicins E, **7a**~**7d**, respectively. These latter intermediates were thoroughly purified and the pure substances (**7a**~**7d**) were then subjected to hydrogenolysis over a 5% palladium on carbon catalyst in 0.2 N methanolic hydrochloric acid to yield 4-*N*-glycylfortimicin E tetrahydrochloride (**8a**), 4-*N*-β-alanylfortimicin E tetrahydrochloride (**8b**), 4-*N*-sarcosylfortimicin E tetrahydrochloride (**8c**), and 4-*N*-(L-2-hydroxy-4-aminobutyryl)fortimicin E tetrahydrochloride (**8d**), respectively. The aqueous solutions of the tetrahydrochlorides **8a**~**8d** were converted to the free bases **9a**~**9d**, respectively, for further characterization of the latter.

The ^{13}C-NMR spectrum of **9a** in D_2O showed two resonances of nearly equal intensity for each cyclitol ring carbon as well as C-1′. The ^{1}H-NMR spectrum of **9a** as well as **8a** in D_2O also showed doubling of the cyclitol *N*- and *O*-methyl resonances. The ^{1}H-NMR spectra revealed that this was a characteristic of the entire series of **8a**~**8d** and **9a**~**9c** as well as their blocked precursors. Variable temperature experiments in D_2O solution indicated that the doubling arose from hindered rotation associated with the 4-*N*-

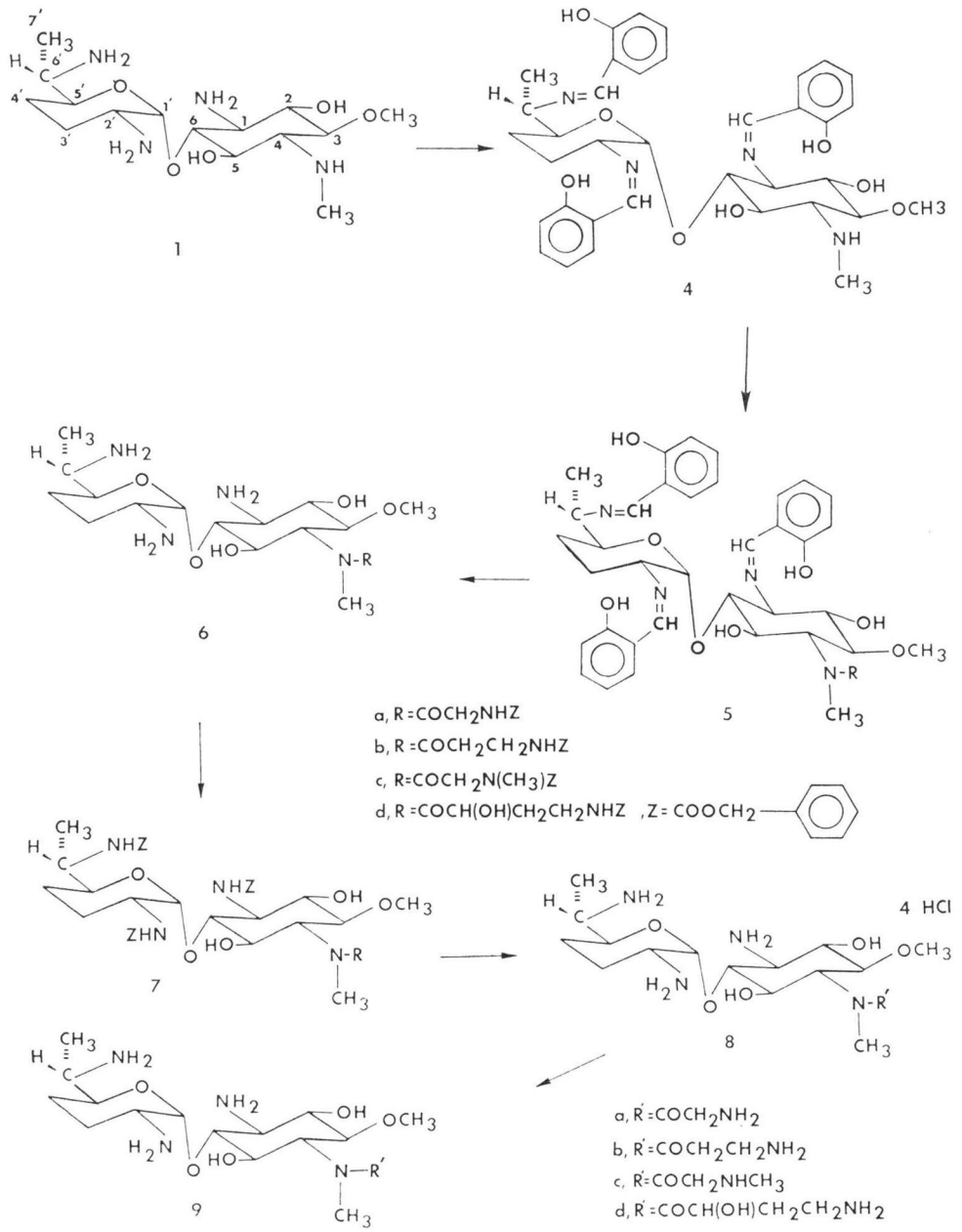
Table 4. ^{13}C Chemical shifts of fortimicin E (**1**) at pD 10.0 and 4-*N*-glycylfortimicin E (**9a**) at pD 12.1.

Carbon	**1**	**9a**	β-Shift of **9a**[a]
C=O (Gly)	—	177.0	
1′	101.9	102.4	−8.0[b]
6	84.6	84.8	−10.3[b]
3	83.5	80.0	
5′	75.1	75.3	−4.4
2	74.2	74.7	−3.8
5	73.6	70.4	
4	62.2	61.3	
3–OCH_3	60.0	58.0	
1	55.5	54.8	
2′	50.9	50.5	
6′	50.4	50.5	
CH_2 (Gly)	—	43.2	
4-*N*-CH_3	33.7	28.7	
4′	27.3	27.3	
3′	26.8	26.9	−5.8
7′–CH_3	18.0	18.6	−3.6

[a] β-shifts were calculated from a complete titration profile from pD 12.1 to pD 1.7.
[b] Previously large β-shifts have been reported for the carbons at the site of the sugar linkage.[3]

amide bond. In D_2O solution the barrier to rotation was sufficiently high to prevent coalescence of the individual resonances; however, population changes and broadening were observed at higher temperature. In DMSO-d_6 solutions singlet signals were observed for the *N*- and *O*-methyl groups at either ambient temperature or at elevated temperature (Tables 2 and 3). In the discussion of the ^{13}C-NMR spectra which follows, the average chemical shift of the two rotamers is used.

When the ^{13}C-NMR spectrum of **9a** is compared with that of **1**, additional resonances arising from glycine are observed at 177.0 and 43.2 ppm. Table 4 shows a comparison of the ^{13}C-NMR



chemical shifts of **1** with those of **9a**. The chemical shift of the 4-*N*-CH_3 in **9a** of 28.7 ppm is upfield from that of the 4-*N*-CH_3 in **1** of 33.7 ppm. This shows that the nitrogen at C-4 has been acylated. These changes parallel those previously seen in the spectra of fortimicins A and B where the 4-*N*-CH_3 resonance shifts from 32.2 ppm for **2** to 35.4 ppm for **3**.[3] The resonances of C-3 and C-5 of **9a** show upfield γ-shifts when compared with those of **1**. These changes offer clear evidence that **9a** is the 4-*N*-glycyl derivative of **1**. The titration curve of **9a** showed β-shifts for the carbons associated with the amines of the purpurosamine sugar, but only for C-6 and C-2 of the cyclitol. The lack of a β-shift for C-3 and C-5 and the 4-*N*-CH_3 confirms that 4-*N*-CH_3 is acylated.

The tetrahydrochlorides **8a**~**8d** showed only weak *in vitro* antibacterial activity against several Gram-positive and Gram-negative microorganisms.

Experimental

General methods

All evaporations were conducted with a rotary evaporator under reduced pressure. Silica gel chromatography was performed on Silica Gel 60, 70~230 mesh (E. Merck, Darmstadt). Optical rotations were determined 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 (Oδ) for the spectra recorded of compounds in deuteriochloroform ($CDCl_3$) solutions and as indicated for spectra recorded of substances in dimethyl sulfoxide (DMSO) solutions. Mass spectra were recorded with an A. E. I. MS-902 mass spectrometer with an ionization energy of 70 eV. ^{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).

4-*N*-(*N*-Benzyloxycarbonylglycyl) fortimicin E (**6a**)

a. 1,2′,6′-Tri-*N*-salicylidenefortimicin E (**4**). A solution of 1.31 g of fortimicin E (**1**) and 1.49 g of salicylaldehyde in 30 ml of methanol was refluxed and stirred for 1 hour. The solvent was evaporated and the residue was dissolved in 30 ml of benzene which was likewise evaporated; this last process was repeated six times. The residue was dried under high vacuum over potassium hydroxide pellets to afford 2.84 g of substance; $\nu_{max}^{CDCl_3}$ 1622, 1575 cm^{-1}.

b. 4-*N*-(*N*-Benzyloxycarbonylglycyl)-1,2′,6′-tri-*N*-salicylidenefortimicin E (**5a**). A solution of 1,2′,6′-tri-*N*-salicylidenefortimicin E (**4**), 2.84 g, and 2.05 g of *N*-benzyloxycarbonylglycine *N*-hydroxysuccinimide active ester[11,12] in 25 ml of tetrahydrofuran was stirred at room temperature overnight. Evaporation of the solvent afforded a residue of 4.86 g of crude coupling product.

c. 4-*N*-(*N*-Benzyloxycarbonylglycyl)fortimicin E (**6a**). The crude coupling product, 4.86 g (**5a**), obtained above was dissolved in 500 ml of chloroform and the solution was shaken with 500 ml of 0.2 N aqueous hydrochloric acid. The layers were separated and the chloroform solution was extracted with three 150-ml portions of 0.2 N hydrochloric acid. The hydrochloric acid layers were washed in series with three 250-ml portions of chloroform. The chloroform solutions were dried over anhydrous sodium sulfate, filtered, combined and evaporated to leave a residue of 1.41 g of nonbasic substances which were not characterized.

The 0.2 N hydrochloric acid extracts were evaporated under reduced pressure at room temperature. The residue was redissolved in 60 ml of methanol and the solvent was evaporated; this last process was repeated six times. The residue was dried under high vacuum over potassium hydroxide pellets to afford 2.74 g of crude 4-*N*-(*N*-benzyloxycarbonylglycyl)fortimicin E trihydrochloride. A partial purification of the above residue of 2.74 g was achieved by chromatography of the substance on 270 g of silica gel using the lower phase of a mixture of chloroform - methanol - concentrated ammonium hydroxide (1 : 1 : 1, v/v) as the eluting solvent to afford, after evaporation of the solvent, 1.43 g of a mixture containing the desired 4-*N*-(*N*-benzyloxycarbonylglycyl)fortimicin E (**6a**). Further chromato-

graphy of this residue on 180 g of silica gel using the lower phase of chloroform - methanol - concentrated ammonium hydroxide - water (2: 2: 1: 1, v/v) mixture as the eluting solvent system led to the isolation of fractions containing the desired 4-*N*-(*N*-benzyloxycarbonylglycyl)fortimicin E (**6a**). Combination and evaporation of the appropriate fractions afforded a residue of 1.08 g containing **6a**. The IR spectrum on a KBr-disc showed ν_{max}^{KBr} 1705 and 1635 cm^{-1}. The ^{1}H-NMR spectrum taken in D_2O showed signals at δ7.85 (Z-Ar), 5.56 (CH_2-Z), 3.91 and 3.85 (*O*-CH_3), 3.35 (4-*N*-CH_3), and 1.45 (7′-CH_3) ppm. This substance was not pure enough for direct hydrogenolytic conversion to **8a**.

Preparation of the additional 4-*N*-(*N*-benzyloxycarbonylaminoacyl)fortimicins E (**6b**, **6c**, and **6d**).

In a similar manner the 1,2′,6′-tri-*N*-salicylidenefortimicin E (**4**) prepared from 1.20 g of fortimicin E (**1**) was coupled with *N*-hydroxy-5-norbornene-2,3-dicarboximide active esters prepared from *N*-carbobenzoxy-β-alanine, *N*-carbobenzoxysarcosine, and *N*-carbobenzoxy-L-α-hydroxy-γ-aminobutyric acid according to FUJINO *et al.*,[13] to give the intermediates **5b**, **5c**, and **5d**, respectively.

Partial deprotection of the latter (**5b**, **5c**, and **5d**) followed by repeated silica gel chromatography employing the lower phases of the solvent mixtures chloroform - methanol - concentrated ammonium hydroxide (1: 1: 1, v/v) and chloroform - methanol - concentrated ammonium hydroxide - water (2: 2: 1: 1, v/v) gave the partially purified 4-*N*-(*N*-benzyloxycarbonylaminoacyl)fortimicins E: **6b** [R = $COCH_2CH_2$–NHZ], 0.79 g, **6c** [R = CO–CH_2–N(CH_3)Z], 0.16 g, and **6d** [R = COCH(OH)–CH_2CH_2NHZ], 0.88 g, respectively.

Tetra-*N*-benzyloxycarbonyl-4-*N*-glycylfortimicin E (**7a**).

A solution containing 1.03 g of 4-*N*-(*N*-benzyloxycarbonylglycyl)fortimicin E (**6a**) and 2.02 g of *N*-benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide[14] in 56 ml of methanol was stirred at room temperature overnight. Evaporation of the solvent left a residue of 3.06 g of product. Chromatography of this material on silica gel using benzene - chloroform - ethylacetate - *n*-propanol (13: 16: 8: 3, v/v) and benzene - methanol - ethanol (1170: 36: 136, v/v) as the eluent gave the analytically pure substance **7a**. The analytical results and physical constants for **7a** are listed in Table 1.

Tetra-*N*-benzyloxycarbonyl-4-*N*-aminoacylfortimicins E (**7b**, **7c**, and **7d**).

The three 4-*N*-(*N*-benzyloxycarbonylaminoacyl)fortimicins E (**6b**, **6c**, and **6d**) prepared above were likewise converted to the corresponding tetra-*N*-benzyloxycarbonyl-4-*N*-aminoacylfortimicins E **7b**, **7c**, and **7d**, respectively. The physical data for these substances and the microanalytical results obtained are listed in Table 1.

4-*N*-Aminoacylfortimicins E tetrahydrochlorides (**8a**, **8b**, **8c**, and **8d**).

The tetra-*N*-benzyloxycarbonyl-4-*N*-aminoacylfortimicins E (**7a**, **7b**, **7c**, and **7d**) were hydrogenolyzed in 0.2 N methanolic hydrochloric acid over 5% Pd/C in the same manner as the corresponding fortimicin A analogs[4] to afford, after filtration and evaporation, the desired tetrahydrochlorides **8a**, **8b**, **8c**, and **8d**, respectively. The physical constants of these tetrahydrochlorides are listed in Table 2.

4-*N*-Aminoacylfortimicins E free bases (**9a**, **9b**, **9c** and **9d**).

The above prepared tetrahydrochlorides **8a**, **8b**, **8c** and **8d** were dissolved in water and filtered through small columns of anion-exchange resin Bio Rad® AG2-X8 (OH^- form). The columns were eluted with water until the pH of the eluent was neutral.

The aqueous solutions were frozen and lyophilized to afford the 4-*N*-aminoacylfortimicins E **9a**, **9b** and **9c**, respectively. In contrast to the findings with the corresponding fortimicin A analogs these three derivatives of fortimicin E were found to be stable in aqueous solution for several days. The fourth substance, **9d**, was found to decompose rapidly to give fortimicin E (**1**). The results of the ^{1}H-NMR spectral studies of the above substances are recorded in Table 3.

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