Semisynthetic Aminoglycoside Antibacterials. 6.[1] Synthesis of Sisomicin, Antibiotic G-52, and Novel 6′-Substituted Analogues of Sisomicin from Aminoglycoside 66-40C

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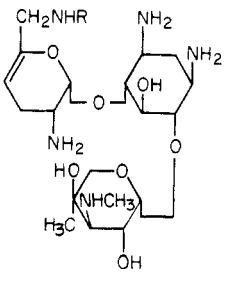
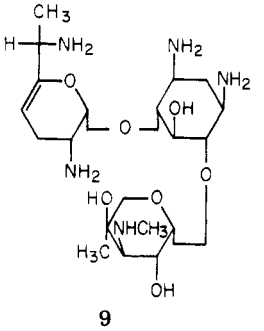
The discovery of aminoglycoside 66-40C, a novel dimeric, unsaturated imine produced by *Micromonospora inyoensis*, afforded a versatile intermediate for the synthesis of a variety of sisomicin analogues modified at the 6′ position. The conversion of 66-40C into sisomicin, antibiotic G-52, and a series of novel 6′-substituted analogues of sisomicin is described, and the biological activity of the products is discussed.

In recent years the structures of a number of novel unsaturated aminoglycoside antibiotics such as sisomicin (**1**),[2] antibiotic G-52 (**2**),[3] verdamicin (**9**),[4] antibiotic 66-40B (**10**),[5] and antibiotic 66-40D (**11**)[5] have been elucidated in these laboratories. The discovery of aminoglycoside 66-40C (**12**)[6] as a minor component in the submerged fermentation of *Micromonospora inyoensis* (NRRL 3292) provided a useful intermediate for the preparation of a number of novel 6′-substituted sisomicin derivatives. The presence of reactive imine functional groups in **12** provided an intermediate that could readily be modified at the 6′ position to provide not only naturally occurring aminoglycoside antibiotics such as **1**[2] and **2**[3] but also a variety of novel 6′-substituted analogues of **1**. The latter would





1, R = H
2, R = CH_3
3, R = $C(CH_3)_3$
4, R = $CH_2CH_2C_6H_5$
5, R = CH_2CH_2OH
6, R = OCH_3
7, R = NH_2
8, R = c-$N(CH_2CH_2)_2O$

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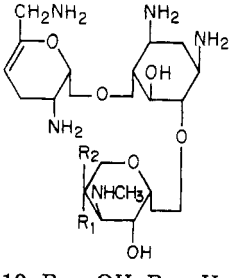
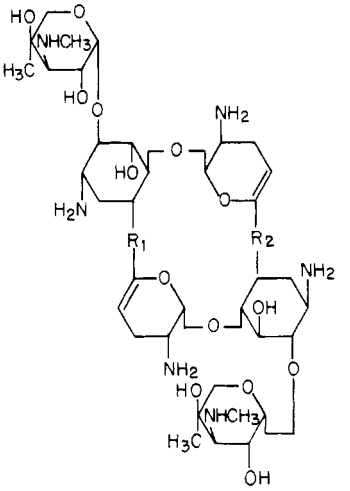
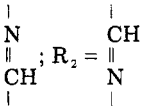


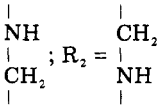
10, R_1 = OH; R_2 = H
11, R_1 = H; R_2 = OH

serve to further expand our knowledge of the effects produced by such modifications on the spectrum of biological activity of **1**. Inactivation of kanamycin A, 3′,4′-dideoxykanamycin B, **14**, and **1** by resistant R-factor bearing strains of *Escherichia coli* and *Pseudomonas aeruginosa* has been demonstrated to result from N-acetylation of the primary 6′-amino group in these molecules.[7-11] However, **15**, **16**, **2**, and **9** were not inactivated by these same resistant strains.[11-13] These differences in resistance profile may be attributed to the presence of either 6′-*N*-alkyl and/or 6′-*C*-alkyl groups in the latter molecules.[11-13]

Chemistry. The imine groups in **12** provided reactive sites for carrying out transamination reactions using a variety of primary amines. When **12** was treated with



12, R_1 =  ; R_2 =

13, R_1 =  ; R_2 =

ammonia in methanol and the mixture was heated in a bomb, transamination to give the monomeric imine **17** occurred. The latter was not isolated but was reduced in situ with sodium borohydride to give **1**.[2] The sample was identical in all respects with an authentic sample. The synthesis of **2**[3] was achieved in a similar manner by treating **12** with methylamine in the presence of phosphoric acid as a catalyst. The initially formed imine **18** was reduced in situ with sodium borohydride to give **2**,[3] which was identical in all respects with an authentic sample. In order to study the effects of changing the nature of the 6′-*N*-alkyl substituent upon the biological activity, three novel 6′-*N*-alkyl derivatives of **1** were prepared. The use of

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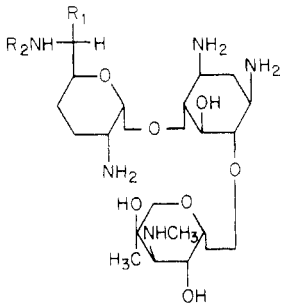
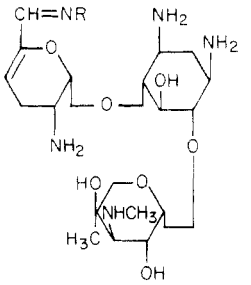
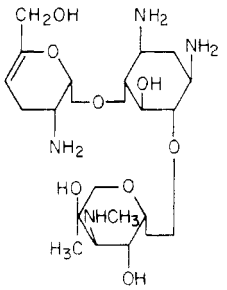
tert-butylamine in the above procedure afforded the imine **19** which was directly reduced to give 6′-*N*-*tert*-butylsi-



14, $R_1 = R_2 = H$
15, $R_1 = R_2 = CH_3$
16, $R_1 = CH_3$; $R_2 = H$



17, R = H
18, R = CH_3
19, R = $C(CH_3)_3$
20, R = $CH_2CH_2C_6H_5$
21, R = CH_2CH_2OH
22, R = OCH_3
23, R = NH_2
24, R = c-$N(CH_2CH_2)_2O$

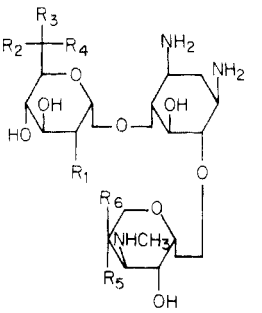


25

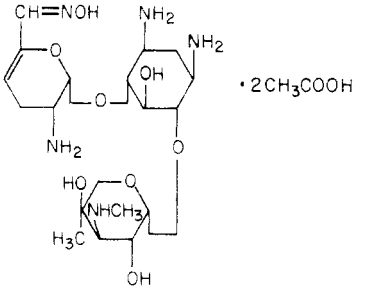
somicin (**3**). Similarly when 2-phenylethylamine was used, the imine **20** was obtained, which on reduction gave 6′-*N*-(2-phenylethyl)sisomicin (**4**). The use of ethanolamine afforded the imine **21** which on reduction gave 6′-*N*-(2-hydroxyethyl)sisomicin (**5**). The physical data lent support to the proposed structures of **3**–**5**. In particular, the ^1H NMR spectra revealed singlets integrating for two protons at δ 3.32, 3.11, and 3.16 for **3**, **4**, and **5**, respectively, due to the 6′-methylene groups in the above molecules. The expected signals for the *tert*-butyl group, the 2-phenylethyl group, and the 2-hydroxyethyl group were present in the ^1H NMR spectra of **3**–**5**, respectively. The mass spectra of these 6′-*N*-alkylsisomicin analogues showed the expected fragment ions for unsaturated aminoglycosides of the sisomicin type.[2,14,15] The presence of the ion D_5 at *m/e* 430 due to loss of the 6′-alkylamino group,[15] as well the ion D_9 at *m/e* 362 due to the retrodiene cleavage of the eno-pyranoside unit,[2,15] clearly located the newly generated alkylamine group at the 6′ position in these molecules. Compound **4** also exhibited prominent ions at *m/e* 460 (20%) (M − $C_6H_5CH_2$·) and *m/e* 442 (6%) (M − $C_6H_5CH_2$· − NH_3) in the mass spectrum, characteristic of the aryl-alkylamino chain at the 6′ position.

During the course of the elucidation of the structure of **12**,[6] it was demonstrated that acidification of a solution of **12** generated the 6′-aldehyde in situ. Reduction of the above aldehyde at pH 3–4 using sodium cyanoborohydride provided the 6′-hydroxy analogue (**25**) of sisomicin. The ^1H NMR spectrum of **25** gave rise to a signal at δ 3.99 due to a 6′-methylene group bearing a hydroxy group. The mass spectrum was also consistent with structure **25**. Compound **25** was of particular interest in view of the antiprotozoal activity of compounds such as gentamicin X_2 (**26**)[16] and antibiotic G-418 (**27**).[17]

The preparation of other novel 6′-substituted sisomicin derivatives was undertaken as follows. The 6′-oxime **33**



26, $R_1 = NH_2$; $R_2 = R_3 = H$; $R_4 = R_6 = OH$; $R_5 = CH_3$
27, $R_1 = NH_2$; $R_2 = R_6 = OH$; $R_3 = R_5 = CH_3$; $R_4 = H$
28, $R_1 = NH_2$; $R_2 = R_3 = R_6 = H$; $R_4 = R_5 = OH$
29, $R_1 = NH_2$; $R_2 = R_3 = R_5 = H$; $R_4 = R_6 = OH$
30, $R_1 = R_6 = OH$; $R_2 = R_3 = H$; $R_4 = NH_2$; $R_5 = CH_3$
31, $R_1 = R_4 = NH_2$; $R_2 = R_3 = H$; $R_5 = CH_3$; $R_6 = OH$
32, $R_1 = R_4 = NH_2$; $R_2 = H$; $R_3 = R_5 = CH_3$; $R_6 = OH$



33

was prepared by treatment of **12** with hydroxylamine hydrochloride in methanol at ambient temperature. The product was purified by adsorption onto an Amberlite IRA 401S (OH^-) resin, followed by elution with aqueous acetic acid affording the diacetate salt of the oxime **33**. The ^1H NMR spectrum of **33** showed a signal at δ 1.92 integrating for six protons of the diacetate salt. The 6′-methine proton gave rise to a singlet at δ 7.65. The 6′-methoxyazylene analogue **22** was prepared in a similar manner by treatment of **12** with methoxyamine hydrochloride. Compound **22** was purified as the free base which showed a singlet at δ 3.92 due to the methoxyazylene group and a singlet at δ 7.70 due to the 6′-methine proton. The mass spectrum of **22** showed prominent fragment ions in the high mass region at *m/e* 444 (3%) due to loss of a methoxyl radical from the molecular ion and at *m/e* 427 (7%) due to loss of ammonia from the ion at *m/e* 444. Reduction of the methoxyazylene derivative **22** with sodium borohydride afforded the 6′-*N*-methoxy derivative (**6**) of sisomicin. The ^1H NMR spectrum of **6** showed a singlet at δ 3.53 due to the methoxyazyl group and a singlet at δ 3.47 for the 6′-methylene group, consistent with structure **6**. The mass spectrum of **6** showed prominent ions in the high mass region of the spectrum at *m/e* 445 (1%) due to loss of methanol from the molecular ion and at *m/e* 428 (10%) due to subsequent loss of ammonia from the ion at *m/e* 445. The loss of methoxyamine to give the ion at *m/e* 430 (1%) was very weak compared with the corresponding fragmentations in the 6′-*N*-alkyl derivatives **2**–**5**.

The 6′-hydrazone analogue (**23**) of sisomicin was prepared by treating **12** with anhydrous hydrazine. The ^1H NMR spectrum of **23** showed the 6′-methine proton as a singlet at δ 7.88. Reduction of **23** with sodium borohydride gave the hydrazine derivative **7**. The latter was found to be somewhat unstable and although a satisfactory mass spectrum could not be obtained, the ^1H NMR spectrum was recorded and was in agreement with the proposed structure.

The 6′-morpholinoazylene analogue (**24**) of sisomicin was

Table I. Minimal Inhibitory Concentrations (MIC, μg/mL) and LD_{50} (mg/kg)

Organism		Inactivating enzyme	1	2	3	4	5	25
E. coli	ATCC 10536		0.75	0.3	0.75	0.75	0.075	0.75
	Baker 2	APH(3')-I	0.75	0.75	7.5	7.5	3.0	17.5
	LA290R55	ANT(2'')	7.5	17.5	17.5	>25	7.5	>25
	JR88	AAC(3)	17.5	>25	>25	>25	>25	>25
	HL97/W677	AAC(6')	7.5	0.75	3.0	0.75	0.75	0.3
Ps. aeruginosa	St. M. 762		0.3	0.3	7.5	>25	0.3	3.0
	St. M. 1395		0.3	0.3	17.5	>25	0.3	17.5
	Stone 130	AAC(3)	17.5	>25	>25	>25	>25	>25
	GN 315	AAC(6')	7.5	0.3	7.5	>25	0.3	>25
K. pneumoniae	Adler 17	APH(3')-I	0.3	0.075	7.5	>25	0.075	3.0
	Georgetown 3694	ANT(2'')	7.5	>25	>25	>25	>25	>25
Providence	164	AAC(2')-I	3.0	>25	17.5	>25	>25	>25
P. mirabilis	Harding		0.3	0.75	3.0	>25	0.3	3.0
P. rettgeri	Membel		3.0	3.0	3.0	>25	0.75	>25
	Anderson		>25	>25	>25	>25	>25	>25
S. marcescens	Dalton		0.3	0.3	7.5	>25	0.75	3.0
Sal. typhimurium	Gr. B.		0.3	3.0	3.0	17.5	0.75	3.0
B. subtilis	ATCC 6633		<0.01	<0.01	<0.01	0.03	0.3	0.075
Staph. aureus	209 P		0.03	<0.01	0.03	3.0	0.03	0.3
	Wood		0.03	<0.01	0.03	3.0	0.03	0.3
	Ziegler		0.03	0.03	0.03	7.5	0.03	0.3
	59N		0.03	0.03	0.75	3.0		0.075
Strep. pyogenes	C		17.5	3.0	17.5	>25	>25	>25
	27		3.0	0.3	7.5	17.5		>25
	Group A Cruz		7.5	0.3	3.0	>25	17.5	>25
	Alvarez		7.5	>25	>25	>25	17.5	>25
LD_{50}			34[a]	50[b]	90	30	135	65

[a] See ref 6. [b] See ref 25.

prepared by reaction of **12** with *N*-aminomorpholine. The ^1H NMR spectrum of **24** showed multiplets at δ 3.06 and 3.88 due to the methylene groups of the morpholino ring. The 6'-methine proton gave rise to a singlet at δ 7.38. Reduction of **24** at pH 3.5 with sodium cyanoborohydride afforded the corresponding 6'-morpholinoazyl derivative **8**. The ^1H NMR spectrum of **8** showed multiplets at δ 2.84 and 3.79 due to the methylene groups of the morpholino ring and a signal at δ 3.42 due to the 6'-methylene group. The mass spectrum of **8** showed prominent ions in the high mass region at *m/e* 446 (5%) due to cleavage of the morpholino unit (M − 86) and at *m/e* 429 (9%) due to loss of ammonia from the ion at *m/e* 446. The fragment ion at *m/e* 101 (60%) formed by cleavage of the aminomorpholino moiety was also observed as a prominent ion in the low mass region of the spectrum.

Biological Activity. Compound **12**[6] was found to lack potent antibacterial activity, the MIC values against the organisms listed in Table I being >25 μg/mL in all instances. The tetrahydro derivative **13**[6] in which the imine groups had been reduced was also without substantial activity as an antibacterial agent. As in the case of **1** neither compound exhibited meaningful antiprotozoal activity. The samples of **1** and **2** showed comparable antibacterial activity (Table I) to the naturally occurring antibiotics produced by *Micromonospora*.[18-20] The novel 6'-*N*-alkyl derivatives **3**, **4**, and **5** were all active antibacterial agents (Table I). Increasing the lipophilicity of the 6'-*N*-alkyl group as in the *tert*-butyl analogue **3** produced a marked decrease in antibacterial potency particularly against *Pseudomonas* strains. The 6'-*N*-(2-phenylethyl) analogue **4** was even less potent than **3** having lost all *Pseudomonas* activity. Previous studies on neomycin B[21] have shown that introduction of a 6'-*N*-(2-phenylethyl) substituent into the molecule produces a considerable increase in the in vitro and in vivo potency of the compound. This dramatic increase in potency was not observed in the sisomicin series. The 6'-*N*-(2-hydroxyethyl) derivative **5** exhibited antibacterial potency similar to that of **1**. The alkyl derivatives **2**–**5** were active against 6'-*N*-acetylating resistant strains of *E. coli*. The alkyl derivatives **2**, **3**, and **5** were also active against 6'-N-acetylating resistant strains of *Pseudomonas*, while **4** was not. The alkyl derivatives **2**–**5** were active against gram-positive strains although the potency again decreased in the order **5** > **2** > **3** > **4**. None of the above 6'-*N*-alkyl derivatives showed meaningful antiprotozoal activity.

Several aminoglycoside antibiotics containing either a 4-*O*-(2-amino-2-deoxy-D-glucopyranosyl) structural unit, or its 6'-*C*-alkyl derivative, have been shown in these laboratories to possess antiprotozoal activity in addition to having antibacterial activity. Examples of such antibiotics are **26**,[16] **27**,[11,12] gentamicin A (**28**),[13,22] and gentamicin A_1 (**29**).[13,23] Some aminoglycoside antibiotics having a 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl) unit also exhibit antiprotozoal activity, but it is generally weaker than that of the above compounds. An example of this class of antibiotics would be gentamicin B (**30**).[10,12] In contrast, aminoglycoside antibiotics having amino groups at both the 2' and 6' positions, such as antibiotics JI-20A (**31**)[13,24] and JI-20B (**32**),[13,24] **14**,[12,13] **15**[12,13] and **16**,[12,13] and **1**[18,19] are devoid of meaningful antiprotozoal activity. As no 6'-hydroxy analogues of sisomicin, or the gentamicin C complex, have been isolated to date from fermentation sources, it was of interest to test **25** prepared from **12** in order to ascertain whether it possessed antiprotozoal activity. The MIC values of **25** are given in Table I and it is evident that the compound has reduced antibacterial activity relative to **1**. Compound **25** does, however, show improved activity against resistant *E. coli* carrying the AAC (6') enzyme that normally inactivates **1** by acetylating the 6'-amino group. However, **25**, in contrast to **1**, was found to possess antiprotozoal activity. When tested against *Trichomonas vaginalis* it was found to be trichomonacidal after 48 h at concentrations of <10 μg/mL. It was also found to be active against *Entamoeba histolytica* (JH strain) having an MIC of 10 μg/mL and being amoebacidal at 20 μg/mL. It was also inactive at 20 μg/mL against *Histomonas meleagridis*. These results are comparable to those obtained in our laboratories with

paromomycin. It also exhibited no anthelmintic activity in mice infected with *Nematospiroides dubius*, *Hymenolepis nana*, and *Syphacia obvelata* at a dose level of 0.1% in the diet (195 mg/kg/day for 5 days).

In general, the in vivo potency of **3–5** and **25** paralleled the in vitro activity. The acute toxicity of these compounds in mice, by the intravenous route, expressed in terms of LD_{50} values is given in Table I.

The other novel 6′-substituted sisomicin derivatives prepared above, namely, **33**, **22**, **6**, **23**, **7**, **24**, and **8**, were all devoid of meaningful antibacterial and antiprotozoal activity.

Experimental Section

Unless otherwise stated optical rotations were recorded at 26 °C in water (*c* 0.3%). IR spectra were recorded in a KCl disk on a Perkin-Elmer Model 221 spectrometer. NMR spectra were obtained at 60 or 100 MHz in D_2O solution on either a Varian A-60A or an XL 100-15 spectrometer. Chemical shifts are reported in parts per million downfield from an internal or external DSS standard. Mass spectra were recorded on a Varian MAT CH5 spectrometer. All of the chromatographically pure aminoglycosides were passed over Amberlite IRA 401S (OH^-) resin and lyophilized to afford colorless amorphous solids free of carbon dioxide. In vitro antibacterial tests were carried out using standard tube dilution techniques in Meuller–Hinton broth buffered at pH 7.4. The in vivo activity was determined using albino male mice of the CF-1 strain weighing approximately 20 g each. The acute toxicity in terms of LD_{50} values was calculated by standard procedures. In vitro tests with *T. vaginalis* were done using STS medium, with *E. histolytica* in Shaeffer–Frye medium and *H. meleagridis* in M-199 medium. Anthelmintic tests were done in mice with natural *S. obvelata* and artificially induced *N. dubius* and *H. nana* infections. Treatment was administered in the diet for 5 days starting 2 weeks after infection.

Sisomicin (1). Compound **12** (100 mg) was dissolved in methanol (3 mL) saturated at room temperature with ammonia gas, and the solution was heated in a bomb at 75 °C for 14 h. Sodium borohydride (300 mg) was added to the cooled solution, and the mixture was stirred at 25 °C for 0.5 h. The solution was evaporated and the residue was chromatographed on a silica gel column (110 × 1 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1:1:1) as the eluent to give **1** (40 mg, 38%): $[\alpha]_D$ +186.0° (lit.[2] $[\alpha]_D$ +188.9°). The ^1H NMR, MS, mixed TLC, and antibacterial activity were identical with authentic **1**.[2]

Antibiotic G-52 (2). Compound **12** (100 mg) was dissolved in ethanol (3 mL) saturated at room temperature with methylamine. Phosphoric acid (3 drops) was added and the mixture was heated in a bomb at 100 °C for 16 h. Sodium borohydride (400 mg) was added to the cooled solution and the mixture was stirred at 25 °C for 0.5 h. The mixture was passed through Amberlite IRA 401S (OH^-) resin and concentrated, and the residue was chromatographed on a silica gel column (110 × 1 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1:1:1) as the eluent to give **2** (32 mg, 30%): $[\alpha]_D$ +147.5° (lit.[3] $[\alpha]_D$ +151°). The ^1H NMR, MS, mixed TLC, and antibacterial activity were identical with authentic **2**.[3]

6′-*N*-*tert*-Butylsisomicin (3). Compound **12** (100 mg) was dissolved in a mixture of ethanol (1 mL) and *tert*-butylamine (2 mL). Phosphoric acid (4 drops) was added and the mixture was heated in a bomb at 120 °C for 15 h. Sodium borohydride (50 mg) was added to the cooled solution, and the mixture was stirred at 25 °C for 0.5 h. The mixture was passed through Amberlite IRA 401S (OH^-) resin and concentrated, and the residue was chromatographed on a silica gel column (110 × 1 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1:1:1) as the eluent to give **3** (20 mg, 17%): mp 125–130 °C; $[\alpha]_D$ +136.1°; IR 3350, 2950, 1690, 1070 cm^{-1}; NMR δ 1.21 [s, 12, $(CH_3)_4C$– and 4″-CH_3], 2.52 (s, 3, 3″-NCH_3), 3.32 (s, 2, $H_{6'}$), 4.99 (m, 1, $H_{4'}$), 5.07 (d, J = 4 Hz, 1, $H_{1''}$), and 5.34 (d, J = 2 Hz, 1, $H_{1'}$); m/e 503 ($M^{+\cdot}$). Anal. ($C_{23}H_{45}N_5O_7 \cdot 0.5H_2O$) C, H, N.

6′-*N*-(2-Phenylethyl)sisomicin (4). Compound **12** (500 mg) was dissolved in a solution of 2-phenylethylamine (7 mL) in ethanol (7 mL) and phosphoric acid (2 drops) was added. The mixture was heated in a bomb at 70 °C for 16 h and then at 100 °C for 5 h. Sodium borohydride (500 mg) was added to the cooled solution, and the mixture was stirred at 25 °C for 1 h. The solution was passed through Amberlite IRA 401S (OH^-) resin, and the aqueous solution was extracted with benzene. The aqueous layer was adjusted to pH 9 by addition of acetic acid. The benzene extract was rewashed with water, and the latter was combined with the previous aqueous extract and evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2:1:1) as the eluent to give **4** (67 mg, 10%): $[\alpha]_D$ +137.7°; CD $[\theta]_{288}$ −8230 (TACu) and $[\theta]_{290}$ −6620 (Cupra A); IR (KBr) 3300, 1650, 1055, 1030 cm^{-1}; NMR δ 1.15 (s, 3, 4″-CH_3), 2.47 (s, 3, 3″-NCH_3), 2.77 (s, 4, 6′-$NCH_2CH_2C_6H_5$), 3.11 (s, 2, 6′-CH_2), 4.79 (m, 1, $H_{4'}$), 5.03 (d, J = 4 Hz, 1, $H_{1''}$), 5.20 (d, J = 2 Hz, 1, $H_{1'}$), 7.30 and 7.32 (s, 5, $-C_6H_5$); m/e 551.3333 ($M^{+\cdot}$ theory requires 551.3319). Anal. ($C_{27}H_{45}N_5O_7 \cdot 0.5H_2O$) C, H, N.

6′-*N*-(2-Hydroxyethyl)sisomicin (5). Compound **12** (500 mg) was dissolved in a solution of 2-hydroxyethylamine (2 mL) and ethanol (4 mL), and phosphoric acid (4 drops) was added. The mixture was heated in a bomb at 100 °C for 17 h. The mixture was cooled and diluted with ethanol (100 mL), sodium borohydride (500 mg) was added, and the solution was stirred at 25 °C for 2 h. The solution was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2:1:1) as the eluent to give **5** (100 mg, 17%): $[\alpha]_D$ +143.0°; IR (KBr) 3330, 1670, 1050, 1010 cm^{-1}; NMR δ 1.20 (s, 3, 4″-CH_3), 2.51 (s, 3, 3″-NCH_3), 2.70 (t, J = 5.5 Hz, 2, $-NHCH_2CH_2OH$), 3.68 (t, J = 5.5 Hz, 2, $-NHCH_2CH_2OH$), 3.16 (s, 2, 6′-CH_2), 4.89 (m, 1, $H_{4'}$), 5.09 (d, $J_{1'',2''}$ = 4 Hz, 1, $H_{1''}$), and 5.33 (d, $J_{1',2'}$ = 2 Hz, 1, $H_{1'}$); m/e 491.2953 ($M^{+\cdot}$ theory requires 491.2955).

***O*-2-Amino-2,3,4-trideoxy-α-D-*glycero*-hex-4-enopyranosyl(1→4)garamine (25).** Compound **12** (200 mg) was dissolved in distilled water (20 mL), and the solution was acidified to pH 3–4 by dropwise addition of phosphoric acid. The reaction mixture was stirred at 25 °C for 0.5 h and then reduced with sodium cyanoborohydride (200 mg) for a further 0.5 h. The solution was passed over Amberlite IRA 401S (OH^-) resin, and the strongly basic eluate was acidified carefully with acetic acid and then evaporated to dryness. The product was chromatographed on a silica gel column (70 × 1 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1:1:1) as the eluent to give **25** (70 mg, 33%): $[\alpha]_D$ +163.9°; CD $[\theta]_{290}$ −8400 (TACu); IR 3300, 2900, 1680, 1060 cm^{-1}; NMR δ 1.24 (s, 3, 4″-CH_3), 2.56 (s, 3, 3″-NCH_3), 3.99 (s, 2, 6′-CH_2), 5.06 (m, 1, $H_{4'}$), 5.13 (d, J = 4 Hz, 1, $H_{1''}$), and 5.41 (d, J = 2 Hz, 1, $H_{1'}$); m/e 448 ($M^{+\cdot}$). Anal. ($C_{19}H_{36}N_4O_8 \cdot 0.5H_2O$) C, H, N.

***O*-2-Amino-6-(hydroxyazylene)-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl(1→4)garamine Diacetate Salt (33).** Compound **12** (200 mg) and hydroxylamine hydrochloride (500 mg) in methanol (10 mL) were stirred at 25 °C for 3 h. The product was absorbed onto Amberlite IRA 401S (OH^-) resin, and the impurities were eluted with distilled water. Elution of the resin with 25% aqueous acetic acid followed by evaporation afforded **33** as a pale yellow solid (180 mg, 66%): $[\alpha]_D$ +91.1°; IR 3200, 2930, 1670, 1070, 1030 cm^{-1}; NMR δ 1.30 (s, 3, 4″-CH_3), 1.92 (s, 6, $CH_3COO^-NH^+$), 2.89 (s, 3, 3″-NCH_3), 5.05 (d, J = 4 Hz, 1, $H_{1''}$), 5.41 (m, 1 $H_{4'}$), 5.64 (d, J = 2 Hz, 1, $H_{1'}$), and 7.65 (s, 1, H_6). Anal. ($C_{19}H_{35}N_5O_8 \cdot 2CH_3COOH \cdot CO_2$) C, H, N; H: calcd, 6.93; found, 7.59; N: calcd, 11.19; found, 10.25.

***O*-2-Amino-6-(methoxyazylene)-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl(1→4)garamine (22).** Compound **12** (300 mg) and methoxyamine hydrochloride (500 mg) in methanol (6 mL) were stirred at 45 °C for 3 h. The reaction mixture was concentrated to a gum and chromatographed on a silica gel column (110 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2:1:1) as the eluent to give **22** (200 mg, 60%): $[\alpha]_D$ +100.0°; IR 3300, 2900, 1650, 1050 cm^{-1}; NMR δ 1.23 (s, 3, 4″-CH_3), 2.57 (s, 3, 3″-NCH_3), 3.92 (s, 3, $=NOCH_3$), 5.15 (d, J = 4 Hz, 1, $H_{1''}$), 5.51 (m, 1, $H_{4'}$), 5.51 (d, J = 2 Hz, 1, $H_{1'}$), and 7.70 (s, 1, $H_{6'}$); m/e 476 $(M + 1)^+$. Anal. ($C_{20}H_{37}N_5O_8 \cdot 0.5H_2O$) C, H, N.

***O*-2-Amino-6-(methoxyazyl)-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl(1→4)garamine (6).** Compound **22** (100 mg) was dissolved in water (7 mL) and the pH was adjusted to 1.5 by careful addition of 1 N sulfuric acid. Sodium cyanoborohydride (200 mg) was added, and the solution was stirred at 25 °C for 60 h. The reaction mixture was neutralized with dilute ammonium hydroxide and evaporated to dryness, and the product was chromatographed on a silica gel column (160 × 1 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2:1:1) as the eluent to give **6** (30 mg, 30%): $[\alpha]_D$ +157.4°; IR 3300, 2880, 1680, 1050 cm^{-1}; NMR δ 1.20 (s, 3, 4″-CH_3), 2.52 (s, 3, 3″-NCH_3), 3.47 (s, 2, 6′-CH_2), 3.53 (s, 3, $NOCH_3$), 4.96 (m, 1, $H_{4'}$), 5.10 (d, J = 4 Hz, 1, $H_{1''}$), and 5.37 (d, J = 2 Hz, 1, $H_{1'}$); m/e 477 ($M^{+\cdot}$). Anal. ($C_{20}H_{39}N_5O_8 \cdot CO_2$) C, H, N; C: calcd, 48.36 found, 48.84; H: calcd, 7.54; found, 7.89.

***O*-2-Amino-6-(1,1-diazanediyl)-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl(1→4)garamine (23).** Compound **12** (150 mg) was dissolved in anhydrous hydrazine (10 mL) and after 2.5 h the solution was diluted with water and evaporated to dryness. The product was chromatographed on a silica gel column (110 × 1 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1:1:1) as the eluent to give **23** (92 mg, 57%): mp 125–130 °C dec; $[\alpha]_D$ +125.9°; IR 3300, 2900, 1650, 1060 cm^{-1}; NMR δ 1.21 (s, 3, 4″-CH_3), 2.53 (s, 3, 3″-NCH_3), 5.09 (d, J = 4 Hz, 1, $H_{1''}$), 5.34 (q, 1, $H_{4'}$), 5.45 (d, J = 2 Hz, 1, $H_{1'}$), and 7.88 (s, 1, $H_{6'}$); m/e 461 ($M + 1)^+$. Anal. ($C_{19}H_{36}N_6O_7 \cdot 0.5H_2O$) C, H, N.

***O*-2-Amino-6-diazanyl-2,3,4,6-tetradeoxy-α-D-glycero-hex-4-enopyranosyl(1→4)garamine (7).** Compound **12** (150 mg) was dissolved in anhydrous hydrazine (10 mL) and the solution was stirred at 25 °C for 2.5 h. The solution was diluted with water and evaporated in vacuo. The residue was dissolved in methanol (15 mL) and the pH was adjusted to 5.5 using 50% aqueous acetic acid. Sodium cyanoborohydride (400 mg) was added and the mixture was stirred at 25 °C for 3.5 h. The solution was passed over Amberlite IRA 401S (OH^-) resin, and the aqueous eluate was evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1:1:1) as the eluent to give **7** (30 mg, 20%): NMR δ 1.21 (s, 3, 4″-CH_3), 2.53 (s, 3, 3″-NCH_3), 5.09 (d, J = 4 Hz, 1, $H_{1''}$), 5.33 (d, J = 2 Hz, 1, $H_{1'}$), and 5.33 (m, 1, $H_{4'}$).

***O*-2-Amino-6-(morpholinoazylene)-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl(1→4)garamine (24).** Compound **12** (300 mg) was dissolved in *N*-aminomorpholine (5 mL) and the solution was stirred at 25 °C for 4 h. The mixture was chromatographed on a silica gel column (110 × 2.5 cm) using the lower phase of chloroform–methanol–concentrated ammonium hydroxide solution (2:1:1) as the eluent to give **24** (200 mg, 54%): mp 123–130 °C; $[\alpha]_D$ +90.1°; IR 3320, 2900, 1655, 1060 cm^{-1}; NMR δ 1.20 (s, 3, 4″-CH_3), 2.51 (s, 3, 3″-NCH_3), 3.06 (m, 4, morpholino), 3.88 (m, 4, morpholino), 5.10 (d, J = 4 Hz, 1, $H_{1''}$), 5.46 (m, 1, $H_{4'}$), 5.46 (d, J = 2 Hz, 1, $H_{1'}$), and 7.38 (s, 1, $H_{6'}$); m/e 531 ($M + 1)^+$. Anal. ($C_{23}H_{42}N_6O_8 \cdot 0.5H_2O$) C, H, N.

***O*-2-Amino-6-(morpholinoazyl)-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl(1→4)garamine (8).** Compound **24** (90 mg) was dissolved in methanol–water (3:1) (6 mL) and the pH was adjusted to 3.5 with 1 N sulfuric acid. Sodium cyanoborohydride (300 mg) was added and the mixture was stirred at 25 °C for 2 h. The solution was concentrated and chromatographed on a silica gel column (110 × 1 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2:1:1) as the eluent to give 8 (61 mg, 68%): $[\alpha]_D$ +139.2°; IR 3310, 2900, 1680, 1060 cm^{-1}; NMR δ 1.21 (s, 3, 4″-CH_3), 2.52 (s, 3, 3″-NCH_3), 2.84 (m, 4, morpholino), 3.42 (s, 2, 6′-CH_2), 3.79 (m, 4, morpholino), 4.97 (m, 1, $H_{4'}$), 5.12 (d, J = 4 Hz, 1, $H_{1''}$), and 5.36 (d, J = 2 Hz, 1, $H_{1'}$); m/e 532 ($M^{+\cdot}$). Anal. ($C_{23}H_{44}N_6O_8 \cdot 0.5H_2O$) C, H, N; H: calcd, 8.37; found, 7.73.

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