SYNTHESIS OF DEMETHYL DERIVATIVES OF ISTAMYCIN A

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4-N,6′-N,3-O-Tridemethylistamycin A_0 (**9**) and 6′-N,3-O-didemethylistamycin A_0 (**15**) were synthesized from 3,2′,6′-tri-N-benzyloxycarbonyl-3′,4′-dideoxyneamine 1,6-carbamate (**1**) through an aziridine derivative **6** by an analogous procedure employed in the total synthesis of istamycin A_0 (**19**). Acylation of **15** with glycine at the 4-methylamino group gave 6′-N,3-O-didemethylistamycin A (**18**) having interesting activities especially against pseudomonas, but 4-N,6′-N,3-O-tridemethylistamycin A (**12**) derived from **9** showed only weak activity. Therefore, the 4-N-methyl group of istamycin A (**20**) is essential for the antimicrobial activity.

Istamycins A and B which are produced by *Streptomyces tenjimariensis* are new members of fortimicin-group antibiotics[1]. Recently, we synthesized[2] istamycin A starting from 3′,4′-dideoxyneamine[3,4] by a 17-step procedure. The key step involved an aziridine formation for the stereospecific synthesis of the 1,4-diaminocyclitol moiety. We now wish to report an extension of the total synthesis, which gives useful derivatives having interesting properties. 4-N,6′-N,3-O-Tridemethylistamycin A and 6′-N,3-O-didemethylistamycin A have been synthesized by starting from 3,2′,6′-tri-N-benzyloxycarbonyl-3′,4′-dideoxyneamine 1,6-carbamate[5,6] (**1**) and by using analogous reactions employed in the total synthesis of istamycin A. It has been found that the latter is more active than istamycin A against pseudomonas, but the former has almost no antibacterial activity.

The 5-hydroxyl group of **1** was protected with the tetrahydropyranyl group to afford compound **2** and then the 1,6-cyclic carbamate of **2** was hydrolyzed in barium hydroxide solution to give the amino alcohol **3**. Protection of the 1-amino group in **3** with *tert*-butoxycarbonyl group followed by mesylation of the 6-hydroxyl group in compound **4** with methanesulfonyl chloride yielded compound **5**. Aziridine ring formation of **5** with sodium methoxide in anhydrous tetrahydrofuran gave compound **6** in an excellent yield. The conformation (**6**) of the aminocyclitol with aziridine ring can reasonably be shown by the ^{1}H NMR spectral analysis. The ring opening reaction of aziridine in **6** with sodium benzoate afforded the expected 1,4-diaminocyclitol derivative **7** in a good yield. The numbering of the 1,4-diaminocyclitol compounds is based upon that of fortamine[7]. The removal of the O-benzoyl group in **7** gave compound **8**. Simultaneous removal of N-benzyloxycarbonyl, N-*tert*-butoxycarbonyl and O-tetrahydropyranyl groups in **8** by catalytic hydrogenation in 90% trifluoroacetic acid afforded 4-N,6′-N,3-O-tridemethylistamycin A_0 (**9**) as its sesquicarbonate, which was purified by column chromatography on Amberlite CG-50 resin.

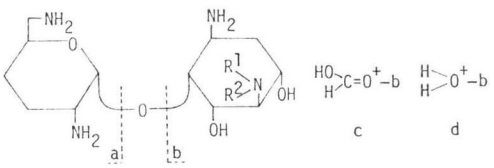
The 4-N-*tert*-butoxycarbonyl and 5-O-tetrahydropyranyl groups in **8** were removed by treatment with 90% trifluoroacetic acid to give 1,2′,6′-tri-N-benzyloxycarbonyl-4-N,6′-N,3-O-tridemethylistamycin A_0 (**10**). Acylation of the 4-amino group in **10** with N-hydroxysuccinimide ester of N-benzyloxycarbonylglycine followed by the removal of N-benzyloxycarbonyl groups in compound **11** afforded 4-N,6′-N,3-O-tridemethylistamycin A (**12**) as its sesquicarbonate, which was purified by column chromato-

graphy on Amberlite CG-50 resin.

The 4-amino group in **10** was acylated with ethoxycarbonyl group to give compound **13**. The removal of N-benzyloxycarbonyl groups in **13** followed by the reduction of the trifluoroacetate salt of compound **14** with diborane in tetrahydrofuran afforded 6′-N,3-O-didemethylistamycin A_0 (**15**) as its monocarbonate, which was purified by column chromatography on Amberlite CG-50 resin.

The amino groups of **15** were protected with benzyloxycarbonyl group to yield the 1,2′,6′-tri-N-benzyloxycarbonyl derivative **16**. Acylation of the 4-methylamino group in **16** with N-hydroxysuccinimide ester of N-benzyloxycarbonylglycine followed by the removal of the N-protective groups of compound **17** afforded 6′-N,3-O-didemethylistamycin A (**18**) as its monocarbonate, which was purified by column chromatography on Amberlite CG-50 resin.

Table 1. MS spectra (m/z) of 4-N,6′-N,3-O-tridemethylistamycins A_0 (**9**) and A (**12**), and 6′-N,3-O-didemethylistamycins A_0 (**15**) and A (**18**).



	9	12	15	18
R^1	H	Gly	H	Gly
R^2	H	H	CH_3	CH_3
$(M+1)^+$	291	348	305	362
M^+	290		304	
c	191	248	205	262
d	163	220	177	234
b	145	202	159	216
a	129	129	129	129

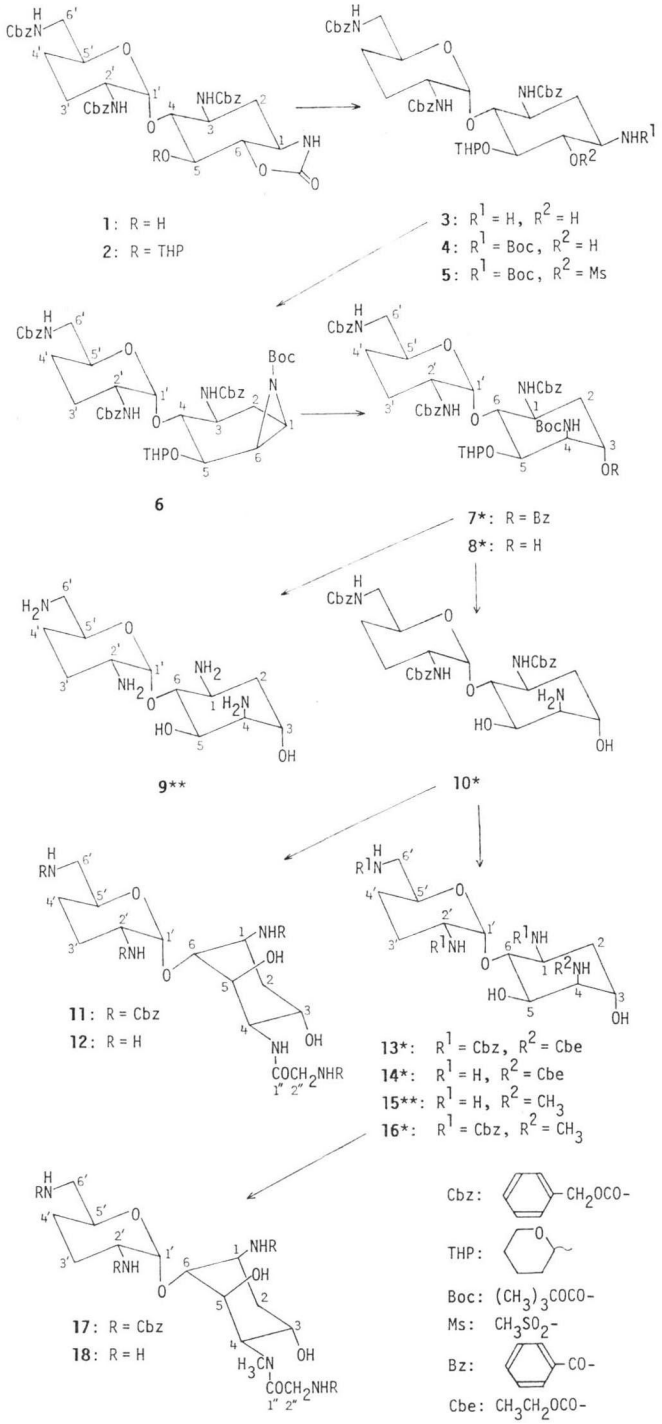
The structures of **9**, **12**, **15** and **18** were confirmed by their spectral analysis. Significant MS spectral fragmentations of these four compounds are shown in Table 1. The chemical shifts of FOURIER-transform ^{13}C NMR spectra of these compounds were assigned by comparing with those of istamycins A_0

Table 2. ^{13}C Chemical shifts (ppm) of 4-N,6′-N,3-O-tridemethylistamycins A_0 (**9**) and A (**12**), 6′-N,3-O-didemethylistamycins A_0 (**15**) and A (**18**), and istamycins A_0 (**19**) and A (**20**).

Carbon	**9** pD 4.8	**12** pD 2.0	**15** pD 2.0	**18** pD 1.0	**19** pD 5.0	**20** pD 5.4
1	49.3	49.4	49.3	49.4	49.2	49.4
2	31.9	32.0	31.9	33.3	28.3	29.2
3	62.4	64.0	62.2*	60.3	72.0	71.3
4	55.3	54.6	62.1*	57.8	61.2	56.5*
5	68.0	70.1	65.3	71.3	65.7	69.8
6	73.1	74.0	73.3	73.4	73.1	73.2
3-OCH_3					57.5	56.6*
4-NCH_3			31.4	31.9	31.6	31.9
1′	95.8	95.8	96.2	95.3	95.8	95.1
2′	50.7	50.6	50.4	51.0	50.1	50.7
3′	21.7	21.7	21.7	21.7	21.9	21.6
4′	26.5	26.7	26.5	26.6	26.8	26.8
5′	66.8	66.8	66.9	66.6	66.4	66.2
6′	43.5	43.5	43.5	43.5	53.0	52.9
6′-NCH_3					34.3	34.2
1″		168.3		168.8		168.7
2″		41.5		41.3		41.2

pD of sample in D_2O was adjusted with DCl. Similar values with asterisks within each column may be interchanged.

Scheme.



* Conformation is uncertain.

** Conformation of the 1,4-diaminocyclitol moiety in the protonated form is converted into another chair conformer.

(**19**) and A (**20**), as shown in Table 2. In NMR spectra of the protonated forms of **9** and **15**, the conformation of the 1,4-diaminocyclitol moiety was confirmed to be a 1,5,6-tri-axial chair conformer as similar to that of **19**.

Table 3. Minimum inhibitory concentrations (μg/ml) of 4-N,6′-N,3-O-tridemethylistamycin A (**12**), 6′-N,3-O-didemethylistamycin A (**18**) and istamycin A (**20**).

Test organism	12	18	20	Test organism	12	18	20
Staph. aureus FDA 209P	>100	1.56	0.78	*E. coli* JR 225[f]	>100	3.13	1.56
Staph. aureus Smith	12.5	<0.20	<0.20	*Kl. pneumoniae* PCI 602	>100	3.13	1.56
Staph. aureus Ap 01[a]	100	1.56	0.78	*Kl. pneumoniae* 22 # 3038[d,e]	>100	12.5	3.13
Staph. epidermidis 109[a]	100	1.56	0.78	*Sh. dysenteriae* JS 11910	>100	12.5	3.13
Micrococcus flavus FDA 16	>100	3.13	3.13	*Sh. flexneri* 4b JS 11811	>100	12.5	6.25
Sarcina lutea PCI 1001	>100	0.78	0.20	*Sh. sonnei* JS 11746	>100	6.25	6.25
B. anthracis	50	0.39	<0.20	*Sal. typhi* T-63	100	3.13	0.78
B. subtilis PCI 219	6.25	<0.20	<0.20	*Sal. enteritidis* 1891	>100	6.25	3.13
B. subtilis NRRL B-558	>100	<0.20	0.39	*Proteus vulgaris* OX 19	100	1.56	0.78
B. cereus ATCC 10702	>100	3.13	3.13	*Proteus rettgeri* GN 311	>100	50	25
Corynebact. bovis 1810	>100	1.56	1.56	*Proteus rettgeri* GN 466	>100	12.5	6.25
Myco. smegmatis ATCC 607	100	0.39	1.56	*Serratia marcescens*	>100	12.5	6.25
E. coli NIHJ	>100	6.25	3.13	*Serratia* sp. SOU	>100	>100	>100
E. coli K-12	>100	6.25	1.56	*Serratia* sp. 4[d]	>100	50	50
E. coli K-12 R 5[b]	>100	100	3.13	*Providencia* sp. Pv 16[g]	>100	12.5	6.25
E. coli K-12 R 388	>100	1.56	0.78	*Providencia* sp. 2991[g]	>100	12.5	25
E. coli K-12 J 5 R 11-2[c]	>100	3.13	3.13	*Ps. aeruginosa* A 3	>100	3.13	6.25
E. coli K-12 ML 1629[c]	>100	3.13	3.13	*Ps. aeruginosa* No. 12	>100	25	100
E. coli K-12 ML 1630	>100	12.5	3.13	*Ps. aeruginosa* H 9[e]	>100	12.5	25
E. coli K-12 ML 1410	>100	6.25	3.13	*Ps. aeruginosa* H 11	>100	50	100
E. coli K-12 ML 1410 R 81[c]	>100	3.13	1.56	*Ps. aeruginosa* TI-13[c]	>100	12.5	25
E. coli K-12 LA 290 R 55[d]	>100	12.5	3.13	*Ps. aeruginosa* GN 315[b]	>100	25	25
E. coli K-12 LA 290 R 56	>100	3.13	1.56	*Ps. aeruginosa* 99[f]	>100	>100	>100
E. coli K-12 LA 290 R 64	>100	3.13	1.56	*Ps. aeruginosa* B-13[c,e]	>100	>100	>100
E. coli W 677	>100	3.13	1.56	*Ps. aeruginosa* 21-75[h]	>100	>100	>100
E. coli JR 66/W 677[d,e]	>100	6.25	3.13	*Ps. aeruginosa* PSTI[f]	>100	50	100
E. coli K-12 C 600 R 135[f]	>100	>100	>100	*Ps. aeruginosa* ROS 134/PU 21[f]	>100	50	100
				Ps. aeruginosa K-Ps 102[i]	>100	12.5	50
				Ps. aeruginosa GN 907[i]	>100	>100	>100

Resistance mechanisms: a) ADD (4′), b) AAC (6′), c) APH (3′)-I, d) AAD (2″), e) APH (3′)-II, f) AAC (3), g) AAC (2′), h) APH (3′)-III, i) permeability.

As shown in Table 3, the minimum inhibitory concentrations of 4-N,6′-N,3-O-tridemethylistamycin A (**12**) and 6′-N,3-O-didemethylistamycin A (**18**) were tested and compared with those of istamycin A (**20**). Compound **12** was almost inactive against all of the microorganisms tested, but compound **18** is more active than **20** against pseudomonas strains. This indicates that the 4-N-methyl group is essential for the antimicrobial activity of istamycin A.

Experimental

General

Melting points were determined in capillary tubes and uncorrected. Optical rotations were measured with a Carl Zeiss LEP A2 polarimeter. ^1H and ^{13}C NMR spectra were obtained on a Varian XL-100 spectrometer. Chemical shifts of ^1H NMR in D_2O were recorded in ppm using tetramethylsilane as an external reference. All chemical shifts of ^1H NMR in other solvents were recorded in ppm downfield from internal tetramethylsilane. Chemical shifts of ^{13}C NMR in D_2O were measured by using dioxane (67.4 ppm) as an internal reference. Mass spectra were recorded with a Hitachi RMU-6M mass spectrometer. Wako gel C-200 (Wako Pure Chemical Industries, Ltd.) was used for column chromatography on silica gel. Preparative TLC was performed on a silica gel plate (Art. 5744 of E. Merck).

3,2′,6′-Tri-N-benzyloxycarbonyl-3′,4′-dideoxy-5-O-tetrahydropyranylneamine 1,6-carbamate (2)

According to the method of UMEZAWA *et al.*[5,6], 3,2′,6′-tri-N-benzyloxycarbonyl-3′,4′-dideoxyneamine 1,6-carbamate (**1**) was derived from 3′,4′-dideoxyneamine. To a solution of **1** (3.7 g) in anhydrous N,N-dimethylformamide (50 ml) were added 3,4-dihydro-2*H*-pyran (1.3 g, 3 equiv.) and anhydrous *p*-toluenesulfonic acid (196 mg, 0.2 equiv.) at room temperature with stirring. After 72 hours, triethylamine (1 ml) was added to the solution and then the solution was concentrated under reduced pressure to give an oil. The oil was chromatographed on a silica gel column (chloroform - ethanol, 100: 1) to give tetrahydropyranyl ether **2** (2.4 g, 57%), $[\alpha]_D^{22}+33°$ (*c* 0.5, chloroform).

Anal. Calcd for $C_{42}H_{50}N_4O_{12}$: C, 62.83; H, 6.28; N, 6.98.
Found: C, 62.94; H, 6.68; N, 6.16.

3,2′,6′-Tri-N-benzyloxycarbonyl-1-N-*tert*-butoxycarbonyl-3′,4′-dideoxy-6-O-methanesulfonyl-5-O-tetrahydropyranylneamine (5)

To a solution of **2** (2.3 g) in dioxane (80 ml) was added 0.05 M aqueous barium hydroxide solution (25 ml). The mixture was stirred at 60°C for 30 minutes. To the resulting neutral solution, additional aliquots of the barium hydroxide solution (30 and 30 ml) were added at intervals of 30 minutes. Carbon dioxide was introduced into the mixture and after filtration the solution was concentrated to give a colorless solid of **3** (2.2 g).

To a solution of **3** in methanol (50 ml) were added triethylamine (0.4 ml) and *tert*-butyl S-4,6-dimethylpyrimid-2-ylthiolcarbonate (1.35 g, 2 equiv.) with stirring at room temperature. The solution was stirred overnight, then concentrated under reduced pressure to give an oil. The oil was triturated with a mixture of toluene and *n*-hexane to afford an yellow solid. The solid was washed with water and dried to give **4**.

A solution of **4** and methanesulfonyl chloride (574 mg, 2 equiv.) in pyridine (50 ml) was allowed to stand overnight. The reaction mixture was concentrated to dryness and the residue was dissolved in chloroform. The solution was washed with potassium hydrogen sulfate solution, sodium hydrogen carbonate solution and water, successively, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with toluene - acetone (5: 1) to give the mesylate **5** (1.7 g, 61% from **2**), $[\alpha]_D^{22}+29°$ (*c* 0.44, chloroform). ^1H NMR ($CDCl_3$): δ 1.43 (Boc), 3.04 (OMs).

Anal. Calcd for $C_{47}H_{62}N_4O_{15}S$: C, 59.11; H, 6.54; N, 5.87; S, 3.36.
Found: C, 59.49; H, 6.57; N, 5.95; S, 3.12.

Preparation of the aziridine derivative 6

To a solution of **5** (1.37 g) in anhydrous tetrahydrofuran (55 ml) was added sodium methoxide (194 mg, 2.5 equiv.) with stirring at 0°C under argon atmosphere. After 30 minutes at 0°C, the mixture was stirred at 10°C for 15 hours. Excess amounts of ammonium chloride and water were added to the mixture and the mixture was concentrated to give a solid. The solid was dissolved in chloroform and the solution was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford an aziridine derivative **6** (1.23 g, quantitatively), $[\alpha]_D^{22}+10°$ (*c* 0.5, chloroform). ^1H NMR ($CDCl_3$); δ 1.44 (Boc), 2.74 (H-1, broad t, $J_{1,2eq}=J_{1,6}$ 5.5 Hz), 2.92 (H-6, dd, $J_{1,6}$ 5.5 and $J_{5,6}$ 4.0 Hz).

Anal. Calcd for $C_{46}H_{58}N_4O_{12}$: C, 64.32; H, 6.81; N, 6.52.
Found: C, 64.51; H, 6.89; N, 6.49.

Aziridine ring opening of **6**

To a solution of **6** (1.12 g) in N,N-dimethylformamide (50 ml) was added sodium benzoate (1.3 g). The mixture was stirred at 105°C for 10 hours and diluted with chloroform (300 ml). The mixture was washed with a brine and the organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford a light brown syrup. The syrup was chromatographed on a column of silica gel with toluene - ethyl acetate (5: 2) to yield a solid of the benzoate **7** (878 mg, 76%), $[\alpha]_D^{22}+61°$ (*c* 0.5, chloroform). ^1H NMR ($CDCl_3$); δ 1.28 (Boc), 7.4 and 8.1 (C_6H_5CO).

Anal. Calcd for $C_{53}H_{64}N_4O_{14}$: C, 64.88; H, 6.58; N, 5.71.
Found: C, 65.15; H, 6.59; N, 5.50.

De-O-benzoylation of **7**

A solution of **7** (860 mg) in 12% methanolic ammonia (20 ml) was kept at room temperature for 40 hours. The solution was concentrated under reduced pressure to give a residue. The residue was chromatographed on a column of silica gel with benzene - ethyl acetate (1: 1) to afford compound **8** (693 mg, 90%), $[\alpha]_D^{22}+44°$ (*c* 0.5, chloroform).

Anal. Calcd for $C_{46}H_{60}N_4O_{13}$: C, 63.00; H, 6.90; N, 6.39.
Found: C, 63.66; H, 6.90; N, 6.50.

4-N,6′-N,3-O-Tridemethylistamycin A_0 (**9**)

A solution of **8** (90 mg) in 90% trifluoroacetic acid (2 ml) was hydrogenated with 5% palladium on charcoal (100 mg) under atmospheric pressure for 4 hours. The catalyst was removed by filtration and the solution was concentrated under reduced pressure to give a glassy solid, which was dissolved in water (10 ml). The pH of the solution was adjusted to 9 with 1 M aqueous ammonia and the solution was then chromatographed on a column of Amberlite CG-50 (NH_4^+, 10 ml). The column was washed with water, 0.2 and 0.4 M aqueous ammonia, successively, and then eluted with 0.5 M aqueous ammonia. Fractions containing the product were combined and evaporated under reduced pressure to give the tridemethylistamycin A_0 (**9**) as a sesquicarbonate (29 mg, 74%), mp 140~145°C (decomp), $[\alpha]_D^{22}+82°$ (*c* 1.2, water).

Anal. Calcd for $C_{12}H_{26}N_4O_4\cdot\frac{3}{2}H_2CO_3$: C, 42.29; H, 7.62; N, 14.61.
Found: C, 42.24; H, 7.00; N, 14.54.

4-N,6′-N,3-O-Tridemethylistamycin A (**12**)

Compound **8** (680 mg) was dissolved in 90% trifluoroacetic acid (10 ml) at 0°C and the solution was kept at that temperature for 2 hours, then concentrated to a brown oil under reduced pressure. The oil was dissolved in chloroform (50 ml) and the solution was washed with 1 N sodium hydroxide and water, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford an yellow solid. The solid was chromatographed on a column of silica gel with chloroform - methanol - 17% ammonium hydroxide (80: 10: 1) to give a colorless solid **10** (350 mg, 65%).

A solution of **10** (48 mg), triethylamine and N-(N-benzyloxycarbonylglycyloxy)succinimide (25 mg, 1.2 equiv.) in dioxane was stirred overnight at room temperature and the solution was concentrated to give a solid. The solid was purified by preparative TLC (chloroform - ethanol, 10: 1) to afford compound **11** (35 mg, 57%).

A solution of **11** (35 mg) in a mixture of acetic acid, methanol and water (2: 1: 1, 4 ml) was hydrogenated with 5% palladium on charcoal (50 mg) under atmospheric pressure for 3 hours. The catalyst was filtered off and the solution was concentrated to give a solid. The solid was chromatographed on a column of Amberlite CG-50 (NH_4^+, 5 ml). The column was washed with water and 0.2 M aqueous ammonia, and eluted with 0.4 M aqueous ammonia. Fractions containing the product were combined and evaporated under reduced pressure to yield the tridemethylistamycin A (**12**, 16 mg, 92%) as a sesquicarbonate, mp 155~158°C (decomp), $[\alpha]_D^{22}+94°$ (*c* 0.61, water).

Anal. Calcd for $C_{14}H_{29}N_5O_5\cdot\frac{3}{2}H_2CO_3$: C, 42.26; H, 7.32; N, 15.90.
Found: C, 42.60; H, 7.10; N, 16.27.

6′-N,3-O-Didemethylistamycin A_0 (**15**)

To a solution of **10** (293 mg) in methanol (5 ml) was added a solution of sodium carbonate (27 mg) in water (0.5 ml) at room temperature. The resulting mixture was cooled at 0°C and to this was added ethyl chloroformate (55 mg, 1.2 equiv.) with stirring. After stirring for 2 hours, the mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in chloroform (30 ml) and the solution was washed with water, dried over anhydrous sodium sulfate and evaporated to afford the tri-N-benzyloxycarbonyl-mono-N-ethoxycarbonyl derivative **13** (318 mg, 98%).

A solution of **13** in a mixture of methanol, water and acetic acid (2: 1: 1, 8 ml) was hydrogenated with 5% palladium on charcoal (100 mg) under atmospheric pressure for 2 hours. After filtration to remove the catalyst, the solution was concentrated under reduced pressure. The residue was chromatographed on a column of Amberlite CG-50 (NH_4^+, 11 ml). The column was washed with water and eluted with 0.2 M aqueous ammonia. Fractions containing the product were combined and evaporated to afford 6′-N,3-O-didemethyl-4-N-ethoxycarbonylistamycin A_0 (**14**, 143 mg). Compound **14** (130 mg) was dissolved in anhydrous trifluoroacetic acid (6 ml) at 0°C. The excess acid was removed by concentration to dryness under high vacuum at room temperature for 3 hours to yield a glassy solid of the trifluoroacetate. This salt was dissolved in anhydrous tetrahydrofuran (3 ml) and a 1 M solution of diborane in tetrahydrofuran (15 ml) was added under argon atmosphere. The resulting solution was heated at 50°C with stirring for 18 hours. The excess diborane was decomposed by the addition of water and the solution was concentrated under reduced pressure to give a residue, which was taken up in methanol (10 ml). The mixture was again evaporated to remove boric acid. The residue was dissolved in water (10 ml) and acidified with 1 N hydrochloric acid to pH 4. The pH of the solution was adjusted to 9 with 1 M aqueous ammonia. The solution was chromatographed on a column of Amberlite CG-50 (NH_4^+, 30 ml). The column was washed with water, 0.2 and 0.4 M aqueous ammonia, successively, and then eluted with 0.5 M aqueous ammonia. Fractions containing the product were combined and evaporated under reduced pressure to afford 6′-N,3-O-didemethylistamycin A_0 (**15**) as a monocarbonate (45 mg, 36% from **13**), mp 115~118°C (decomp.), $[\alpha]_D^{22}$ +102° (*c* 0.65, water).

Anal. Calcd for $C_{13}H_{28}N_4O_4 \cdot H_2CO_3$: C, 45.89; H, 8.25; N, 15.29.
Found: C, 46.15; H, 7.97; N, 15.37.

In this resin chromatography, compound **14** (44 mg) was recovered.

6′-N,3-O-Didemethylistamycin A (**18**)

To a solution of **15** (45 mg) in methanol (3 ml) was added triethylamine and N-benzyloxycarbonyloxysuccinimide (95 mg, 3.1 equiv.) and the solution was allowed to stand for 6.5 hours at room temperature, then concentrated under reduced pressure to give an oil of **16**. The oil was dissolved in dioxane (3 ml) and to this was added N-(N-benzyloxycarbonylglycyloxy)succinimide (68 mg, 1.5 equiv.) and the solution was warmed at 60°C for 6 hours. After concentration of the solution, the residue was dissolved in a mixture (4 ml) of acetic acid, methanol and water (2: 1: 1) and hydrogenated with 5% palladium on charcoal (50 mg) under atmospheric pressure for 4 hours. The catalyst was removed by filtration and the filtrate was evaporated to give a colorless solid. The solid was purified by ion-exchange chromatography on Amberlite CG-50 (NH_4^+, 6 ml). The column was washed with water and 0.2 M aqueous ammonia, and then eluted with 0.4 M aqueous ammonia. Fractions containing the product were combined and evaporated to afford 6′-N,3-O-didemethylistamycin A (**18**) as a monocarbonate (15 mg, 30%), mp 138~145°C (decomp.), $[\alpha]_D^{22}$ +136° (*c* 0.36, water).

Anal. Calcd for $C_{15}H_{31}N_5O_5 \cdot H_2CO_3$: C, 45.38; H, 7.85; N, 16.54.
Found: C, 45.39; H, 7.63; N, 16.43.

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