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ISOLATION AND STRUCTURES OF ISTAMYCIN COMPONENTS*

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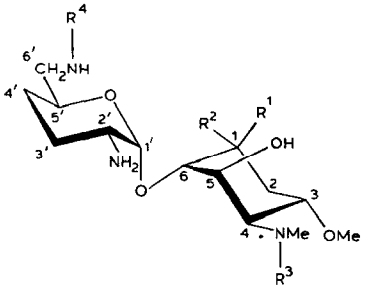
ABSTRACT

In addition to istamycin A and B previously isolated from culture filtrates of *Streptomyces tenjimariensis*, istamycin C, A_0, B_0, C_0, A_1, B_1, C_1, and A_2 which contain the 1,4-diaminocyclitol moiety, have been found as minor components. Isolation and structural elucidation of these antibiotics are described. Istamycin A_0, B_0, and C_0 are deglycyl derivatives, and istamycin A_1, B_1, and C_1 are 2″-*N*-formyl derivatives, of istamycin A, B, and C, respectively. Istamycin A_2 is 2″-*N*-carbamoyl-istamycin A.



		R^1	R^2	R^3 (1″ 2″)	R^4
Istamycin A	1	NH_2	H	$COCH_2NH_2$	Me
Istamycin B	2	H	NH_2	$COCH_2NH_2$	Me
Istamycin C	3	NH_2	H	$COCH_2NH_2$	Et
Istamycin A_0 (in acid)	4	NH_2	H	H	Me
Istamycin B_0	5	H	NH_2	H	Me
Istamycin C_0 (in acid)	6	NH_2	H	H	Et
Istamycin A_1	7	NH_2	H	$COCH_2NHCHO$	Me
Istamycin B_1	8	H	NH_2	$COCH_2NHCHO$	Me
Istamycin C_1	9	NH_2	H	$COCH_2NHCHO$	Et
Istamycin A_2	10	NH_2	H	$COCH_2NHCONH_2$	Me

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.
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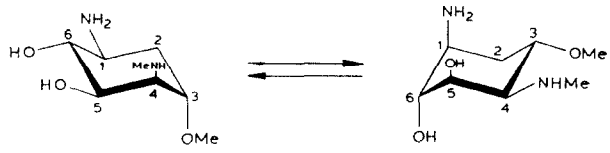
INTRODUCTION

Two 1,4-diaminocyclitol-containing aminoglycoside antibiotics, istamycin A (**1**) and B (**2**), were discovered in culture filtrates of *Streptomyces tenjimariensis* nov. sp., which was isolated from a sample of sea mud[1-4]. These antibiotics show strong activity against Gram-positive and -negative bacteria, except for *Pseudomonas*. The isolation, characterization, and structures of **1** and **2** have been the subject of a preliminary report[1]. Besides these two, eight new, minor components, named istamycins C (**3**), A_0 (**4**), B_0 (**5**), C_0 (**6**), A_1 (**7**), B_1 (**8**), C_1 (**9**), and A_2 (**10**) have been isolated from culture filtrates of this strain. In this paper, we report the isolation and structural elucidation of these ten istamycin components.

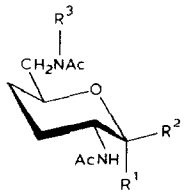
RESULTS AND DISCUSSION

A crude powder, obtained from culture filtrates by adsorption on a column of Amberlite IRC-50 (NH_4^+) and elution with aqueous ammonia, was separated into ten components by column chromatography on Amberlite CG-50 (NH_4^+) with gradient elution by 0.1 and 0.8M ammonium hydroxide followed by chromatography on silica gel developed with chloroform–methanol–aqueous ammonia. Properties of these ten istamycin components are shown in Table I. The ^{1}H-n.m.r. spectra showed that **1** and **2** have two *N*-methyl and one *O*-methyl group, and **3** has one *N*-methyl, one *N*-ethyl, and one *O*-methyl group. The mass-fragmentation patterns indicated that **1**, **2**, and **3** are closely related in their structures to fortimicin A (ref. 5) and sporaricin A (ref. 6).

Alkaline hydrolysis of **1**, **2**, and **3** in barium hydroxide solution gave one mol



11a (pD 11) ⇌ 11b (pD 1)



	R^1	R^2	R^3
12a	OMe	H	Me
12b	H	OMe	Me
14a	OMe	H	Et
14b	H	OMe	Et

TABLE I

PROPERTIES OF ISTAMYCIN COMPONENTS

Analytical data	*Compound*									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
M.p. (dec.)	102–108°	112–124°	[a]	[a]	[a]	[a]	85–115°	87–120°	70–78°	105–135°
$[\alpha]_D$ (water)	+155° at 25°	+165° at 25°	+131° at 25°	+76° at 22°	+160° at 26°	+69° at 23°	+113° at 22°	+112° at 21°	+108° at 21°	+115° at 20°
Formula	$C_{17}H_{35}N_5O_5 \cdot 0.5H_2CO_3$	$C_{17}H_{35}N_5O_5 \cdot 0.5H_2CO_3 \cdot 0.5H_2O$	$C_{18}H_{37}N_5O_5 \cdot H_2CO_3 \cdot 0.5H_2O$	$C_{15}H_{32}N_4O_4 \cdot 0.5H_2CO_3$	$C_{15}H_{32}N_4O_4 \cdot 0.5H_2CO_3 \cdot 0.5H_2O$	$C_{16}H_{34}N_4O_4 \cdot 0.5H_2CO_3$	$C_{18}H_{35}N_5O_6 \cdot H_2CO_3$	$C_{18}H_{35}N_5O_6 \cdot H_2CO_3$	$C_{19}H_{37}N_5O_6 \cdot 0.5H_2CO_3$	$C_{18}H_{36}N_6O_6 \cdot 0.5H_2CO_3 \cdot 0.5H_2O$
Anal. (%)	Calc. Found	Calc. Found	Calc. Found	Calc. Found	Calc. Found	Calc. Found	Calc. Found	Calc. Found	Calc. Found	Calc. Found
C	49.99 50.01	48.94 48.93	48.09 48.02	51.22 50.94	49.98 50.33	52.50 53.01	47.59 47.41	47.59 47.68	50.63 50.41	47.02 47.06
H	8.63 8.70	8.68 9.30	8.50 8.94	9.15 9.21	9.20 9.17	9.36 9.32	7.78 7.60	7.78 7.70	8.28 8.17	8.10 7.80
N	16.65 16.75	16.31 16.39	14.76 14.66	15.42 15.26	15.04 15.27	14 84 15.02	14.60 14.45	14.60 14.55	15.14 14.88	17.79 17.47
T.l.c. (R_F)	0.17	0.11	0.26	0.36	0.14	0.57	0.24	0.12	0.32	0.15
P.e. (R_M)	2.1	2.1	2.1	2.1	2.1	2.1	1.8	1.7	1.6	1.6
I.r. ν_{max}^{KBr} (cm^{-1})	1630, 1575, 1470, 1095, 1020	1630, 1580, 1470, 1090, 1020	1640, 1450, 1090, 1020	1580, 1480, 1100, 1040	1580, 1480, 1090, 1020	1620, 1480, 1100, 1040	1680, 1640, 1580, 1470, 1110, 1040	1680, 1640, 1580, 1470, 1110, 1020	1680, 1640, 1580, 1480, 1100, 1030	1640, 1540, 1480, 1100, 1020
Mass spectrum (m/z),										
e.i. M^+	389	389	403	332	332	346	417	417	431	
cyclitol moiety	230	230	230	173	173	173	258	258	258	230
hexose moiety	143	143	157	143	143	157	143	143	157	143
f.d.: MH^+										433
^{1}H-N.m.r. (δ, J in Hz)	(pD 5.4)	(pD 5.4)	(pD 2.3)	(pD 5.0)	(pD 5.0)	(pD 2.3)	(pD 3.0)	(pD 3.5)	(pD 2.0)	(pD 3.0)
6′-NCH_2CH_3			1.80 (t, 7.5)			1.67 (t, 7.5)			1.82 (t, 7.5)	
6′-NCH_3	3.21 (s)	3.26 (s)		3.23[b] (s)	3.21[b] (s)		3.29 (s)	3.29 (s)		3.29 (s)
4-NCH_3	3.57 (s)	3.59 (s)	3.62 (s)	3.28[b] (s)	3.28[b] (s)	3.12 (s)	3.69 (s)	3.64 (s)	3.67 (s)	3.67 (s)
3-OCH_3	3.89 (s)	3.95 (s)	3.94 (s)	3.98 (s)	3.91 (s)	3.83 (s)	3.97 (s)	3.97 (s)	3.95 (s)	3.97 (s)
NCH_2CO	4.50 (ABq)	4.57 (ABq)	4.55 (ABq)				4.75 (ABq)	~4.7	~4.7	4.56 (ABq)
H-1′	5.80 (d, 3.5)	5.96 (d, 3.5)	5.85 (d, 3.5)	5.86 (d, 3.5)	5.92 (d, 3.5)	5.72 (d, 3.5)	5.84 (d, 3.5)	5.96 (d, 3.5)	5.82 (d, 3.5)	5.84 (d, 3)
NC*H*O							8.70 (s)	8.71 (s)	8.69 (s)	
Bioactivity (% of **2**)	76	100	68	0.4	1	0.3	3	9	3	45

[a]Not definite. [b]Assignments within any vertical column may be reversed.

TABLE II

^{1}H-N.M.R. CHEMICAL SHIFTS AND COUPLING CONSTANTS FOR THE TWO CONFORMERS OF DIAMINOCYCLITOL **11** IN D_2O

	Chemical shifts (δ)		*Coupling constants (Hz)*		
	11a (pD 11)	**11b** (pD 1)		**11a**	**11b**
H-1	~3.4	4.16	$J_{1,2ax}$	11	3
			$J_{1,2eq}$	4	4.5
H-2ax	2.19	2.57	$J_{2ax,eq}$	14.5	15
H-2eq	2.59	2.92	$J_{2ax,3}$	3	10
			$J_{2eq,3}$	4	4.5
H-3	~4.3	4.45	$J_{3,4}$	4	10
H-4	3.52	3.92	$J_{4,5}$	4.5	3.5
H-5	4.32	4.85	$J_{5,6}$	9	4
H-6	3.97	4.59	$J_{1,6}$	9	4.5
NCH_3	2.90	3.28			
OCH_3	3.90	3.95			

TABLE III

^{13}C-N.M.R. SPECTRA OF ISTAMYCIN A COMPONENTS IN D_2O

Carbon atom	**1**		**4**		**7**		**10**
	pD 11	*pD 5.4*	*pD 11*	*pD 5.0*	*pD 11*	*pD 3.0*	*pD 2.0*
1	50.1[a]	49.4[a]	49.2[a]	49.2[a]	49.7[a]	49.4[a]	49.4[a]
2	33.0	29.2	31.2	28.3	31.9	29.2	29.2
3	73.6	71.3[b]	76.2	72.0	72.5	71.5	71.5
4	55.9	56.5	63.3	61.2	55.9	56.6	56.6
5	67.3	69.8	68.6	65.7	66.0	69.9	69.9
6	77.4	73.2[b]	84.1	73.1	76.9	73.2	73.2
3-OCH_3	57.6	56.5	56.9	57.5	57.7	56.6	56.6
4-NCH_3	32.1	31.9	34.7	31.6	32.4	32.1	32.0
1′	100.0	95.1	101.6	95.8	98.6	95.1	95.1
2′	51.4[a]	50.7[a]	50.4[a]	50.1[a]	51.6[a]	50.9[a]	50.9[a]
3′	26.4	21.6	26.9	21.9	25.1	21.7	21.6
4′	28.4	26.8	28.7	26.8	27.8	26.7	26.7
5′	70.9	66.2	71.1	66.4	71.3	66.3	66.3
6′	54.5	52.9	55.4	53.0	53.3	52.9	52.9
6′-NCH_3	34.8	34.2	35.5	34.3	34.1	34.3	34.4
1″	175.9	168.7			171.4	171.5	173.2
2″	43.2	41.2			41.1	41.2	42.9
NCHO					165.4	165.6	
NCON							162.4

[a,b]Assignments within any vertical column may be reversed.

each of glycine and a pseudodisaccharide. The latter, obtained from **1**, **2**, or **3**, was identical with **4**, **5**, or **6**, respectively. Tetra-*N*-acetylistamycin A_0, prepared by *N*-acetylation of **4** with acetic anhydride in methanol, was treated with anhydrous hydrogen chloride in methanol. Reacetylation gave the di-*N*-acetyl derivative of a diaminocyclitol (**11**) and two anomeric methyl glycosides (**12a** and **12b**) identical with methyl 2,6-diacetamido-2,3,4,6-tetradeoxy-6-*N*-methyl-α- and -β-D-*erythro*-hexopyranosides (methyl 2,6-di-*N*-acetyl-6-*N*-methyl-α- and -β-purpurosaminide C) derived from 3′,4′-dideoxy-6′-*N*-methylkanamycin B (ref. 7).

The ^{1}H-n.m.r. spectra of the diaminocyclitol (**11**, Table II) suggested that it had the structure **11a** or its enantiomer. In acid solution, **11a** underwent conformational change to **11b**. From ^{1}H- and ^{13}C-n.m.r. spectra of **4** (Tables I and III), it has been shown that, in **4**, the diaminohexose (**12**) is linked to the 6-hydroxyl group of **11** through an α-glycosidic linkage. As reported previously[8], we synthesized **4** and **1** starting from 3′,4′-dideoxyneamine via an aziridine intermediate, and the stereochemistry of **11** was conclusively proved to be that of 2-deoxyfortamine (**11a**

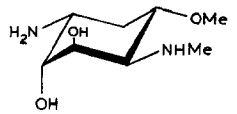


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TABLE IV

^{13}C-N.M.R. SPECTRA OF ISTAMYCIN B COMPONENTS IN D_2O

Carbon atom	**2**		**5**		**8**	
	pD 11	*pD 5.5*	*pD 11*	*pD 5.2*	*pD 11*	*pD 5.5*
1	46.6	47.2	46.6	46.8	47.4	47.3
2	31.6	29.3	33.2	28.1	31.0	29.3
3	73.1	71.9	78.1	74.4	73.1	72.0
4	55.9	56.5	61.4	60.3	56.0	56.6
5	67.0	68.3	65.3	62.0	66.0	68.3
6	77.5	73.6	77.2	71.4	76.6	73.6
3-O*C*H$_3$	56.7	56.5	57.0	57.5	56.9	56.4
4-N*C*H$_3$	34.2	32.0	33.3	31.6	32.1	32.1
1′	97.6	93.1	97.8	91.7	98.4	93.0
2′	50.2	49.6	50.4	49.0	50.1	49.5
3′	25.7	21.4	26.8	21.5	25.5	21.4
4′	28.4	26.7	28.7	26.4	28.3	26.6
5′	69.9	66.5	68.3	66.7	70.4	66.5
6′	54.3	53.0	55.2	52.9	53.5	52.9
6′-N*C*H$_3$	34.7	34.4	35.5	34.4	34.2	34.4
1″	175.8	168.7			171.5	171.6
2″	43.2	41.3			41.1	41.2
N*C*HO					165.4	165.6

TABLE V

^{13}C-N.M.R. SPECTRA OF ISTAMYCIN C COMPONENTS IN D_2O

Carbon atom	**3**		**6**		**9**	
	pD 11	*pD 2.0*	*pD 11*	*pD 2.0*	*pD 11*	*pD 2.0*
1	49.0[a]	49.4[a]	49.1[a]	49.3[a]	49.7[a]	49.4[a]
2	31.8	29.2	31.2	29.1	31.9	29.1
3	72.6	71.3	76.3	72.5	72.5	71.4
4	54.5	56.6	63.4	61.6	55.9	56.6
5	67.4	69.8	68.9	66.1	65.8	69.9
6	76.6	73.1	84.7	74.3	76.7	73.1
3-OCH_3	56.3	56.6	56.9	57.3	57.7	56.6
4-NCH_2	30.8	31.9	34.9	32.0	32.4	32.1
1′	99.2	95.1	101.9	96.3	98.8	95.1
2′	50.1[a]	50.7[a]	50.5[a]	50.0[a]	51.1[a]	50.9[a]
3′	25.6	21.6	27.0	22.6	25.4	21.6
4′	27.7	26.9	28.9	27.0	28.0	26.8
5′	69.7	66.2	71.3	66.5	71.3	66.3
6′	51.9	50.7	53.3	50.8	51.7	50.8
6′-NCH_2CH_3	42.0[b]	44.3	44.0	44.2	43.9	44.4
6′-NCH_2CH_3	12.8	11.0	14.1	11.0	11.3	11.1
1″	174.9	168.6			171.4	171.5
2″	42.6[b]	41.3			41.1	41.2
2″-N*C*HO					165.4	165.6

[a,b]Assignments within any vertical column may be reversed.

or **11b**). After our paper on istamycin had been published[1], Watanabe *et al.*[9] reported on the structures of sannamycin A and B, which were found to be identical with istamycin A and A_0, respectively.

Methanolysis of tetra-*N*-acetylistamycin B_0 gave the di-*N*-acetyl derivative of a diaminocyclitol **13** and two anomers, **12a** and **12b**. Compound **13** was confirmed[6] to be identical with the diaminocyclitol obtained from sporaricin A. By ^{1}H- and ^{13}C-n.m.r. analysis (Tables I and IV) the structures of **5** and **2** were determined.

Methanolysis of tetra-*N*-acetylistamycin C_0 gave the di-*N*-acetyl derivative of **11** and two anomers of a new methyl glycoside (**14a** and **14b**). Compounds **14a** and **14b** were shown by their optical rotations to be D sugars. From ^{13}C- and ^{1}H-n.m.r. spectra, **14a** and **14b** were determined to be methyl 2,6-diacetamido-2,3,4,6-tetradeoxy-6-*N*-ethyl-α- and -β-D-*erythro*-hexopyranosides, respectively. By these spectral analyses (Tables I and V) the structures of **6** and **3** were established.

Mild acid hydrolysis of **7**, **8**, and **9** in 0.5M hydrogen chloride for 4 h at 60° afforded **1**, **2**, and **3**, respectively, whose identities were confirmed by p.e. and t.l.c. By ^{1}H- and ^{13}C-n.m.r. analysis, the structures of **7**, **8**, and **9** were determined to be the 2″-*N*-formyl derivatives of **1**, **2**, and **3**, respectively.

Alkaline hydrolysis of **10** in 0.05M barium hydroxide for 1 h at 100° gave

hydantoic acid and **4**. By ^{13}C-n.m.r. analysis and synthesis of **10**, the structure was confirmed to be 2″-*N*-carbamoylistamycin A.

Among these istamycin components, **2** is the most active in inhibiting growth of bacteria, and **1**, **3**, and **10** are fairly active (Table I). However, the deglycyl (**4**, **5**, and **6**) and 2″-*N*-formyl (**7**, **8**, and **9**) derivatives show very weak activity.

EXPERIMENTAL

General methods. — Melting points were determined with a Yamato apparatus and are uncorrected. Optical rotations were measured with a Carl Zeiss LEP A2 polarimeter. I.r. spectra were recorded with a Hitachi Model 260-10 infrared spectrophotometer. The ^{1}H- and ^{13}C-n.m.r. spectra were obtained with a Varian XL-100 or EM-390 spectrometer. ^{1}H-N.m.r. chemical shifts in D_2O refer to an external standard of tetramethylsilane ($\delta = 0$). ^{1}H-N.m.r. chemical shifts in $CDCl_3$ and ^{13}C-n.m.r. shifts in D_2O refer to Me_4Si as the internal standard. Mass spectra were recorded with a Hitachi RMU-6M spectrometer for electron-impact ionization or RMU-7M for field-desorption. T.l.c. for istamycin components was performed on plates of Silica Gel 60 (E. Merck) developed with 2:1:1 chloroform–methanol–17% aqueous ammonia; zones of compounds were detected by ninhydrin spray. High-voltage paper electrophoresis (p.e.) was performed on Toyo No.51 paper with 1:3:36 formic acid–acetic acid–water electrolyte with a Savant Model LT-48A instrument at 3,000 V for 15 min, with cooling below 20°; components on the paper were detected by ninhydrin and the mobilities expressed relative to alanine ($R_M = 1.0$). Bioactivities of antibiotics were determined by the cylinder-plate method against *Bacillus subtilis* PCI219 with pure istamycin B (**2**) as the assay standard (as 1,000 μg/mg or 100%).

Isolation of istamycin components. — *Streptomyces tenjimariensis* SS-939 on an agar slant was transferred into a 100-L fermentor containing 50 L of a seed medium (1.0% starch, 0.2% glucose, 1.0% soybean meal, 0.3% sodium chloride, 0.1% potassium hydrogenphosphate, and 0.1% magnesium sulfate heptahydrate, adjusted to pH 7.2) and cultured for 24 h at 28° under agitation at 200 r.p.m. and aeration at 100% vol per min. The seed culture (6 L) thus prepared was inoculated into a 570-L fermentor containing 300 L of a production medium (4.0% starch, 0.4% glucose, 5.0% wheat germ, 0.3% sodium chloride, 0.6% calcium carbonate and 0.0005% cobalt dichloride hexahydrate) and cultured for 89 h at 28° under agitation at 280 r.p.m. and aeration at 100% vol per min.

The cultured broths in two fermentors were combined, and filtered twice at pH 2.5 and 7.0. Antibiotics in the filtrate (350 L, pH 6.7, bioactivity 30 μg/mL) were adsorbed on a column of Amberlite IRC-50 (NH_4^+, 70 L), which was washed with water (140 L), and the antibiotics were eluted with 0.84M ammonium hydroxide (15-L fractions). The active eluate (fractions 3–8) was evaporated, yielding 29.1 g (bioactivity 260 μg/mg) of a crude powder. A solution of this powder in 1 L of water was passed through a column of Amberlite CG-50 (NH_4^+, 400 mL). The column

was washed with water (800 mL) and the antibiotics were eluted by a linear gradient from 0.1 to 0.8M ammonium hydroxide (3 L of each), collecting 20-mL fractions. Fractions 81–90 were combined and evaporated, yielding powder (3.2 g) containing **7**, **8**, and **9**. Fractions 91–98 contained **10** (1.48 g); nos. 108–118 contained **2**, **3**, and **6** (816 mg); nos. 119–132 contained **1** and **2** (1.55 g); nos. 133–152 contained **1** and **4** (1.57 g), and nos. 153–179 contained **5** (520 mg). The appropriate fractions from the previous column were separated into their individual components by chromatography on silica gel (Kieselgel 60, Merck). The eluting solvents were as follows: 2:1:1 chloroform–methanol–8.5% ammonia for **7**, **8**, and **9**, and 2:1:1 chloroform–methanol–17% ammonia for the others. For compounds **7**, **8**, **9**, and **10**, further purification on Amberlite CG-50 (NH_4^+) with elution by ammonium hydroxide afforded 80, 112, 22, and 37 mg, respectively. Compounds **1**, **2**, **3**, **4**, **5**, and **6** were obtained in amounts of 1034, 740, 68, 626, 212, and 10 mg, respectively.

Properties of these istamycin components are shown in Table I, and ^{13}C-n.m.r. spectra of istamycin A, B, and C components are shown in Tables III, IV, and V, respectively.

Crystalline istamycin B_0 (**5**) *disulfate dihydrate.* — An aqueous solution (0.5 mL) of **5** (monocarbonate, 200 mg) was adjusted to pH 6.5 with M sulfuric acid (0.5 mL), and then methanol (6 mL) was added. After being kept overnight at 5°, colorless crystals were filtered off and 150 mg of crystalline **5** disulfate dihydrate was obtained, m.p. 215–244° (decomp.), $[\alpha]_D^{15}$ +88° (*c* 1.0, water); ν_{max}^{KBr} 1630, 1540, 1470, and 1100 cm^{-1}.

Anal. Calc. for $C_{15}H_{32}N_4O_4 \cdot 2\ H_2SO_4 \cdot 2\ H_2O$: C, 31.91; H, 7.14; N, 9.92; S, 11.36; Found: C, 31.31; H, 6.88; N, 9.19; S, 11.19.

Alkaline hydrolysis of istamycin A (**1**), *B* (**2**), *and C* (**3**). — A solution of **2** (720 mg) in 0.25M barium hydroxide (25 mL) was boiled under reflux for 2.5 h and made neutral with carbon dioxide. The precipitate was filtered off, and washed with water. The combined filtrate was passed through a column of Amberlite CG-50 (NH_4^+, 35 mL). The column was washed with water and eluted with 0.4M ammonium hydroxide. Fractions containing **5** were combined and evaporated to give colorless, solid **5** (hemicarbonate, 593 mg, 96%). The identity was confirmed by p.e., t.l.c., optical rotation, and ^{1}H- and ^{13}C-n.m.r. spectra.

The effluent solution from the column of Amberlite CG-50 (NH_4^+) contained glycine, whose identity was confirmed by p.e. (R_M 1.10).

Alkaline hydrolysis of **1** (hemicarbonate, 40 mg), followed by column chromatography on Amberlite CG-50 (NH_4^+, 5 mL) eluted with 0.4M ammonium hydroxide gave a colorless solid of **4** (hemicarbonate, 31.5 mg, 91%). Alkaline hydrolysis of **3** (carbonate hemihydrate, 16 mg) followed by column chromatography on Amberlite CG-50 (NH_4^+, 2.5 mL) gave **6** as a colorless solid (hemicarbonate, 12.3 mg, 97%).

*Tetra-*N*-acetylistamycin A_0, B_0, and C_0.* — To a solution of **4** (hemicarbonate, 100 mg) in methanol (4 mL) was added acetic anhydride (1 mL) at room temperature. The solution was kept for 1 h at this temperature, and then evaporated to afford a colorless solid, tetra-*N*-acetylistamycin A_0 (120 mg), $[\alpha]_D^{20}$ +174° (*c* 1, methanol);

m/z 500 (M^+), 303, 275, 257, and 227; ^{1}H-n.m.r. ($CDCl_3$): δ 1.97 (s, 6 H, NAc × 2), 2.03, 2.06, 2.10, and 2.13 (total 6 H, NAc × 2, rotamers), 2.62, 2.90, 3.10, and 3.12 (total 6 H, NMe × 2, rotamers), and 3.33 (3 H, OMe).

Anal. Calc. for $C_{23}H_{40}N_4O_8$: C, 55.18; H, 8.06; N, 11.19. Found: C, 55.46; H, 8.11; N, 11.14.

Starting from **5** (hemicarbonate hemihydrate, 481 mg), tetra-*N*-acetylistamycin B_0 (645 mg) was obtained, $[\alpha]_D^{25}$ +159° (*c* 0.5, methanol); *m/z* 500 (M^+), 303, 275, 257, and 227; ^{1}H-n.m.r. ($CDCl_3$): δ 1.92 (s, 3 H, NAc), 2.00 (s, 3 H, NAc), 2.08 (s, 3 H, NAc), 2.12 (s, 3 H, NAc), 3.08 (s, 3 H, NMe), 3.12 (s, 3 H, NMe), and 3.37 (s, 3 H, OMe).

Anal. Calc. for $C_{23}H_{40}N_4O_8 \cdot H_2O$: C, 53.27; H, 8.16; N, 10.81. Found: C, 53.98; H, 7.93; N, 10.47.

Starting from **6** (hemicarbonate, 300 mg), tetra-*N*-acetylistamycin C_0 (390 mg) was obtained, $[\alpha]_D^{25}$ +184° (*c* 1, methanol); *m/z* 514 (M^+), 303, 275, 257, and 241; ^{1}H-n.m.r. ($CDCl_3$): δ 1.07 and 1.13 (t, total 3 H, *J* 7.5 Hz, NCH_2CH_3 rotamers), 1.95 (s, 6 H, NAc), 2.02, 2.04, 2.08, and 2.14 (total 6 H, NAc × 2 rotamers), 2.73 and 3.11 (total 3 H, NMe rotamers), and 3.32 (3 H, OMe).

Anal. Calc. for $C_{24}H_{42}N_4O_8$: C, 56.01; H, 8.23; N, 10.89. Found: C, 55.44; H, 8.06; N, 10.57.

*Methanolysis of tetra-*N*-acetylistamycin A_0, B_0, and C_0.* — A solution of tetra-*N*-acetylistamycin B_0 (645 mg) in 6M hydrogen chloride in methanol (15 mL) was boiled for 17 h under reflux. The solution was evaporated to give a yellowish solid after repeated addition and evaporation of methanol. A solution of the solid in water (5 mL) was passed through a column of Dowex 1-X2 (OH^-, 15 mL) and the effluent was evaporated. The residue was reacetylated with acetic anhydride (0.7 mL) in methanol (5 mL) overnight at room temperature and the solution evaporated. The residue was separated into four compounds by column chromatography on silica gel (25 g) developed with 3:1 acetone–chloroform and then 6:2:1 acetone–chloroform–methanol. From the eluate with the former solvent-mixture were obtained two compounds having R_F 0.50 and 0.41 in t.l.c. (silica gel, 6:2:1 acetone–chloroform–methanol) and from the eluate with the latter, two compounds having R_F 0.36 and 0.25.

Methyl 2,6-diacetamido-2,3,4,6-tetradeoxy-6-*N*-methyl-α-D-*erythro*-hexopyranoside (**12a**, 99 mg) had R_F 0.50; $[\alpha]_D^{25}$ +170° (*c* 0.52, methanol); *m/z* 258 (M^+); ^{1}H-n.m.r. ($CDCl_3$): δ 1.98 (s, 3 H, NAc), 2.19, and 2.22 (total 3 H, NAc rotamers), 2.97 and 3.11 (total 3 H, NMe rotamers), 3.93 (s, 3 H, OMe), and 4.58 (d, 1 H, *J* 3.5 Hz, H-1).

Anal. Calc. for $C_{12}H_{22}N_2O_4$: C, 55.79; H, 8.58; N, 10.85. Found: C, 56.39; H, 8.44; N, 10.15.

The β anomer (**12b**, 57 mg) had R_F 0.41; $[\alpha]_D^{25}$ +6° (*c* 1.0, methanol); *m/z* 258 (M^+); ^{1}H-n.m.r. ($CDCl_3$): δ 1.97 (s, 3 H, NAc), 2.20 and 2.23 (total 3 H, NAc rotamers), 2.98 and 3.13 (total 3 H, NMe rotamers), 3.92 and 3.94 (total 3 H, OMe rotamers), and 4.18 and 4.24 (d, total 1 H, *J* 8 Hz, H-1 rotamers). Compounds **12a**

and **12b** were confirmed to be identical with the α and β anomers obtained by methanolysis of 3′,4′-dideoxy-6′-*N*-methylkanamycin B (ref. 7).

The di-*N*-acetyl derivative of diaminocyclitol **13** (172 mg) had R_F 0.36; $[\alpha]_D^{25}$ +94° (*c* 0.5, methanol); *m/z* 274 (M^+); ^{1}H-n.m.r. (D_2O): δ 2.15 (bq, 1 H, *J* 12 Hz, H-2ax), 2.54 (s, 3 H, NAc), 2.66 and 2.68 (total 3 H, NAc rotamers), 2.81 (dt, 1 H, *J* 4 and 12 Hz, H-2eq), 3.51 and 3.63 (total 3 H, NMe rotamers), 3.90 and 3.93 (total 3 H, OMe rotamers), and 5.00 (dd, 1 H, *J* 3 and 11 Hz).

Anal. Calc. for $C_{12}H_{22}N_2O_5 \cdot 0.5\ H_2O$: C, 50.87; H, 8.18; N, 9.89. Found: C, 50.58; H, 7.70; N, 9.25.

Unreacted tetra-*N*-acetylistamycin B_0 (153 mg), R_F 0.25, was recovered.

Methanolysis of tetra-*N*-acetylistamycin A_0 (300 mg) gave **12a** (54 mg), **12b** (30 mg), and the di-*N*-acetyl derivative of diaminocyclitol **11** (90 mg) showing R_F 0.32; $[\alpha]_D^{25}$ +122° (*c* 1.0, water); *m/z* 274 (M^+); ^{1}H-n.m.r. (D_2O): δ 2.35 (m, 1 H, *J* 4, 10.5, and 13.5 Hz, H-2ax), 2.53 (s, 3 H, NAc), 2.66 and 2.68 (total 3 H, NAc rotamers), 2.82 (dt, 1 H, *J* 4, 4 and 13.5 Hz, H-2eq), 3.54 and 3.66 (total 3 H, NMe rotamers), 3.88 and 3.90 (total 3 H, OMe rotamers), and 5.05 (dd, 1 H, *J* 3 and 10.5 Hz).

Anal. Calc. for $C_{12}H_{22}N_2O_5 \cdot 0.5\ H_2O$: C, 50.87; H, 8.18; N, 9.89. Found: C, 50.82; H, 7.86; N, 9.93.

Unreacted tetra-*N*-acetylistamycin A_0 (70 mg) was recovered.

Methanolysis of tetra-*N*-acetylistamycin C_0 (300 mg) gave the new methyl glycosides **14a** (86 mg, R_F 0.66) and **14b** (48 mg, R_F 0.51). The di-*N*-acetyl derivative of **11** (167 mg) and unreacted tetra-*N*-acetylistamycin C_0 (71 mg) were also obtained.

Methyl 2,6-diacetamido-2,3,4,6-tetradeoxy-6-*N*-ethyl-α-D-*erythro*-hexopyranoside (**14a**) had $[\alpha]_D^{24}$ +170° (*c* 1.0, methanol); *m/z* 272 (M^+); ^{1}H-n.m.r. ($CDCl_3$): δ 1.12 and 1.18 (total 3 H, *J* 7 Hz, NCH_2CH_3 rotamers), 1.97 and 1.98 (total 3 H, NAc rotamers), 2.10 and 2.11 (total 3 H, NAc rotamers), 3.32 and 3.33 (total 3 H, OMe rotamers), 3.46 (q, 2 H, *J* 7 Hz, NCH_2CH_3), and 4.56 (bd, 1 H, *J* 3 Hz, H-1).

Anal. Calc. for $C_{13}H_{24}N_2O_4$: C, 57.33; H, 8.88; N, 10.29. Found: C, 56.44; H, 8.57; N, 9.89.

The β anomer (**14b**) had $[\alpha]_D^{24}$ +11° (*c* 1.0, methanol); *m/z* 272 (M^+); ^{1}H-n.m.r. ($CDCl_3$): δ 1.12 and 1.18 (total 3 H, *J* 7 Hz, NCH_2CH_3 rotamers), 1.96 and 1.97 (total 3 H, NAc rotamers), 2.11 and 2.12 (total 3 H, NAc rotamers), 3.42 (3 H, OMe), 3.47 (q, 2 H, *J* 7 Hz, NCH_2CH_3), and 4.16 and 4.24 (total 1 H, *J* 8 Hz, H-1).

Diaminocyclitols **11** *and* **13**. — The di-*N*-acetyl derivative of **11** (167 mg) in 4M sodium hydroxide (1 mL) was heated for 7 h at 110°, and made neutral with 6M hydrochloric acid. The solution was diluted to 10 mL with water and charged onto a column of Amberlite CG-50 (NH_4^+, 15 mL). After washing with 0.2M ammonium hydroxide, the column was eluted with 0.5M ammonium hydroxide. Ninhydrin-positive fractions were combined and evaporated, yielding **11** as a colorless solid (83 mg) $[\alpha]_D^{24}$ −43° (*c* 1, water); *m/z* 190 (M^+); ^{13}C-n.m.r. (D_2O pD 1.0): δ 72.0

(C-3), 68.4 (C-5), 67.6 (C-6), 61.2 (C-4), 57.5 (3-OCH_3), 50.8 (C-1), 31.6 (4-NCH_3), and 27.4 (C-2); ^{1}H-n.m.r. see Table II.

Hydrolysis of the di-*N*-acetyl derivative of **13** (40 mg) gave **13** as a colorless solid (26 mg), $[\alpha]_D^{25}$ +97° (*c* 0.38, water) (lit.[6] $[\alpha]_D$ +75°).

Synthesis of istamycin B (**2**) *from* B_0 (**5**). — To a solution of **5** (hemicarbonate hemihydrate, 1.86 g, 5.0 mmol) in methanol (84 mL), was added zinc diacetate dihydrate (4.8 g, 22 mmol). After stirring overnight at room temperature, *N*-benzyloxycarbonyloxysuccinimide (4.44 g, 17.5 mmol) was added and the mixture was stirred overnight at room temperature. After further stirring for 4 h at pH 10–11 (adjusted with aqueous ammonia), the mixture was evaporated. A solution of the residue in chloroform (130 mL) was washed twice with M ammonium hydroxide (70 mL × 2) and twice with water (80 and 60 mL), and evaporated, yielding the tri-*N*-benzyloxycarbonyl derivative of **5** as a crude powder (3.92 g). The powder was dissolved in 1,4-dioxane (80 mL), and to the solution triethylamine (0.93 mL) and the *N*-hydroxysuccinimide ester (2.82 g, 9.2 mmol) of *N*-benzyloxycarbonylglycine were added. The solution was stirred overnight at 60° and evaporated. A solution of the residue in chloroform (100 mL) was washed twice with water (50 mL), dehydrated with anhydrous sodium sulfate, and evaporated, yielding a syrup (6.35 g). The syrup was dissolved in a mixture of methanol (30 mL), acetic acid (1.32 mL), and water (8 mL), and hydrogenated with 5% palladium-on-carbon (1.2 g) under a hydrogen stream at atmospheric pressure for 11 h. The catalyst was filtered off and the filtrate evaporated. A solution of the residue in water (60 mL) was adjusted to pH 8.0 with aqueous sodium hydroxide and passed through a column of Amberlite CG-50 (NH_4^+, 60 mL), which was then washed with water (60 mL) and 0.25M ammonium hydroxide (250 mL). The column was then eluted with 500 mL of 0.3M ammonium hydroxide (2.3-mL fractions). Fractions 52–160 were combined and evaporated, yielding 1.52 g (66%) of **2** as the carbonate hemihydrate. The synthetic **2** was identical with natural **2** in all respects.

Synthesis of istamycin C (**3**) *from* C_0 (**6**). — To a solution of **6** (hemicarbonate, 150 mg, 0.40 mmol) in methanol (4 mL) was added zinc diacetate dihydrate (366 mg, 1.68 mmol). The mixture was stirred overnight at room temperature and *N*-benzyloxycarbonyloxysuccinimide (306 mg, 1.24 mmol) was added. Stirring was continued overnight at room temperature and for 4 h at pH 11 (adjusted with aqueous ammonia) and the mixture was then evaporated. A solution of the residue in chloroform (30 mL) was washed with M ammonium hydroxide and then water, and evaporated to yield a crude powder (330 mg) of the tri-*N*-benzyloxycarbonyl derivative of **6**. The powder was dissolved in 1,4-dioxane (5 mL), and to the solution triethylamine (0.082 mL) and the *N*-hydroxysuccinimide ester of *N*-benzyloxycarbonylglycine (183 mg, 0.6 mmol) were added. The solution was stirred for 6 h at 55° and evaporated. The residue was dissolved in a mixture of methanol (6 mL), acetic acid (1 mL), and water (3 mL), and hydrogenated with 5% palladium-on-carbon under a hydrogen stream at atmospheric pressure for 3 h. After removal of the catalyst by filtration, the filtrate was evaporated (425 mg). A solution of the residue in water (4 mL) was

adjusted to pH 8.5 with aqueous ammonia, and passed through a column of Amberlite CG-50 (NH_4^+, 15 mL). The column was washed with 30 mL of water and 60 mL each of 0.1 and 0.2M ammonium hydroxide, and eluted with 60 mL of 0.3M ammonium hydroxide (2.5-mL fractions). Fractions 10–24 were combined and evaporated, yielding 92 mg (48%) of **3** as the carbonate hemihydrate. The synthetic **3** was identical with natural **3** in all respects.

Mild acid hydrolysis of istamycin A_1 (**7**), B_1 (**8**), *and* C_1 (**9**). — A solution of **8** (12 mg) in 0.5M hydrochloric acid (1 mL) was heated for 3 h at 30°. The solution was made neutral with M sodium hydroxide and passed through a column of Amberlite CG-50 (NH_4^+, 2.5 mL). The column was washed with water (10 mL) and then eluted with 0.4M ammonium hydroxide (13-mL fractions). Fractions 5–8 were combined and evaporated, yielding 7.2 mg (64%) of **2**. The identity of **2** was confirmed by p.e., t.l.c., and ^{1}H-n.m.r. spectroscopy.

Compounds **1** and **3** (1 mg of each) in the hydrolyzates of **7** and **9** with 0.5M hydrochloric acid (0.5 mL) were detected by p.e. and t.l.c.

Alkaline hydrolysis of istamycin A_2 (**10**). — A solution of **10** (12 mg) in 0.05M barium hydroxide (2 mL) was boiled for 1 h under reflux, and made neutral with carbon dioxide. The precipitate formed was removed by filtration and washed with water (2 mL). The combined filtrates were passed through a column of Amberlite CG-50 (NH_4^+, 5 mL). The column was washed and eluted with water (20 mL), 0.2M (40 mL), and 0.4M ammonium hydroxide (40 mL). The eluate was collected in 3-mL fractions. Fractions 2–3 were combined and evaporated, yielding the barium salt of hydantoic acid (15 mg), which was identified by t.l.c. on silica gel developed with 3:1:1 1-butanol–acetic acid–water (R_F 0.44). Fractions 24–29 were combined and evaporated, yielding 7.0 mg (84%) of **4**. The identity of **4** was confirmed by p.e., t.l.c., and by its ^{1}H-n.m.r. spectrum.

Synthesis of istamycin A_2 (**10**) *from* A_0 (**4**). — To a solution of **4** (monocarbonate, 300 mg, 0.76 mmol) in methanol (12 mL), triethylamine (0.18 mL) and *N*-benzyloxycarbonyloxysuccinimide (570 mg, 2.28 mmol) were added at 5°. The mixture was kept overnight at 5° and evaporated. A solution of the residue in chloroform (80 mL) was washed 3 times with water (35 mL) and evaporated, yielding as a crude powder (563 mg) the tri-*N*-benzyloxycarbonyl derivative of **4**. The powder (222 mg) was dissolved in *N*,*N*-dimethylformamide (6 mL), and to the solution triethylamine (0.065 mL) and the *N*-hydroxysuccinimide ester of hydantoic acid (96.8 mg, 0.45 mmol) were added. The mixture was stirred for 5 h at 60° and then evaporated. The residue was dissolved in a mixture of methanol (5 mL), acetic acid (0.5 mL), and water (1 mL), and hydrogenated with 5% palladium-on-carbon under a hydrogen stream at atmospheric pressure for 3 h. The catalyst was filtered off and the filtrate evaporated. A solution of the residue in water (6 mL) was made neutral with aqueous ammonia and passed through a column of Amberlite CG-50 (NH_4^+, 15 mL). The column was washed successively with 45 mL of water, 0.1 and 0.15M ammonium hydroxide, and then eluted with 90 mL of 0.2M ammonium hydroxide

(6-mL fractions). Fractions 3–7 were combined and evaporated, yielding 83 mg (59%) of **10**. The identity of **10** was confirmed by p.e., t.l.c., optical rotation, and by ^{1}H- and ^{13}C-n.m.r. spectra.

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