4-*N*-AMINOACYLATION OF SUBSTANCES DERIVED FROM LYSINOMICIN

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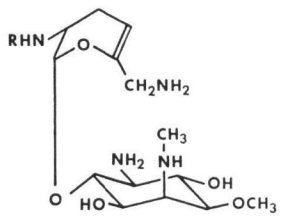
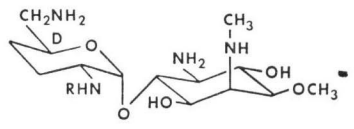
The preparations of 4-*N*-glycyllysinomicin and several 4-*N*-aminoacyl derivatives of compounds prepared from lysinomicin are presented. The new substances have lower antimicrobial activities than the original 4-*N*-unsubstituted lysinomicin derivatives. This result indicates that the structure-activity relationship observed with fortimicin A and fortimicin B does not apply to the lysinomicin derivatives studied.

The structure and stereochemistry of the aminoglycoside antibiotic lysinomicin (**1**) was recently determined in this laboratory.[1] In the course of the structure proof of **1**, 2′-de-*N*-L-β-lysyllysinomicin (**2**), 3-*epi*-2′-*N*-L-β-lysyl-6′-de-*C*-methylfortimicin B (**3**), and 3-*epi*-6′-de-*C*-methylfortimicin B (**4**) were obtained and found to exhibit good antimicrobial activities.[1]



1 R = $COCH_2CH(CH_2)_3NH_2$ (NH$_2$)

2 R = H



3 R = $COCH_2CH(CH_2)_3NH_2$ (NH$_2$)

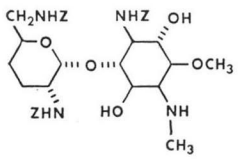
4 R = H

On the basis of the structure activity relationship discovered with fortimicin A and fortimicin B[2] it was of interest to examine the effect of 4-*N*-aminoacylation on some of the above substances (**1**～**4**). It was found earlier that 4-*N*-acylation of 3,5′-di-*epi*-6′-de-*C*-methylfortimicin B, which contains the same 3-*epi*-fortamine residue as the above compounds **1**～**4**, could not be achieved with acetic anhydride in methanolic solution or with *N*-acetoxy-5-norbornene-2,3-dicarboximide,[1] thus indicating that the sterically hindered 4-*N*-CH_3 group was difficult to acylate. In addition it was known that sterically hindered substances derived from fortimicin B could undergo 5-*O*-acylation under the base catalyzed influence of the sterically hindered 4-*N*-CH_3 group.[3,4] In the course of work carried out in connection with the preparation of fully *N*-protected lysinomicin derivatives in these laboratories it was found that the use of anhydrides in the presence of base may be the method of choice in the preparation of 4-*N*-acyllysinomicin derivatives.

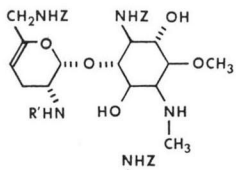
In order to examine the effect of 4-*N*-aminoacylation in the lysinomicin series it was decided to attempt the preparation of the 4-*N*-glycyl and the 4-*N*-L-β-lysyl derivatives of **4** and also to prepare the 4-*N*-glycyl derivatives of **1** and **2**. The biological evaluation of these substances would allow us to determine if such compounds would exhibit better antimicrobial properties than the original substances.

The suitably protected intermediates **5**, **6**, and **7** were obtained from **4**, **1**, and **2**, respectively, in good yield by reacting the latter with *N*-(benzyloxycarbonyloxy)succinimide as previously described.[5] The reactions of **5**, **6**, and **7** with *N*-benzyloxycarbonylglycyl anhydride[8] in the presence of a small amount of triethylamine in tetrahydrofuran solutions led to the isolation of the protected 4-*N*-glycyl derivatives, **8**, **10**, and **11**, respectively, in good yield.

The attempted preparation of *N*,*N*′-dibenzyloxycarbonyl-L-β-lysyl anhydride by the published procedure[6] led to the isolation of a product which did not exhibit the characteristic anhydride bands in the IR spectrum at 1840 and 1765 cm^{-1}. The desired *N*,*N*′-dibenzyloxycarbonyl-L-β-lysyl anhydride was prepared from *N*,*N*′-dibenzyloxycarbonyl-L-β-lysine with *N*,*N*′-dicyclohexylcarbodiimide in tetrahydrofuran solution and the anhydride was used without isolation for coupling at the 4-*N*-CH_3 group of **5** in the presence of a small amount of triethylamine to afford the desired protected 4-*N*-L-β-lysyl derivative **9** in poor yield. The observations of the typical ^1H NMR signals of the substituted 4-*N*-CH_3 group between 3.08 and 3.12 ppm of the substances **8**~**11**, together with the absence of ester bands in their IR spectra established that the reaction products were indeed the desired 4-*N*-substituted substances **8**~**11**.

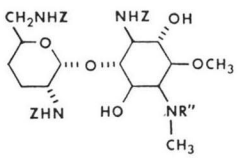


5



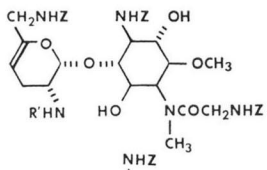
6 R′ = $COCH_2CH(CH_2)_3NHZ$

7 R′ = Z



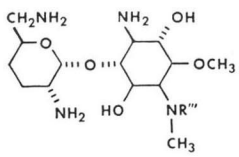
8 R″ = $COCH_2NHZ$

9 R″ = $COCH_2CH(CH_2)_3NHZ$



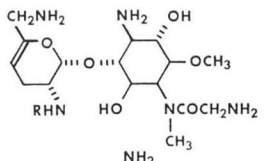
10 R′ = $COCH_2CH(CH_2)_3NHZ$

11 R′ = Z



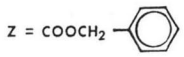
12 R‴ = $COCH_2NH_2$

13 R‴ = $COCH_2CH(CH_2)_3NH_2$



14 R = $COCH_2CH(CH_2)_3NH_2$

15 R = H



Z = $COOCH_2$

Table 1. ^{13}C NMR chemical shifts of **12** and the aminocyclitol of **1** in acidic and basic D_2O solutions.*

Assignment	12		1	
	pD 1.9	pD 10.2	pD 1.8	pD 11.0
C-1′	95.0	101.2		
C-3	81.8	81.6	79.0	81.4
C-6	75.5	82.9	75.7	82.6
C-5	75.7	72.0	68.1	72.5
C-2	69.2	73.8	69.3	73.5
C-5′	66.6	70.1		
O-CH_3	59.3	59.1	59.7	57.8
C-1	55.0	56.0	54.7	56.0
C-4	54.8	53.8	60.4	60.5
C-2′	49.1	50.4		
C-6′	43.5	45.5		
Gly-CH_2	41.6	43.3		
N-CH_3	35.9	35.1	36.8	38.5
C-4′	26.6	28.2		
C-3′	21.7	26.6		

* The ^{13}C NMR spectra were determined in D_2O solutions, chemical shifts are reported downfield from tetramethylsilane and were measured from internal dioxane (67.4 ppm). The spectrum of **12** was recorded on the JEOL FX 90Q instrument at 22.5 MHz while the spectrum of **1** was obtained with a Varian Associates XL-100/NTC TT-100 spectrometer system at 25.2 MHz.

Deprotection of the saturated compounds **8** and **9** by hydrogenolysis in methanolic hydrochloric acid under the usual conditions[5] led to the isolation of the tetrahydrochloride salt of **12** and the pentahydrochloride salt of **13**, respectively. Again the structures were shown to be the desired 4-*N*-substituted substances by spectral means.

The results of the comparison of the ^{13}C NMR spectra of **12** with the aminocyclitol residue of **1** in acidic and basic D_2O solutions are summarized in Table 1. With the exception of the glycyl carbonyl signal the resonances of all the carbons of **12** are included. The aminocyclitol carbon resonances of **12** are compared with those of **1**.[1] It should be noted that the 4-*N*-CH_3 group of **12** is acylated while the 4-*N*-CH_3 group of **1** is not substituted. When the chemical shifts of the carbon atoms are compared, at both acidic and basic pD, the signals are easily assigned. The resonances at C-4 and the *N*-CH_3 would be expected to be different for the two compounds. The comparison of the chemical shift assignments of the aminocyclitol residue of **12** with those of **1** suggests that the two compounds may have the same aminocyclitol conformation. The remaining resonances are readily assigned. The resonance at 101.2 ppm has to be due to C-1′ and the resonance at 70.1 ppm is caused by C-5′. The resonance at 45.5 ppm is assigned to C-6′ since it is a triplet in the off-resonance spin decoupling experiment. The methylene resonances at 28.2 and 26.6 ppm can be distinguished. Since the signal at 26.6 ppm shows a β-titration shift it is assigned to C-3′.

The above titration sample of **12** was allowed to stand in D_2O solution at pD 10.2 for one week. It was noted that the signal for C-1′ had shifted from 101.2 ppm to 99.1 ppm and C-3′ had shifted from 26.6 ppm to 23.7 ppm while the resonance for C-4 had shifted from 53.8 ppm to 60.4 ppm. The *N*-CH_3 signal had shifted from 35.1 ppm to 38.4 ppm. Slight differences in the other chemical shift values were observed as well. However, the major changes were observed for the resonances due to C-1′, C-3′, C-4, and the *N*-CH_3. The upfield (γ-shift) for C-1′ and C-3′ support the contention that **12** had undergone an isofortimicin rearrangement[7~9] in which the glycyl residue at the 4-*N*-CH_3 group of **12** was transferred to the 2′-amino group in 3-*epi*-2′-*N*-glycyl-6′-de-*C*-methylfortimicin B. The downfield shifts of the signals of C-4 and the *N*-CH_3 group also support this contention. This type of rearrangement was previously observed on fortimicin A and some 4-*N*-acyl fortimicin B free bases.[7~9]

The deprotection of the unsaturated intermediates **10** and **11** was carried out in methanol containing a small amount of glacial acetic acid over a prereduced 10% Pd-C catalyst for 1 hour to afford the pentaacetate salt of **14** and the tetraacetate salt of **15**, respectively. This method of deprotection of unsaturated substances was developed to effect deprotection with a minimal amount of double bond reduction.[10] The residue obtained from the deprotection of **10** was estimated to contain at least 60% of the pentaacetate salt of **14** (^1H NMR, TLC; the saturated 5′-*epi*-derivative was the main contaminant). The residue obtained after the deprotection of **11** contained about 85% of the tetraacetate salt of **15** (^1H NMR, TLC; the saturated 5′-*epi*-derivative was the main contaminant). No attempt was made to isolate the two compounds in pure form since the substances were expected to rearrange and/or decompose in the form of the free bases.

The comparison of the biological activities of the hydrochloride salts of **12** and **13** and the acetate salts of **14** and **15** with the antimicrobial activity of fortimicin A is summarized in Table 2. This study indicates that the structure-activity relationship observed with fortimicin A and fortimicin B[2] does not apply to lysinomicin derivatives; lysinomicin (**1**) was shown to be a slightly more potent antibiotic than fortimicin A.[11]

Table 2. *In vitro* antimicrobial activity of **12, 13, 14** and **15** compared to that of fortimicin A (MIC* μg/ml).

Organism	Fortimicin A	**12**	**13**	**14****	**15****
Staphylococcus aureus Smith	0.78	6.2	6.2	3.1	50
Streptococcus faecalis 10541	50	>100	>100	>100	>100
Enterobacter aerogenes 13048	3.1	12.5	50	25	>100
E. coli JUHL	3.1[a], 6.2[b]	12.5	50	50	>100
E. coli BL 3676 (Res)	25	100	>100	>100	>100
E. coli 76–2	3.1	12.5	50	25	100
Klebsiella pneumoniae 10031	1.56	12.5	25	12.5	100
Klebsiella pneumoniae KY 4262	6.2	25	100	50	>100
Providencia 1577	1.56	100	50	25	>100
Pseudo. aeruginosa BMH #10	0.78	6.2	25	12.5	50
Pseudo. aeruginosa KY 8512	6.2	25	100	100	>100
Pseudo. aeruginosa KY 8516	6.2	>100	>100	100	>100
Pseudo. aeruginosa 209	>100	>100	>100	>100	>100
Pseudo. aeruginosa 27853	6.2[b], 12.5[a]	25	>100	100	>100
Salmonella typhimurium Ed. #9	3.1	25	50	25	100
Serratia marcescens 4003	1.56[b], 3.1[a]	12.5	25	12.5	50
Shigella sonnei 9290	6.2	25	100	50	>100
Proteus rettgeri U6333	12.5	>100	>100	100	>100
Proteus vulgaris JJ	3.1	12.5	50	25	100
Proteus mirabilis Fin. #9	3.1[a], 6.2[b]	12.5	50	50	100

* The method was a two-fold dilution test using Mueller-Hinton agar at 10 ml/plate. The inoculum of approximately 10^5 organisms was applied to the agar surface by a Steers replicating device. The plates were incubated at 35°C for 24 hours. The control was fortimicin A. Since the table represents the results of two different assays the MIC values given in the fortimicin A controls for **12** and **13** are indicated by [a)] while the fortimicin A controls for **14** and **15** are indicated by [b)] where values were found to differ.

** **14** and **15** were contaminated with the corresponding saturated 5′-*epi* compounds (see Experimental) which were previously shown to be inactive since they contain a saturated L-diaminosugar residue.

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 Perkin-Elmer Model 241 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 XL-100/NTC-TT-100 spectrometer. Chemical shifts are reported in ppm from internal tetramethylsilane (0 δ) for the spectra recorded of compounds in deuteriochloroform ($CDCl_3$) solutions, and in ppm from sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (0 δ) for the spectra recorded of compounds in deuterium oxide (D_2O) solutions. Mass spectra were recorded with an A.E.I. MS-902 mass spectrometer with an ionization energy of 70 eV.

Preparation of the Partially Protected Intermediates **5**, **6**, and **7**

The substances **4**, **1**, and **2** were treated in aqueous methanolic solutions with an excess of *N*-(benzyloxycarbonyloxy)succinimide and the reaction mixtures were worked up as previously described[5)] to afford the desired compounds **5**, **6**, and **7**, respectively. The analytical and spectral data obtained on these substances are listed in Table 3.

Table 3. Physical constants of the partially protected intermediates **5**, **6**, and **7**.

Measurement	**5**	**6**	**7**
Sum formula	$C_{38}H_{48}N_4O_{11}$	$C_{52}H_{64}N_6O_{14}$	$C_{38}H_{46}N_4O_{11}$
Anal. Calcd.	C 61.94, H 6.57, N 7.60%	C 62.63, H 6.47, N 8.43%	C 62.11, H 6.31, N 7.63%
Found	C 61.63, H 6.61, N 7.56%	C 62.35, H 6.39, N 8.38%	C 61.94, H 6.40, N 7.61%
Optical rotation (c in CH_3OH)	$[\alpha]_D^{21}+37°$ (1.03)	$[\alpha]_D^{21}+51°$ (0.93)	$[\alpha]_D^{20}+72°$ (0.94)
IR $\tilde{\nu}_{max}^{CDCl_3}$ cm^{-1}	1712, 1515	1712, 1665, 1513	1714, 1513
^1H NMR ($CDCl_3$) δ ppm	7.29(Ar-Z), 5.06(CH_2-Z), 3.44(*O*-CH_3),2.50(4-*N*-CH_3)	7.32(Ar-Z), 5.07(CH_2-Z), 3.37(*O*-CH_3),2.51(4-*N*-CH_3)	7.31(Ar-Z), 5.07(CH_2-Z), 3.43(*O*-CH_3),2.52(4-*N*-CH_3)

Table 4. Physical constants of the fully protected 4-*N*-substituted intermediates **8**~**11**.

Measurement	**8**	**9**	**10**	**11**
Sum formula	$C_{48}H_{57}N_5O_{14}$	$C_{60}H_{72}N_6O_{16}$	$C_{62}H_{73}N_7O_{17}$	$C_{48}H_{55}N_5O_{14}$
Anal. Calcd.	C 62.12, H 6.19, N 7.55%	C 63.59, H 6.40, N 7.42%	C 62.66, H 6.19, N 8.25%	C 62.26, H 5.99, N 7.56%
Found	C 62.22, H 6.49, N 7.58%	C 63.34, H 6.44, N 7.11%	C 62.78, H 6.41, N 8.23%	C 62.11, H 6.22, N 7.53%
Optical rotation (c in CH_3OH)	$[\alpha]_D^{26}+43°$ (1.00)	$[\alpha]_D^{23}+26°$ (0.98)	$[\alpha]_D^{22}+50°$ (0.98)	$[\alpha]_D^{22}+62°$(1.02)
IR $\tilde{\nu}_{max}^{CDCl_3}$ cm^{-1}	1712, 1647, 1511	1711, 1633, 1511	1715, 1655, 1512	1716, 1648, 1511
^1H NMR ($CDCl_3$) δ ppm	7.29 (Ar-Z), 5.05 (CH_2-Z), 3.38 (*O*-CH_3), 3.09 (4-*N*-CH_3)	7.28 (Ar-Z), 5.04 (CH_2-Z), 3.35 (*O*-CH_3), 3.12 (4-*N*-CH_3)	7.28 (Ar-Z), 5.05 (CH_2-Z), 3.32 (*O*-CH_3), 3.15 (4-*N*-CH_3)	7.30 (Ar-Z), 5.08 (CH_2-Z), 3.35 (*O*-CH_3), 3.08 (4-*N*-CH_3)

Reactions of **5**, **6**, and **7** with *N*-Benzyloxycarbonylglycyl Anhydride

The above prepared intermediates, **5**, **6**, and **7** were allowed to react with 3 mmole of *N*-benzyloxycarbonylglycyl anhydride[6] in the presence of 3 mmole of triethylamine per mmole of protected derivative in tetrahydrofuran for 2 hours at room temperature and then under a gentle reflux for 20 hours. The reaction mixtures were evaporated under reduced pressure and the residues obtained were purified by chromatography on silica gel columns in CH_2Cl_2 - CH_3OH - conc. NH_4OH (950: 50: 5, v/v) solutions to afford the desired substances, **8**, **10**, and **11** in good yields. The analytical data for these substances are recorded in Table 4.

4-*N*-(*N*,*N*′-Dibenzyloxycarbonyl-L-β-lysyl)-3-*epi*-6′-de-*C*-methyl-1,2′,6′-tri-*N*-benzyloxycarbonyl fortimicin B(**9**)

A solution of 1.951 g of *N*,*N*′-dibenzyloxycarbonyl-L-β-lysine and 0.486 g of *N*,*N*′-dicyclohexylcarbodiimide in 40 ml of tetrahydrofuran was stirred at room temperature for 3 hours. The precipitated *N*,*N*′-dicyclohexylurea was collected on a filter and the filtrate containing the anhydride was used in the coupling reaction with 0.578 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*epi*-6′-de-*C*-methylfortimicin B (**5**) in the presence of 0.245 g of triethylamine first at room temperature for 3 hours and then under a gentle reflux for 22 hours. Evaporation of the solvent afforded a residue of 4.265 g which was purified by chromatography on 215 g of silica gel in CH_2Cl_2 - CH_3OH - conc. NH_4OH (950: 50: 5, v/v) to afford 0.236 g of the pentaprotected substance. An analytical sample of **9** amounting to 0.127 g was obtained after further chromatography of the above sample. The analytical data obtained on this compound are recorded in Table 4.

4-*N*-Glycyl-3-*epi*-6′-de-*C*-methylfortimicin B (**12**) Tetrahydrochloride Salt

A solution of 0.169 g of the tetraprotected substance **8** in 82 ml of CH_3OH and 18 ml of 0.2 N HCl in CH_3OH was hydrogenolyzed over 0.170 g of 5% Pd-C in the usual manner for 4 hours.[5] The filtrate obtained after removal of the catalyst was evaporated to dryness under reduced pressure. The

residue was repeatedly redissolved in CH_3OH and the solvent was evaporated. The residue was dried over KOH-pellets under high vacuum: 0.109 g of the tetrahydrochloride salt of **12**. The sample had the following physical constants: $[\alpha]_D^{22}+61°$ (*c* 1.02, CH_3OH); $\tilde{\nu}_{max}^{KBr}$ 1650, 1600, 1487 cm^{-1}; ^1H NMR (D_2O) 5.66 (anom. H), 4.11 (Gly-CH_2), 3.47 (*O*-CH_3), 3.15 (4-*N*-CH_3) ppm; ^{13}C NMR see Table 1; MS [$C_{16}H_{33}N_5O_6-OH$]$^+$ Calcd. for $C_{16}H_{32}N_5O_5$: 374.2404, Found *m/z*: 374.2424.

4-*N*-L-*β*-Lysyl-3-*epi*-6′-de-*C*-methylfortimicin B (**13**) Pentahydrochloride Salt

The hydrogenolysis of 0.145 g of **9** was carried out in the same manner as described above[5] to afford 0.102 g of the pentahydrochloride salt of **13** with the following physical constants: $[\alpha]_D^{25}+63°$ (*c* 1.04, CH_3OH); $\tilde{\nu}_{max}^{KBr}$ 1620, 1485 cm^{-1} (the strong salt bands cover the region of the tertiary amide absorption); ^1H NMR (D_2O) 5.65 (anom. H), 3.47 (*O*-CH_3), 3.18 (4-*N*-CH_3) ppm; MS[$C_{20}H_{42}N_6O_6-C_4H_{12}N_2O$]$^+$ Calcd. for $C_{16}H_{30}N_4O_5$: 358.2216, Found *m/z*: 358.2193.

4-*N*-Glycyllysinomicin (**14**) Pentaacetate Salt

A solution of 0.262 g of the pentaprotected intermediate **10** in 160 ml of CH_3OH containing 0.067 ml of glacial acetic acid was hydrogenolyzed over 0.262 g of prereduced 10% Pd-C for 1 hour.[10] The catalyst was collected on a filter and the filtrate was evaporated under reduced pressure. The residue was repeatedly redissolved in CH_3OH and the solvent was evaporated. The residue was dried over KOH pellets under high vacuum to afford 0.158 g of reaction mixture. The content of the desired unsaturated pentaacetate salt of **14** in the above residue was estimated to be at least 60% (^1H NMR, TLC: the contaminating product was the 5′-*epi* compound). Since the compound (**14**) was not expected to be stable as the free base, chromatographic purification was avoided. The substance had the following physical constants: $\tilde{\nu}_{max}^{KBr}$ 1648, 1565, 1403 cm^{-1} (absence of ester bands); ^1H NMR (D_2O) 3.46 (*O*-CH_3), 3.07 (4-*N*-CH_3) ppm; MS (**14**, [M]$^+$) Calcd. for $C_{22}H_{43}N_7O_7$: 517.3224, Found *m/z*: 517.3244; MS (5′-*epi*-[M]$^+$) Calcd. for $C_{22}H_{45}N_7O_7$: 519.3381, Found *m/z*: 519.3358.

4-*N*-Glycyl-2′-de-*N*-L-*β*-lysyllysinomicin (**15**) Tetraacetate Salt

A solution of 0.250 g of **11** in 160 ml of CH_3OH containing 0.066 ml of glacial acetic acid was hydrogenolyzed over 0.250 g of prereduced 10% Pd-C as in the above case[10] to afford a residue of 0.155 g containing at least 85% of the desired tetraacetate salt of **15** (^1H NMR, TLC). Since the substance (**15**) was not expected to be stable as the free base chromatographic purification was not attempted. The substance had the following physical constants: $\tilde{\nu}_{max}^{KBr}$ 1635, 1560, 1403 cm^{-1} (absence of ester bands); ^1H NMR (D_2O) 3.47 (*O*-CH_3), 3.08 (4-*N*-CH_3) ppm; MS (**15**, [M]$^+$) Calcd. for $C_{16}H_{31}N_5O_6$: 389.2274, Found *m/z*: 389.2258; MS (5′-*epi*-[M]$^+$) Calcd. for $C_{16}H_{33}N_5O_6$: 391.2431; Found *m/z*: 391.2440.

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