LYSINOMICIN, A NEW AMINOGLYCOSIDE ANTIBIOTIC

II. STRUCTURE AND STEREOCHEMISTRY

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The structure of lysinomicin, a new aminocyclitol antibiotic, was established as 3-*epi*-2′-*N*-(L-β-lysyl)-4′,5′-didehydro-6′-de-*C*-methylfortimicin B (**1**) on the basis of spectral evidence and chemical degradation of the antibiotic. In the course of the degradation of **1**, three additional compounds with interesting biological properties were obtained: 3-*epi*-2′-*N*-(L-β-lysyl)-6′-de-*C*-methylfortimicin B (**4**), 3-*epi*-4′,5′-didehydro-6′-de-*C*-methylfortimicin B (**6**) and 3-*epi*-6′-de-*C*-methylfortimicin B (**7**).

The taxonomy of the lysinomicin* producing organism, the fermentation, isolation, and biological properties of the new antibiotic were disclosed[1] and an account of the present work was likewise presented.[2] This paper deals with the structure and stereochemistry of lysinomicin and some of its degradation products. The isolation procedure,[1] together with spectral properties, discussed below, indicated that lysinomicin is a novel aminocyclitol antibiotic which is acylated with a six-carbon diamino acid.

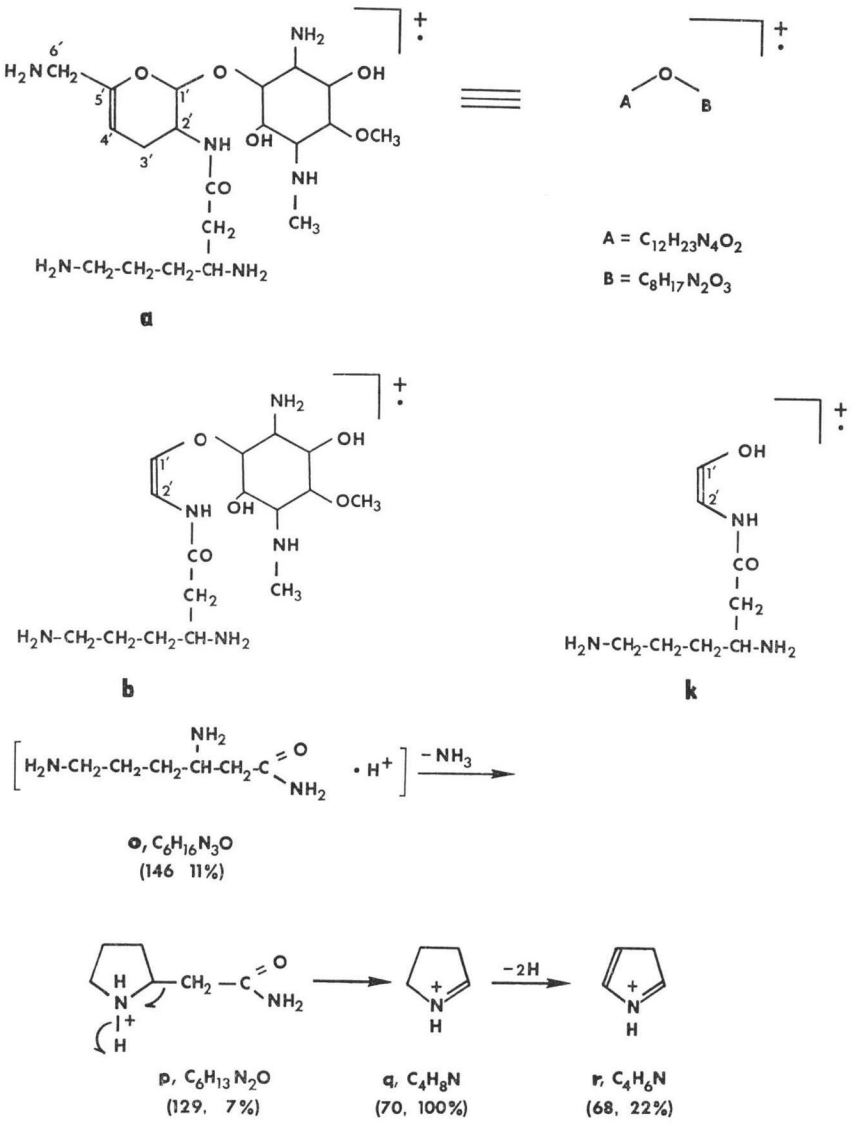
The IR spectrum of the free base of the new antibiotic showed a band at 1690 cm^{-1} which is characteristic of an enol ether, while the bands at 1645 and 1545 cm^{-1} can be assigned to a secondary amide. The results of the mass spectral studies of the new substance are summarized in Scheme 1 and Table 1. The fragmentation of the new antibiotic under electron impact can be explained by the assignment of the gross structure **a** to the molecular ion of the antibiotic. The letter A is used in the description of fragments associated with the substituted diamino sugar portion of the molecule while the letter **B** is used to describe ions arising from the fortamine residue of the antibiotic.

The ions **d, e,** and **f,** all containing 12 carbon atoms, are members of the first group, while the ions **g, h, i, j, l, m,** and **n** are representatives of the second group. In the course of the mass spectral fragmentation of fortimicin B free base ions of *m/z* 235, 217, 207, and 189, respectively, were observed.[3] These fragments have the same composition as the fragments **g, h, i,** and **j** respectively, of the new antibiotic. This finding suggests that lysinomicin may contain an unsubstituted fortamine portion very similar to that of fortimicin B.

The ions **b** and **k** arising from **a** and **d**, respectively, by the loss of $CH_2=CHCOCH_2NH_2$, (C_4H_7NO), are of particular interest in connection with the structure of the new antibiotic. The same fragment was lost from sisomicin by a retro Diels-Alder cleavage in the mass spectral fragmentation of that antibiotic.[4] These findings suggested that the amino sugar part (A) of lysinomicin may be the same as

* Lysinomicin was originally called AX-127B-1 (J. Antibiotics 35: 82–128, 1982).

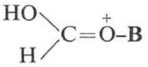
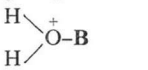
Scheme 1. Mass spectral fragmentation of **1**.



that found in sisomicin with an amino acid residue $C_5H_{13}N_2CO$ bound to the C-2′ amino group. This contention is supported by the observation of a fragment **o**, representing a protonated amino acid amide, which arose by elimination of the C-2′ amino acyl residue. The loss of ammonia from **o** to afford the protonated fragment **p** which gave rise to **q** and **r** upon further fragmentation is compatible with the hypothesis that the substituent at the C-2′ amino group is a β-lysyl residue. Additional ions at *m/z* 86 (C_4H_8NO, 82%), 87 (C_4H_9NO, 41%), and 88 ($C_4H_{10}NO$, 47%) were observed in the mass spectrum of **1**.

The results of the 270 MHz ^1H NMR spectrum of **1** sulfate in D_2O are recorded in Table 2. The singlet resonances at 4.01 and 3.40 ppm are assigned to the OCH_3 and $NHCH_3$ groups, respectively, of the 3-*epi*-fortamine residue of **1**. The assignment of the other resonances and coupling constants

Table 1. Significant mass spectral fragmentation of lysinomicin free base.

Ion	*m/z*	Relative intensity* (%)	Formula**	Identification***
a	460	1	$C_{20}H_{40}N_6O_6$	$[M]^+$
b	375	1.6	$C_{16}H_{33}N_5O_5$	Retro Diels-Alder of **a**
c	344	3.5	$C_{18}H_{24}N_4O_3$	**a**−(CH_3NH_2, CH_3OH, NH_3, $2H_2O$)
d	273	18.6	$C_{12}H_{25}N_4O_3$	**A**−OH protonated
e	272	16.2	$C_{12}H_{24}N_4O_3$	**A**−OH
f	238	3.1	$C_{12}H_{20}N_3O_2$	**A** (−NH_3)
g	235	4	$C_9H_{19}N_2O_5$	
h	217	11	$C_9H_{17}N_2O_4$	**g**−H_2O
i	207	30	$C_8H_{19}N_2O_4$	
j	189	25	$C_8H_{17}N_2O_3$	**B**
k	187	7.6	$C_8H_{17}N_3O_2$	Retro Diels-Alder of **d**
l	172	8.8	$C_8H_{14}NO_3$	**j**−NH_3
m	171	8.7	$C_8H_{15}N_2O_2$	**j**−H_2O
n	154	7	$C_8H_{12}NO_2$	**j**−NH_3−H_2O

* Approximate values.
** Determined by high resolution mass measurements.
*** **A**=diaminosugar part, **B**=3-*epi*-fortamine part, see Scheme 1.

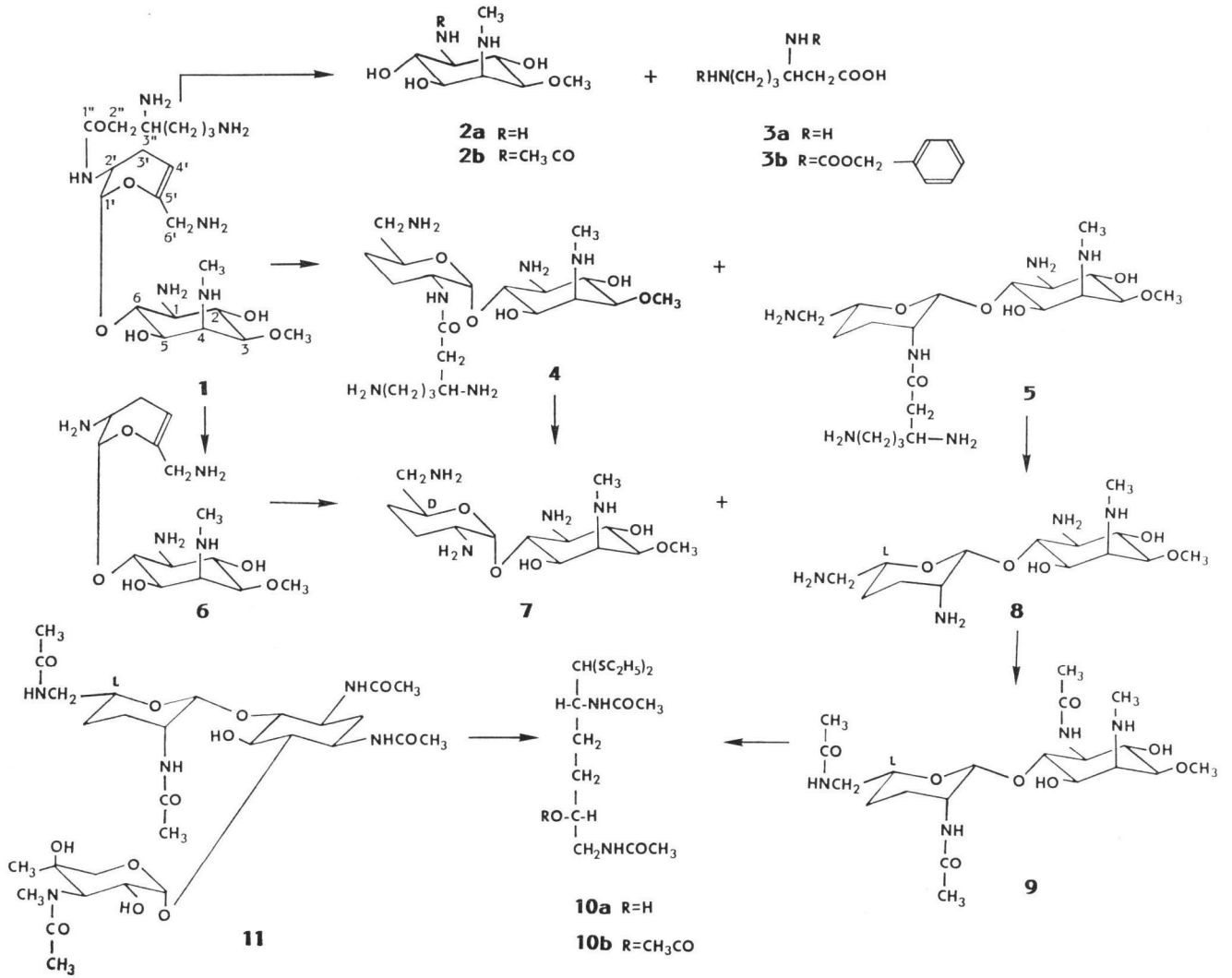
Table 2. ^1H NMR (270 MHz) spectrum of lysinomicin sulfate in D_2O.

	Chemical shifts	Coupling constants		Chemical shifts	Coupling constants
H-1	3.71	$J_{1,2}$=10.0	H-2′	4.77	
H-2	4.53	$J_{2,3}$=10.0	CH_2-3′	2.68, 2.89	$J_{3',4'}$=~4.0
H-3	4.10	$J_{3,4}$= 4.0	H-4′	5.60	
H-4	4.44	$J_{4,5}$= 4.5	CH_2-6′	4.1~4.2	
H-5	4.65	$J_{5,6}$= 9.5	CH_2-2″	3.16, 3.32	$J_{2'',2''}$=16.5
H-6	4.80	$J_{6,1}$= 9.5	H-3″	4.1~4.2	$J_{3'',2''}$=8.0, 4.5
OCH_3	4.01		CH_2-4″	2.2~2.3	
NCH_3	3.40		CH_2-5″	2.2~2.3	
H-1′	5.96	$J_{1',2'}$=1.5	CH_2-6″	3.50	

of the 3-*epi*-fortamine residue were obtained from the 270 MHz spectrum, together with some spin decoupling experiments performed at 100 MHz. The four large coupling constants $J_{1,2}$=10, $J_{2,3}$=10, $J_{5,6}$=9.5, and $J_{6,1}$=9.5 Hz, together with the CD spectral evidence presented below, support the formulation of the 3-*epi*-fortamine part of **1** (Scheme 2), in which the 4-$NHCH_3$ group is the only axial substituent while all the other substituents are equatorial.

The spectrum shows an anomeric resonance at 5.96 ppm for H-1′. A spin decoupling experiment revealed that this proton is coupled to the proton H-2′ at 4.77 ppm. The small coupling constant, $J_{1',2'}$=1.5 Hz, is consistent with an α-glycosidic bond, often seen in aminoglycoside antibiotics, and an equatorial aminoacyl group as formulated in **1**. The chemical shifts of H-1′ at 5.96 ppm and H-6 at 4.80 ppm are very close to the corresponding chemical shifts observed for fortimicin B sulfate which were found to be at 6.01 ppm (H-1′) and 4.81 ppm (H-6),[3] respectively. This finding confirms the presence of an α-glycosidic linkage between C-1′ and C-6 in **1**. These assignments were confirmed in

Scheme 2. Chemical degradation of **1**.



the course of the degradation work on **1** discussed below. The ^1H NMR spectrum shows a triplet resonance with a small coupling at 5.6 ppm (H-4′). This observation, together with the absence of a signal for a proton at C-5′ led to the formulation of **1** with a double bond between C-4′ and C-5′. Other resonances associated with the unsaturated diaminosugar residue in **1** are listed in Table 2. Carbon atoms of the β-lysyl ($C_5H_{13}N_2CO$) side chain are numbered 1″ through 6″.

The CD spectrum of lysinomicin in Cupra A solution[5] exhibits an intense positive rotation at short wave lengths (290~450 nm) and a less intense negative rotation at longer wave lengths (580~660 nm). Since a very similar CD spectrum was obtained on fortimicin B,[3] one may conclude that the stereochemistry of the vicinal amino alcohol groups in the fortamine portion of lysinomicin is the same as that determined for the fortamine part of fortimicin B.

The hydrolysis of the sulfate salt of lysinomicin in 1 M hydrochloric acid led to the isolation of 3-*epi*-fortamine (**2a**) the structure and stereochemistry of which was confirmed by the usual spectral and analytical methods. The observation of four large coupling constants, $J_{1,2}=J_{2,3}=J_{5,6}=J_{6,1}=10.2$ Hz, in the 100 MHz ^1H NMR spectrum of **2a** is of particular interest in connection with the conformational formulation of the aminocyclitol ring of lysinomicin since it confirms the earlier conclusion that the aminocyclitol of the antibiotic **1** contains five axial protons.

L-β-Lysine (**3a**) was the second product isolated from the above hydrolysis mixture. The reaction of **3a** with *N*-(benzyloxycarbonyloxy)succinimide afforded *N,N′*-(dibenzyloxycarbonyl)-L-β-lysine (**3b**) the melting point and optical rotation of which corresponded to those reported in the literature[6] for **3b**.

The catalytic reduction of the free base **1** over a 20% Pd-C (50% wet) catalyst in methanolic solution led to a mixture from which 3-*epi*-2′-*N*-(L-β-lysyl)-6′-de-*C*-methylfortimicin B (**4**) and 3,5′-di-*epi*-2′-*N*-(L-β-lysyl)-6′-de-*C*-methylfortimicin B (**5**) were isolated in yields of about 10% and 40%, respectively. The stereochemical assignments of the structures **4** and **5** were the result of spectral evidence as well as additional chemical evidence obtained by the degradation work discussed below.

The ^{13}C NMR chemical shifts of **1** (SFORD experiment), **4** and **5** at basic pD in D_2O are recorded in Table 3. The double bond carbon signals at 150.9 ppm (C-5′) and 96.3 ppm (C-4′) of **1** are expected from the presence of an unsaturated aminosugar residue in the antibiotic (**1**). The assignments of the ^{13}C NMR signals of **1** have been confirmed by comparison with model compounds and single frequency decoupling experiments. The chemical shifts of the diaminocyclitol resonances of **1**, **4**, and **5** are close, if not identical, which is expected since the 3-*epi*-fortamine residue is present in all three compounds.

A comparison of the amino sugar resonances in Table 3 shows that the double bond of **1** is not present in **4** and **5**. The resonances of **4** are different from those of **5** due to the difference in stereochemistry at C-5′ in **4** and **5**. The chemical shift C-5′ of **4** is similar to that of the purpurosamine C residue of gentamicin C_{1a} (Table 5) which suggests that the diaminosugar of **4** has the same relative configuration at C-5′ as that determined for gentamicin C_{1a}. The carbonyl absorptions around 175 ppm as well as the signals caused by the β-lysyl side chain around 48 (3″-CH), 44 (2″-CH_2), 41 (6″-CH_2), 34 (4″-CH_2), and about 28 (5″-CH_2) ppm are very similar for the three substances (**1**, **4**, and **5**). The ^{13}C NMR titration of **1** confirms a sisomicin-like sugar which is acylated at the C-2′ amino group since no β-shifts are observed for C-1′ and C-3′. This finding is in agreement with the mass spectral fragmentation (Scheme 1 and Table 1) of **1** discussed above. The formulation of the 3-*epi*-fortamine residue in **1** as a 1,4-diaminocyclitol is supported by the small but significant β-shifts of the amino-

Table 3. ^{13}C NMR chemical shifts of **1**, **4**, and **5** in D_2O.

Carbon	**1**** (pD 11.0)	β-Shift*	**4** (pD 11.0)	**5** (pD 10.8)
C=O	s 175.3		174.6	175.5
5′	s 150.9	7.1	71.6	78.6
1′	d 98.5		99.8	102.7
4′	d 96.3		28.1	27.4
3	d 82.6	3.6	82.6	82.6
6	d 81.3	5.7	83.7	82.6
2	d 73.5	4.2	73.3	73.1
5	d 72.5	4.4	72.6	72.9
4	d 60.4		60.4	59.9
OCH_3	q 57.8		57.8	57.8
1	d 55.9		56.3	55.9
3″	d 48.8		48.8	47.8
2′	d 46.7		50.3	48.9
2″	t 44.1	2.5	44.3	44.1
6′	t 43.3		45.7	45.6
6″	t 41.1		41.2	41.1
NCH_3	q 38.5		38.3	38.3
4″	t 34.3	4.6	34.4	34.0
5″	t 27.9	3.8	27.6	27.4
3′	t 23.1		24.0	23.0

* β-Shift for acidification from pD 11 to pD 2.
** SFORD experiment.

cyclitol resonances of C-2, C-3, C-5, and C-6.

Hydrazinolysis of **1** under the conditions previously employed in the cleavage of an amide bond in lincomycin[7] led to the formation of 3-*epi*-4′,5′-didehydro-6′-de-*C*-methylfortimicin B (**6**). The CD spectrum of **6** in Cupra A solution[5] exhibited an intense positive rotation at short wave lengths (290~450 nm) and a less intense negative rotation at longer wave lengths (580~660 nm). This evidence further supports the stereochemical assignments to the 3-*epi*-fortamine residue of the molecule. The IR spectrum of **6** revealed a band at 1684 cm^{-1} which is characteristic of an enol ether group in the molecule while the secondary amide bands observed in the IR spectrum of **1** were missing.

When the mass spectral fragmentation pattern of **6** was compared to that of **1**, the absence of the ions derived from the β-lysyl side chain of **1** (**o**~**r**) was noted in the mass spectrum of **6**. The following ions resulting from the 3-*epi*-fortamine residue of **1** (see Table 1) were likewise observed in the spectrum of **6** (relative intensities of ions from **6** in parenthesis): **g** (7.8%), **h** (54%), **i** (9.6%), **j** (47.5%), **l** (44%), **m** (9%), and **n** (10.6%), The ions at *m/z* 88 ($C_4H_{10}NO$, 62.4%), 87 (C_4H_9NO, 45%), and 86 (C_4H_8NO, 100%) were even more prominent in the spectrum of **6** than in that of **1**. The same fragments were observed in the mass spectra of fortimicin A and fortimicin B. Since the structural elements of the latter two compounds include a saturated diaminosugar residue rather than an unsaturated diaminosugar residue as in **1** and **6**, one may conclude that these ions (*m/z* 88, 87, and 86) originate from the very similar fortamine residues in the four compounds. The ions **d**′~**l**′ (Table 4) all containing six carbon atoms, indicate that the unsaturated diaminosugar residue in **1** and **6** is a C_6-diaminohexose. The ions **c**′ and **m**′ are retro Diels-Alder fragments of the ions **a**′ and **e**′, respectively. These ions (**c**′ and **m**′) indicate that the unsaturated diaminosugar residue ($C=C_6H_{11}N_2O$) contained in **6** is of the same gross structure as that found in sisomicin.[4]

Hydrazinolysis[7] of **4** or **5** afforded 3-*epi*-6′-de-*C*-methylfortimicin B (**7**) or 3,5′-di-*epi*-6′-*C*-methyl-

Table 4. Significant mass spectral fragments including parts of the unsaturated diaminosugar of **6**.

Ion	*m/z*	Relative intensity* (%)	Formula**	Identification***
a′	332	7.2	$C_{14}H_{28}N_4O_5$	$[M]^+$
b′	315	12.6	$C_{14}H_{25}N_3O_5$	**a′** $(-NH_3)$
c′	247	21.2	$C_{10}H_{21}N_3O_4$	Retro Diels-Alder of **a′**
d′	145	7.4	$C_6H_{13}N_2O_2$	C−OH protonated
e′	144	10.7	$C_6H_{12}N_2O_2$	C−OH
f′	129	3.3	$C_6H_{11}NO_2$	**d′** $(-NH_2)$
g′	128	2.5	$C_6H_{10}NO_2$	**d′** $(-NH_3)$
h′	127	28	$C_6H_{11}N_2O$	C
i′	127	11.3	$C_6H_9NO_2$	**e′** $(-NH_3)$
j′	126	4.6	$C_6H_{10}N_2O$	**e′** $(-H_2O)$
k′	126	12.6	$C_6H_8NO_2$	C−O $(-NH_3)$
l′	110	12.6	C_6H_8NO	**h′** $(-NH_3)$
m′	59	7.4	C_2H_5NO	Retro Diels-Alder of **e′**

* Approximate values.
** Determined by high resolution mass measurements.
*** Unsaturated diaminosugar residue $C=C_6H_{11}N_2O$.

fortimicin B (**8**), respectively. Catalytic hydrogenation of the double bond in **6** gave a reaction mixture from which, upon chromatography, a small amount of **7** could be separated from the main product **8**. The saturated pseudodisaccharides, **7** and **8**, obtained by the two routes were shown to be identical by their chromatographic behavior and spectral properties.

The ^{13}C NMR spectral results of the purpurosamine C residue of gentamicin C_{1a},[8] **6**, **7**, and **8** in D_2O are summarized in Table 5. The diaminocyclitol resonances of **6**, **7**, and **8** are easily assigned by comparison with the cyclitol resonances of **1**, **4**, and **5** (Table 3). A comparison of the diaminosugar resonances of C-4′ and C-5′ of **6** with the corresponding signals of **7** and **8** clearly indicates that the pseudodisaccharide **6** has a double bond between C-4′ and C-5′ while **7** and **8** are saturated compounds. The difference in chemical shifts observed at C-5′ of **7** and **8** is the result of different stereochemistry at that center. Small differences observed in the resonances of the remaining carbons of the three com-

Table 5. ^{13}C NMR chemical shifts of the purpurosamine C of gentamicin C_{1a}, **6**, **7**, and **8** in D_2O.

Carbon	Purpurosamine C of gentamicin C_{1a}* (pD 11.0)	**6**	**7**		**8**
		(pD 10.9)	(pD 10.9)	β-Shift	(pD 9.9)
1′	102.2	100.9	102.5	7.4	102.9
3		82.7	82.9	4.2	82.5
6		81.2	83.0	9.5	80.4
2		73.6	73.6	5.1	73.4
5		72.5	72.7	5.9	72.8
5′	71.5	150	71.3	4.5	76.6
4		60.4	59.9		60.2
OCH_3		57.8	57.8		57.8
1		55.9	56.0		55.7
2′	51.0	47.3	50.5		48.0
6′	46.0	43.3	45.9		44.9
NCH_3		38.4	38.4		38.3
4′	28.4	96.8	28.3		28.2
3′	27.1	25.5	27.1	5.7	22.2

* Taken from ref 8.

pounds may be understood as the result of the steric deformation of the diaminosugar of **6** due to the presence of a double bond in the ring as well as the opposite C-5′ stereochemistry of **7** and **8**. Comparison of the chemical shifts of C-1′ at 102.5 ppm and C-6 at 83 ppm of **7** with the corresponding values recorded[8] for C-1′ at 102.5 ppm and C-6 at 84.2 ppm in the case of fortimicin B agree with the earlier ^{1}H NMR finding of a glycosidic linkage between C-1′ and C-6.

A comparison of the diaminosugar resonances of **7** with those of the purpurosamine C residue of gentamicin C_{1a} shows that they are almost identical in every case which suggests that the diaminosugar of **7** is identical with purpurosamine C of gentamicin C_{1a}. The results of the ^{13}C NMR titration of **7** fully support the placement of the amino groups on C-1, C-4, C-2′, and C-6′ of the molecule since upon protonation the expected β-shifts at C-2, C-3, C-5, C-6, C-1′, C-3′, and C-5′ are observed.

The structures **4** and **7** were formulated with a D-diaminosugar by analogy to gentamicin C_{1a} and an L-diaminosugar residue in **5** and **8** was postulated by analogy to dihydrosisomicin (5′-*epi*-gentamicin C_{1a}).[4] In order to establish the absolute stereochemistry of the diaminosugar residues in **7** and **8** it was necessary to subject one of the two compounds to further study. The more easily available substance **8** was chosen for this purpose.

Acetylation of **8** with acetic anhydride or *N*-acetoxy-5-norbornene-2,3-dicarboximide[9] in methanol gave rise to 1,2′,6′-tri-*N*-acetyl-3,5′-di-*epi*-6′-de-*C*-methylfortimicin B (**9**). Compound **9** was subjected to mercaptolysis followed by reacetylation under the conditions of the Schering group[4] to afford 2,6-diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexose diethyldithioacetal (**10a**) which was found to be identical in every respect to one of the mercaptolysis products of penta-*N*-acetyldihydrosisomicin (**11**).[4] Acetylation of **10a** afforded 5-*O*-acetyl-2,6-diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexose diethyldithioacetal (**10b**) which had the expected physical properties.[4] This proves that the diaminosugar residue in **5** and **8** is of the L-configuration and is identical with 5-*epi*-purpurosamine C.[4] Since the double bond reduction of **1** and **6** resulted in the introduction of a new center of asymmetry at C-5′ of the reduction products **4** and **7** as well as in the reduction products **5** and **8**, respectively, it follows that the diaminosugar residue of **4** and **7** must have the D-configuration, and in fact is identical with D-purpurosamine C,[10~13] the diaminosugar residue contained in gentamicin C_{1a}.

The evidence presented in this paper permits the assignment of structure **1**, representing the absolute stereochemistry, to the new antibiotic lysinomicin. At the same time the structures and absolute stereochemistry of **4**, **5**, **6**, **7** and **8** are established.

Lysinomicin (**1**) was shown to be a slightly more potent antibiotic than fortimicin A[1]; a comparison of the respective MIC values observed for the two substances is presented in Table 6. In the fortimicin series 4-*N*-aminoacylation was previously considered a requirement for good antimicrobial activity. Isofortimicin (2′-*N*-glycylfortimicin B) has activity orders of magnitude less than fortimicin A (4-*N*-glycylfortimicin B).[14] The preparation of the 4-*N*-glycylderivatives of **1** and **6** has been reported to afford substances of lower microbiological activity than that of the parent compounds, **1** and **6**.[15]

The new substances **4**, **6**, and **7**, which were obtained in the course of the degradation of **1**, were submitted for the determination of their *in vitro* antibacterial activity. The substances were evaluated against 20 organisms and the results are given in Table 6. Comparison of Dreiding molecular models of the unsaturated diaminosugar residue of sisomicin with that of the purpurosamine C residue of gentamicin C_{1a} revealed that the spatial relationship of the functional groups in the two diaminosugar residues is very similar. Since both substances, sisomicin and gentamicin C_{1a}, have good antibiotic properties it was not surprising that **4**, which contains a purpurosamine C residue, retained good antimicrobial

Table 6. *In vitro* antimicrobial activity of fortimicin A, lysinomicin (**1**), **4**, **6** and **7** (MIC, μg/ml).*

Organism	Fortimicin A**	**1**	**4**	**6**	**7**
Staphylococcus aureus Smith	0.39[c], 0.78[a,b]	0.39	0.39	1.56	3.1
Streptococcus faecalis 10541	25[a], 50[b,c]	50	50	100	>100
Enterobacter aerogenes 13048	3.1	1.56	1.56	6.2	12.5
Escherichia coli JUHL	3.1[a,c], 6.2[b]	3.1	3.1	6.2	12.5
E. coli BL 3676 (Res)	12.5[a,c], 25[b]	12.5	12.5	25	25
E. coli 76-2	1.56[a,c], 3.1[b]	1.56	1.56	3.1	6.2
Klebsiella pneumoniae 10031	1.56	0.78	0.78	6.2	12.5
K. pneumoniae KY-4262	3.1[a], 6.2[b,c]	1.56	1.56	25	50
Providencia sp. 1577	0.78[c], 1.56[a,b]	1.56	1.56	50	100
Pseudomonas aeruginosa BMH #10	0.78	0.39	0.78	1.56	3.1
P. aeruginosa KY-8512	3.1[a,c], 6.2[b]	3.1	3.1	6.2	6.2
P. aeruginosa KY-8516	3.1[a,c], 6.2[b]	3.1	12.5	50	100
P. aeruginosa 209	>100	6.2	6.2	12.5	25
P. aeruginosa 27853	6.2	3.1	3.1	6.2	12.5
Salmonella typhimurium Ed. #9	1.56[c], 3.1[a,b]	1.56	1.56	3.1	6.2
Serratia marcescens 4003	1.56[b,c], 3.1[a]	1.56	1.56	3.1	6.2
Shigella sonnei 9290	6.2	3.1	3.1	6.2	12.5
Proteus rettgeri U6333	12.5	25	25	50	100
P. vulgaris JJ	3.1	3.1	3.1	6.2	6.2
P. mirabilis Fin. #9	3.1	6.2	3.1	6.2	6.2

* 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.

** Since the Table represents the results of three different assays the MIC values given for the fortimicin A controls for **1** and **4** are indicated by [a], the fortimicin A controls for **6** are indicated by [b], while the fortimicin A controls for **7** are indicated by [c] where the reference values were found to differ.

activity. The biological activity of **6**, which no longer carries the 2′-*N*-L-β-lysyl substituent, was surprising since this compound was the first unsubstituted pseudodisaccharide related to the fortimicin group of antibiotics to show good biological activity. Consideration of the relationship of sisomicin and gentamicin C_{1a} outlined above, together with the observed biological activity of **6**, suggested that the pseudodisaccharide **7** which contains a purpurosamine C residue, would also exhibit antimicrobial activity. This was indeed found to be the case. The two compounds **5** and **8** contain an L-diaminosugar residue in place of the D-purpurosamine C residue of **4** and **7** and, as expected, were found to be devoid of significant antimicrobial activity.

While this work was in progress the structures of fortimicin KO and fortimicin KO_1 were disclosed; both substances contain a 3-*epi*-fortamine residue.[16,17] Sannamycin C was shown to contain a 2-deoxy-3-*epi*-fortamine residue[18] of the same conformation as the 3-*epi*-fortamine residue contained in lysinomicin (**1**).

Experimental

General Methods

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. The ^{1}H NMR spectrum of the sulfate salt of **1** was determined at 270 MHz on a Bruker HX-270 spectrometer. The other ^{1}H NMR spectra were measured at 90 MHz with a Jeol-FX-90Q instrument or at 100 MHz with a

Varian Associates HA-100 spectrometer in deuterated solvents. Chemical shifts are reported in ppm downfield from internal TMS in case of non-aqueous solutions or from sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 in case of D_2O solutions; coupling constants are reported in Hz. ^{13}C NMR spectra were determined at 25.2 MHz with a Varian Associates XL-100/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. Microanalytical results are reported for those products which could be prepared free of solvent and carbonates formed by atmospheric exposure.

3-*epi*-Fortamine (**2a**)

A solution of 12.5 g of the sulfate salt of **1** in 250 ml of 1 M HCl was heated (75~80°C), with stirring for 1.5 hours. The reaction mixture was cooled and evaporated under reduced pressure at 45°C to a residue. Excess HCl was removed by co-evaporation with absolute EtOH. The residue was taken up in 250 ml of distilled H_2O and passed over a column (4.3×38 cm) of Bio Rad AG 1 X2 (OH^- form) resin. Elution of the column with H_2O provided, after evaporation of the basic fractions under reduced pressure, crude 3-*epi*-fortamine (**2a**). The latter was crystallized from absolute EtOH to afford 2.54 g of **2a** (70% of theory) with the following physical constants: mp 157~160°C (dec), $[\alpha]_D^{23}$ +23° (*c* 1.01, H_2O); ^1H NMR (pyridine-d_5) 2.72 (s, NCH_3), 2.89 (q, H-1), 3.34 (q, H-5), 3.47 (q, H-4), 3.50 (s, OCH_3), 3.83 (q, H-3), 4.08 (q, H-6), 4.10 (q, H-2) ppm, $J_{1,2}=J_{2,3}=J_{5,6}=J_{6,1}=10.2$ Hz, $J_{3,4}=3.4$ Hz, $J_{4,5}=3.8$ Hz; MS Calcd for $C_8H_{18}N_2O_4$: 206.1273, $[M]^+$, found *m/z*: 206.1273.

Anal Calcd for $C_8H_{18}N_2O_4$: C 46.59, H 8.79, N 13.59, O 31.03.
Found: C 45.85, H 9.02, N 13.24, O 31.22.

N,*N*′-Dibenzyloxycarbonyl-L-β-lysine (**3b**)

Further elution of the above column with 2 M AcOH provided, after evaporation of the fractions combined on the basis of TLC, 5.0 g of crude L-β-lysine (**3a**). The latter (**3a**) was dissolved in a mixture of H_2O (15 ml), acetonitrile (15 ml), and triethylamine (5 ml) and treated with 13.5 g of *N*-(benzyloxycarbonyloxy)succinimide with stirring at room temperature for 16 hours. The mixture was evaporated to a residue under reduced pressure. The above residue was purified by chromatography on silica gel in CH_2Cl_2 - CH_3OH (95: 5).

Combination and evaporation of the appropriate fractions and recrystallization of the residue from EtOAc afforded 1.9 g (26% of theory) of **3b** with the following physical constants: mp 151~153°C; $[\alpha]_D^{23}$ −9°(*c* 0.581, CH_3OH) [reported[6] mp 152~153°C; $[\alpha]_D^{23}$ −9±3°(*c* 0.33, CH_3OH)]; IR (KBr) 1723, 1690, 1650, 1547 cm^{-1}; ^1H NMR (CD_3OD - $CDCl_3$, 1: 1) 1.53 (m, 4- and 5-CH_2), 2.46 (d, 2-CH_2, J=7.0 Hz), 3.12 (m, 6-CH_2), 3.33 (m, 3-CH), 4.61 (s, CH_2-Z), 7.30 (s, Ar-Z) ppm; ^{13}C NMR (CD_3OD - $CDCl_3$, 1: 1) 26.9 (t, C-5), 32.2 (t, C-4), 40.1 (t, C-6), 41.1 (t, C-2), 48.7 (d, C-3), 67.0 (CH_2-Z), 128.2~128.9 (Ar-Z), 174.7 (CO) ppm.

Anal Calcd for $C_{22}H_{26}N_2O_6$: C 63.75, H 6.32, N 6.76, O 23.16.
Found: C 63.51, H 6.49, N 6.78, O 22.94.

1-*N*-Acetyl-3-*epi*-fortamine (**2b**)

A mixture of 0.28 g of **2a**, 15 ml of CH_3OH, and 1 ml of acetic anhydride was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure and the residue was dried over KOH-pellets under high vacuum to afford 0.46 g which was chromatographed on 50 g of silica gel in the lower phase of CH_3OH - CH_2Cl_2 - conc NH_4OH (1: 1: 1) to yield, after combination of the appropriate fractions and evaporation of the solvent, a residue of 0.26 g of **2b**. Rechromatography of the above residue (0.26 g) on 22 g of silica gel in the lower phase of CH_3OH - CH_2Cl_2 - conc NH_4OH - H_2O (2: 2: 1: 1) afforded 0.15 g of pure **2b** which was recrystallized from CH_3OH: mp 235~236°C; $[\alpha]_D^{26}$ +1.6° (*c* 0.94, CH_3OH); IR (KBr) 1685, 1543 cm^{-1}; ^1H NMR (CD_3OD, 100 MHz) 3.45 (s, OCH_3), 2.45 (s, NCH_3), 2.00 (s, $COCH_3$) ppm.

Anal Calcd for $C_{10}H_{20}N_2O_5$: C 48.37, H 8.12, N 11.28.
Found: C 48.18, H 8.15, N 11.21.

3-*epi*-2′-*N*-(L-β-Lysyl)-6′-de-*C*-methylfortimicin B (**4**)

A solution of 3.08 g of the free base **1**, prepared from 5.12 g of the sulfate salt in 150 ml of CH_3OH

was hydrogenated over 6 g of a 20% Pd-C (50% wet) catalyst for 72 hours. The catalyst was collected on a filter and washed with CH_3OH. Evaporation of the solvent from the filtrate afforded a residue of 3.04 g which was purified by chromatography on 210 g of silica gel in the lower phase of a CH_2Cl_2 - CH_3OH - conc NH_4OH (1: 1: 1) solvent mixture. The early fractions were discarded. Later fractions yielded chromatographically pure **4** (0.31 g): $[\alpha]_D^{26}+76°$ (*c* 1.00, CH_3OH); IR (KBr) 1653, 1544 cm^{-1}; ^1H NMR (D_2O, pD 11.04, 90 MHz) 5.1 (anomeric H), 3.44 (OCH_3), 2.50 (NCH_3), 3.20 (3″-CH), 2.70 (6″-CH_2), 2.30 (2″-CH_2), 2.0~1.2 (4″, 5″-CH_2, 3′, 4′-CH_2) ppm; MS Calcd for $C_{20}H_{42}N_6O_6$: 462.3166, $[M]^+$, found *m/z*: 462.3146.

3,5′-Di-*epi*-2′-*N*-(L-β-lysyl)-6′-de-*C*-methylfortimicin B (**5**)

Further elution of the above chromatogram led to the isolation of 1.44 g of **5**: $[\alpha]_D^{24}+44°$ (*c* 1.00, CH_3OH); IR (KBr) 1645, 1543 cm^{-1}; ^1H NMR (D_2O, pD 10.77, 90 MHz) 4.82 (anomeric H, $J_{1',2'}=$ 2.0 Hz), 4.18 (2′-H), 3.42 (OCH_3), 2.48 (NCH_3), 3.20 (3″-CH), 2.73 (6″-CH_2), 2.40 (2″-CH_2), 2.0~1.2 (4″, 5″-CH_2, 3′, 4′-CH_2) ppm; MS Calcd for $C_{20}H_{42}N_6O_6$: 462.3166, $[M]^+$, found *m/z*: 462.3153.

3-*epi*-4′,5′-Didehydro-6′-de-*C*-methylfortimicin B (**6**)

A solution of 2.71 g of **1**, prepared from 4.7 g of the sulfate salt, in 25 ml of hydrazine hydrate of bp 120.1°C (Aldrich Chemical Company, Inc.) was refluxed for 22 hours. Evaporation of the hydrazine hydrate under reduced pressure afforded a residue of 2.76 g which was chromatographed on 140 g of silica gel in the lower phase of a CH_3OH - CH_2Cl_2 - conc NH_4OH (1: 1: 1) solvent mixture. Rechromatography of the early fractions, (1.32 g) on 135 g of silica gel in the same solvent system led to the recovery of 1.22 g of the chromatographically pure compound **6** which had the following physical properties: $[\alpha]_D^{23}+120°$ (*c* 1.05, CH_3OH); IR (KBr) 1684 (C=C), 1594 (NH_2) cm^{-1}; ^1H NMR (D_2O, pD 10.92, 90 MHz) 5.16 (anomeric H, $J_{1',2'}=2.69$ Hz), 3.43 (OCH_3), 2.50 (NCH_3) ppm; MS Calcd for $C_{14}H_{28}N_4O_5$: 332.2060, $[M]^+$, found *m/z* 332.2079; CD (Cupra A, *c* 0.047) $[\theta]_{292}+2404$; $[\theta]_{560}-331$. 3-*epi*-Fortamine (**2a**, 0.14 g) was obtained from the later fractions of the first chromatogram.

3-*epi*-6′-De-*C*-methylfortimicin B (**7**)

A solution of 0.23 g of **4** in 2.2 ml of hydrazine hydrate was refluxed with stirring for 22 hours. Evaporation of the hydrazine hydrate afforded a residue of 0.25 g which was chromatographed on 23 g of silica gel in the lower phase of a CH_3OH - CH_2Cl_2 - conc NH_4OH (1: 1: 1) solvent mixture to afford 0.18 g which after further purification gave 0.08 g of **7**: $[\alpha]_D^{27}+77°$ (*c* 1.02, CH_3OH); IR (KBr) 1635, 1600 cm^{-1}; ^1H NMR (D_2O, pD 11.11, 90 MHz) 5.03 (anomeric H, $J_{1',2'}=3.6$ Hz), 3.44 (OCH_3), 2.48 (NCH_3); MS Calcd for $C_{14}H_{30}N_4O_5$: 334.2216, $[M]^+$, found *m/z*: 334.2228.

3,5′-Di-*epi*-6′-de-*C*-methylfortimicin B (**8**)

A solution of 1.38 g of **5** in 13 ml of hydrazine hydrate was stirred and refluxed for 24 hours. Evaporation of the hydrazine hydrate and drying over KOH-pellets under high vaccum afforded a residue of 1.34 g which was chromatographed on 100 g of silica gel in the lower phase of a CH_3OH - CH_2Cl_2 - conc NH_4OH (1: 1: 1) solvent mixture to afford 0.77 g of **8**. Rechromatography of the substance on 80 g of silica gel in the same solvent system afforded a pure sample of **8**: $[\alpha]_D^{25}+60°$ (*c* 0.95, CH_3OH); IR (KBr) 1640, 1600 cm^{-1}; ^1H NMR (D_2O, pD 10.28, 100 MHz) 4.76 (anomeric H), 3.45 (OCH_3), 2.50 (NCH_3), 2.0~1.3 (3′, 4′-CH_2) ppm; MS Calcd for $C_{14}H_{30}N_4O_5$: 334.2216, $[M]^+$, found: *m/z* 334.2238.

Preparation of **7** and **8** by Catalytic Hydrogenation of 2-*epi*-4′,5′-Didehydro-6′-de-*C*-methylfortimicin B (**6**)

A solution of 0.99 g of the unsaturated pseudodisaccharide **6** in 50 ml of CH_3OH was hydrogenated over 2 g of a 20% Pd-C (50% wet) catalyst for 72 hours. The catalyst was collected on a filter and washed with CH_3OH. Evaporation of the filtrate afforded a residue of 0.89 g which was chromatographed on 90 g of silica gel in the lower phase of CH_3OH - CH_2Cl_2 - conc NH_4OH (1: 1: 1) solvent mixture. Early fractions afforded 0.08 g of **7** identical with the product obtained by hydrazinolysis of **4**. Later fractions afforded 0.61 g of **8** which was found to be identical with the above product obtained by hydrazinolysis of **5**.

1,2′,6′-Tri-*N*-acetyl-3,5′-di-*epi*-6′-de-*C*-methylfortimicin B (**9**)

A solution of 0.60 g of **8** and 1.70 g of *N*-acetoxy-5-norbornene-2,3-dicaroximide[9] in 10 ml of CH_3OH which was obtained by gentle warming of the mixture, was stirred at room temperature overnight. Evaporation of the solvent left a residue of 2.56 g which was chromatographed on 100 g of silica gel in the lower phase of a CH_3OH - CH_2Cl_2 - conc NH_4OH - H_2O (2: 2: 1: 1) solvent mixture. The more polar fractions afforded 0.73 g of **9**. An analytical sample had the following physical properties: $[\alpha]_D^{32}+0.7°$ (*c* 1.08, CH_3OH); IR (KBr) 1650, 1550 cm^{-1}; ^1H NMR (D_2O, pD 9.15, 90 MHz) 4.84 (anomeric H), 3.45 (OCH_3), 2.50 (NCH_3), 2.05 ($COCH_3$), 2.3~1.2 (3′, 4′-CH_2) ppm; MS Calcd for $C_{20}H_{36}N_4O_8$: 460.2533, $[M]^+$, found *m/z*: 460.2542.

Anal Calcd for $C_{20}H_{36}N_4O_8 \cdot \frac{1}{2}H_2O$: C 51.16, H 7.94, N 11.93.
Found: C 51.15, H 8.33, N 11.55.

The same tri-*N*-acetyl derivative **9** was obtained when **8** was acetylated with acetic anhydride in CH_3OH.

2,6-Diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexose Diethyldithioacetal (**10a**)

Mercaptolysis of 1,2′,6′-Tri-*N*-Acetyl-3,5′-di-*epi*-6′-de-*C*-methylfortimicin B (**9**): The general procedure of REIMANN *et al.*[4] was followed. A solution of 0.73 g of **9** in 4.5 ml of thioethanol and 4.5 ml of 6 N HCl was stirred at room temperature for 68 hours. The mixture was transferred into a beaker containing 180 ml of H_2O. The aqueous suspension was neutralized by the addition of basic lead carbonate, the insoluble residue was collected on a filter and washed with 100 ml of H_2O. The filtrate was lyophilized to leave a residue of 1.3 g. The latter was repeatedly treated with CH_3OH and CH_3OH insoluble material was collected on a filter. Evaporation of the filtrate afforded a residue of 0.98 g which was stirred with 1.5 ml of acetic anhydride in 25 ml of CH_3OH at room temperature for 1.5 hours. Examination of this mixture by TLC revealed the presence of **10a** as well as the expected 1-*N*-acetyl-3-*epi*-fortamine (**2b**) which was found to be identical with the reference sample prepared from **2a**. The volatile part of the above mixture was evaporated under reduced pressure and the residue was dried under high vacuum overnight to afford 1.04 g of crude reaction mixture. The latter was suspended four times in 5 ml portions of $CHCl_3$. The $CHCl_3$ solutions were pipetted off from the residue, filtered, and evaporated to leave 0.36 g of $CHCl_3$ soluble material which was chromatographed on 50 g of silica gel in a mixture of CH_2Cl_2 - CH_3OH (85: 15). Combination of the appropriate fractions and evaporation of the solvent afforded 0.21 g of **10a** with the following physical constants: $[\alpha]_D^{28}+28°$ (*c* 1.04, CH_3OH); IR ($CDCl_3$) 1665, 1515 cm^{-1}; ^1H NMR (C_5D_5N, 100 MHz) 4.75 (2-CH), 4.42 (1-CH, $J_{1,2}$= 4.0 Hz), 4.16 (5-CH), 4.0~3.3 (6-CH_2), 2.78 and 2.75 (2 Et-CH_2), 2.16 and 2.08 (2 $COCH_3$), 1.27 and 1.21 (2 Et-CH_3) ppm.

Anal Calcd for $C_{14}H_{28}N_2O_3S_2$: C 49.97, H 8.39, N 8.32.
Found: C 49.96, H 8.43, N 8.14.

Mercaptolysis of 1,3,2′,6′,3″-Penta-*N*-acetyldihydrosisomicin (**11**): The catalytic hydrogenation of sisomicin was carried out as above and the resulting dihydrosisomicin was converted to the penta-*N*-acetyl derivative **11** according to the procedure of REIMANN *et al.*[4] The mercaptolysis of **11** followed the previously published procedure[4] to afford, after chromatography of the $CHCl_3$ soluble reacetylated product, the desired pure sample of **10a** with the following physical constants: $[\alpha]_D^{25}+28°$ (*c* 1.01, CH_3OH) [reported[4] $[\alpha]_D^{26}+32°$ (CH_3OH)]; IR ($CDCl_3$) 1665, 1515 cm^{-1}; ^1H NMR (C_5D_5N, 100 MHz) 4.74 (2-CH), 4.41 (1-CH, $J_{1,2}$=4.0 Hz), 4.15 (5-CH), 3.9~3.3 (6-CH_2), 2.78 and 2.74 (2 Et-CH_2), 2.14 and 2.06 (2 $COCH_3$), 1.25 and 1.21 (2 Et-CH_3) ppm.

Anal Calcd for $C_{14}H_{28}N_2O_3S_2$: C 49.97, H 8.39, N 8.32.
Found: C 50.05, H 8.57, N 8.31.

The identity of the above samples (**10a**) prepared from **9** and **11**, respectively, was demonstrated by their TLC as well as their mass spectral behavior. Both samples of **10a** crystallized on standing, mp 86~88°C (reported[4] mp 81~84°C); a mixture of the two samples had the same mp 86~88°C.

5-*O*-Acetyl-2,6-diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexose Diethyldithioacetal (**10b**)

A solution of 0.16 g of the above prepared **10a** (from **9**) in 3 ml of pyridine and 1 ml of acetic anhydride was allowed to stand at room temperature overnight.[4] Evaporation of the solution under

high vacuum afforded a residue of 0.20 g which was chromatographed on 20 g silica gel in a CH_2Cl_2-CH_3OH (85: 15) mixture. Combination and evaporation of the appropriate fractions led to the isolation of 0.11 g of oily **10b** with the following physical constants: $[\alpha]_D^{28}+23°$ (*c* 1.07, CH_3OH) [reported[4] $[\alpha]_D^{26}+22°$ (CH_3OH)]; IR ($CDCl_3$) 1736, 1672, 1512 cm^{-1}; ^1H NMR (C_5D_5N, 100 MHz) 5.33 (5-CH), 4.7 (2-CH), 4.4 (1-CH, $J_{1,2}=4.0$ Hz), 4.0~3.3 (6-CH_2), 2.77 and 2.74 (2 Et-CH_2), 2.12 and 2.04 (2 ($NHCOCH_3$), 1.88 ($OCOCH_3$), 1.25 and 1.2 (2 Et-CH_3) ppm.

Anal Calcd for $C_{16}H_{30}N_2O_4S_2$: C 50.76, H 7.99, N 7.40.
Found: C 50.86, H 8.34, N 7.51.

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