SOME CHEMICAL MODIFICATIONS OF ISTAMYCIN B

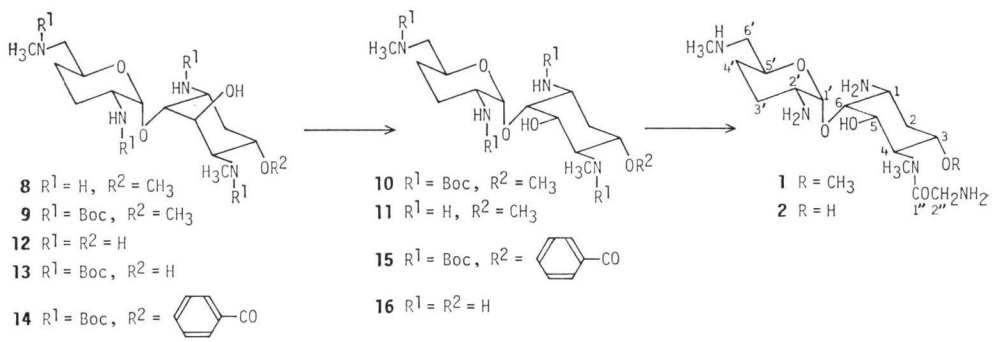
Sir:

As reported in our previous paper,[1] 6′-*N*,3-*O*-didemethylistamycin A which was synthesized starting from 3′,4′-dideoxyneamine through an aziridine intermediate showed stronger activity than istamycin A against pseudomonas strains. 3-*O*-Demethylistamycin B and its 2″-*N*-formimidoyl derivative synthesized from istamycin B_0 also exhibited excellent activities against Gram-positive and -negative bacteria including pseudomonas.[2] In this communication, we wish to report some chemical modifications of istamycin B at the 3- and 5-positions. These include the synthesis and activity of 5-*epi* (**1**), 3-*O*-demethyl-5-*epi* (**2**), 3-*O*-demethyl-3-*epi* (**3**), 3-demethoxy (**4**) and 3-demethoxy-2″-*N*-formimidoyl (**5**) derivatives of istamycin B.* Compounds **4** and **5** are more active than istamycin B (**6**) or 3-*O*-demethylistamycin B (**7**).

The free amino and methylamino groups of istamycin B_0 (**8**) were protected with *tert*-butoxycarbonyl (Boc) groups by reaction with *tert*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate (Kokusan Chemical Works) in methanol to yield 1,4,2′,6′-tetra-*N*-Boc-istamycin B_0 (**9**, $C_{35}H_{64}N_4O_{12}$), in quantitative yield, mp 101~112°C (decomp.), $[\alpha]_D^{22}$ +100° (*c* 0.67, methanol).

Epimerization of the 5-hydroxyl group of **9** by oxidation with dimethyl sulfoxide, trifluoroacetic anhydride and triethylamine in dichloromethane at −70°C under an argon atmosphere, followed by sodium borohydride reduction in methanol gave preferentially 1,4,2′,6′-tetra-*N*-Boc-5-*epi*-istamycin B_0 (**10**) in 83% yield. Removal of the Boc groups of **10** with 90% trifluoroacetic acid, followed by column chromatography on Amberlite CG-50 (NH_4^+) afforded 5-*epi*-istamycin B_0 (**11**) as the hemicarbonate ($C_{15}H_{32}N_4O_4 \cdot \frac{1}{2}H_2CO_3$), in 90% yield, $[\alpha]_D^{21}$ +89° (*c* 0.87, water), EI-MS: *m/z* 332 (M^+). The structure of **11** was confirmed by its ^1H NMR spectrum (Table 1). 5-*Epi*-istamycin B (**1**) was synthesized in 38% yield from **11** by the selective 1,2′,6′-tri-*N*-protection of **11** with 2-(Boc-oxyimino)-2-phenylacetonitrile (Boc-ON, Aldrich, 3 equiv.) in the presence of $Ni(OAc)_2 \cdot 4H_2O$ (2 equiv.), acylation with the *N*-hydroxysuccinimide ester of *N*-Boc-glycine (2 equiv.) in dioxane at 50°C, followed by removal of the Boc groups with 90% trifluoroacetic acid. By column chromatography on Amberlite CG-50 (NH_4^+) eluted with 0.2~0.4 M NH_4OH, **1** was obtained as the sesquicarbonate ($C_{17}H_{35}N_5O_5 \cdot \frac{3}{2}H_2CO_3$), mp 110°C (decomp.), $[\alpha]_D^{18}$ +68° (*c* 0.3, water), EI-MS: *m/z* 389 (M^+).

Protection of the amino and methylamino groups of 3-*O*-demethylistamycin B_0[2] (**12**) with the Boc group gave 1,4,2′,6′-tetra-*N*-Boc-3-*O*-demethylistamycin B_0 [**13**, $C_{34}H_{62}N_4O_{12} \cdot H_2O$, mp 120~122°C (decomp.), $[\alpha]_D^{23}$ +76° (*c* 1, chloroform)] in 93% yield. Preferential *O*-acylation of **13** with benzoyl chloride (2 equiv.) in pyridine at room temperature for 6 hours yielded the 3-*O*-benzoyl derivative **14** in 96% yield. 3-*O*-Demethyl-5-*epi*-istamycin B_0 [**16**, $C_{14}H_{30}N_4O_4 \cdot \frac{3}{2}H_2CO_3$, mp 80~100°C (decomp.), $[\alpha]_D^{21}$ +76° (*c* 0.23, water), EI-MS: *m/z* 318 (M^+), 69% yield from **14** through **15**] and 3-*O*-demethyl-5-*epi*-istamycin B [**2**, $C_{16}H_{33}N_5O_5 \cdot H_2CO_3$, mp 105°C (decomp.), $[\alpha]_D^{18}$ +67° (*c* 0.26, water), EI-MS: *m/z* 375 (M^+), 82% yield from **16**] were synthesized by the same method described for the

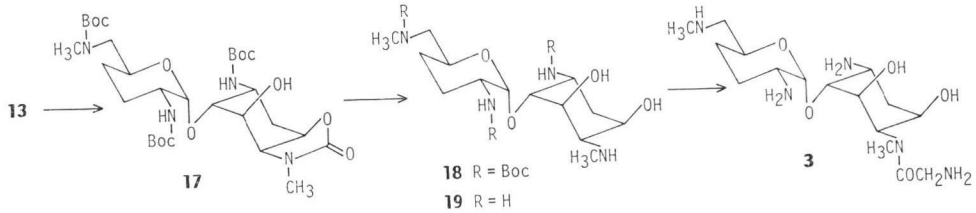


* Satisfactory elemental analyses and reasonable NMR spectral data were obtained for all the compounds cited in this report.

Table 1. Significant ^1H NMR chemical shifts and coupling constants of compounds **11**, **16** and **19**.

Proton	**11** (pD 3)		**16** (pD 2)		**19** (pD 3)	
	δ, ppm	J, Hz	δ, ppm	J, Hz	δ, ppm	J, Hz
1	~4.1		~4.1		4.64	11.2, 6.3, 3.4
2a	2.38	12, 12, 10.5	~2.3		2.76	14.2, 11.2, 3.2
2e	3.09	12, 4.5, 4, <1	2.84	11, 5, 4, <1	2.71	14.2, 6.3, 3.2, 1
3	4.28	10.5, 10, 4.5	4.57	11, 10.5, 5	4.99	3.9, 3.2, 3.2, 1.2
4	~4.2		~4.2		4.04	3.9, 3.7
5	4.72	11, 2.8	4.70	11, 2.8	5.14	3.7, 3.4, 1.2
6	5.07	2.8, 2.8, <1	5.04	2.8, 2.8, <1	4.91	3.4, 3.4, 1
1′	6.23	3.5	6.22	3.5	6.01	3.7
OMe	4.02					
NMe	3.31		3.31		3.34	
	3.29		3.29		3.29	

The ^1H NMR spectra were determined in D_2O and TMS was used as the external reference.



synthesis of **11** and **1**. The structure of **16** was confirmed by its ^1H NMR spectrum (Table 1).

Treatment of **13** with *p*-toluenesulfonyl chloride (3 equiv.) in pyridine at 100°C for 3 hours gave 1, 2′,6′-tri-*N*-Boc-4-*N*,3-*O*-carbonyl-3-*O*-demethyl-3-*epi*-istamycin B_0 (**17**, IR: 1745 cm^{-1}) in 93% yield. Treatment of **17** with $Ba(OH)_2 \cdot 8H_2O$ (2 equiv.) in 50% aqueous dioxane at 100°C for 2 hours gave 1,2′,6′-tri-*N*-Boc-3-*O*-demethyl-3-*epi*-istamycin B_0 (**18**) in 78% yield. Compound **18** was acylated with the *N*-hydroxysuccinimide ester of *N*-Boc-glycine (1.5 equiv.) in dioxane at 60°C for 16 hours and then the Boc groups were removed with 90% trifluoroacetic acid to yield 3-*O*-demethyl-3-*epi*-istamycin B (**3**, 25% yield) as the disulfate trihydrate $[C_{16}H_{33}N_5O_5 \cdot 2H_2SO_4 \cdot 3H_2O$, mp 209~231°C (decomp.) $[\alpha]_D^{20}+59°$ (*c* 1, water), EI-MS: *m/z* 375 $(M^+)]$, which was purified by column chromatography on carbon and Amberlite IRA-400 (SO_4^{2-}). Compound **3** in alkaline solution was readily changed into 3-*O*-demethyl-3-*epi*-istamycin B_0 (**19**), $[\alpha]_D^{20}+91°$ (*c* 1, water), EI-MS: *m/z* 318 (M^+). The structure of **19** was confirmed by its ^1H NMR spectrum (Table 1).

1,2′,6′-Tri-*N*-Boc-*O*-demethylistamycin B_0[2] (**20**) which was derived from **12** was treated with carbonyldiimidazole (1.1 equiv.) in toluene at 60°C for 2.5 hours to afford the 4,5-carbamate (**21**, IR: 1755 cm^{-1}) in 81% yield. Chlorination of **21** with sulfuryl chloride (3 equiv.) in pyridine at −30°C for 10 minutes and then at −10°C for 3.5 hours, followed by reduction with tributylstannane in toluene under an argon atmosphere in the presence of α,α'-azobisisobutyronitrile at 120°C for 3 hours afforded the 3-demethoxy

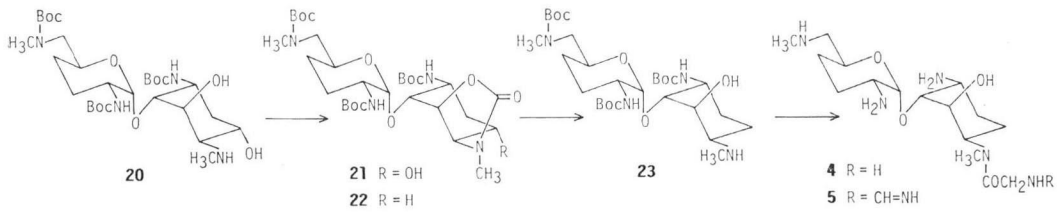


Table 2. Minimum inhibitory concentrations on Mueller-Hinton agar plates.

Test organism	Minimum inhibitory concentrations*, μg/ml						
	1 $\frac{3}{2}H_2CO_3$	2 H_2CO_3	3 $2H_2SO_4 \cdot 3H_2O$	4 $2H_2O$	5 $2H_2SO_4 \cdot 4H_2O$	6 $\frac{1}{2}H_2CO_3 \cdot \frac{1}{2}H_2O$	7 $\frac{3}{2}H_2CO_3$
Staphylococcus aureus 209P	1.56	1.56	0.78	<0.20	0.39	0.39	0.39
S. aureus Smith	0.78	0.78	0.39	0.20	<0.20	0.39	0.39
S. aureus Ap01[a]	3.13	3.13	1.56	0.39	0.39	0.78	0.39
S. epidermidis 109[a]	1.56	0.78	0.78	<0.20	0.20	0.39	0.39
Micrococcus flavus FDA16	6.25	6.25	12.5	3.13	0.39	12.5	3.13
M. luteus PCI1001	1.56	1.56	0.78	<0.20	0.39	0.39	0.39
Bacillus anthracis	1.56	1.56	0.78	0.39	0.39	0.78	0.78
B. subtilis PCI219	0.78	0.78	0.39	<0.20	0.20	0.39	0.39
B. subtilis NRRL B-558	0.78	0.78	0.78	<0.20	<0.20	0.39	0.39
B. cereus ATCC10702	6.25	12.5	3.13	1.56	1.56	6.25	1.56
Corynebacterium bovis 1810	1.56	1.56	0.39	<0.20	<0.20	0.39	0.39
Mycobacterium smegmatis ATCC607	3.13	3.13	1.56	0.39	0.39	0.78	0.78
Escherichia coli NIHJ	1.56	1.56	0.78	0.39	0.20	0.39	0.39
E. coli K-12	1.56	1.56	1.56	0.78	0.39	0.78	0.78
E. coli K-12 R5[b]	3.13	6.25	3.13	1.56	0.78	1.56	3.13
E. coli K-12 R388	1.56	3.13	1.56	0.39	0.39	0.78	0.78
E. coli K-12 J5R11-2[e]	3.13	6.25	6.25	0.78	0.78	1.56	1.56
E. coli K-12 ML1629[e]	3.13	3.13	3.13	0.78	0.39	1.56	1.56
E. coli K-12 ML1630	3.13	12.5	6.25	3.13	1.56	3.13	3.13
E. coli K-12 ML1410	3.13	6.25	3.13	1.56	1.56	3.13	3.13
E. coli K-12 ML1410 R81[e]	3.13	6.25	3.13	0.78	0.39	1.56	1.56
E. coli K-12 LA290 R55[d]	3.13	3.13	1.56	1.56	0.78	1.56	0.78
E. coli K-12 LA290 R56	1.56	3.13	1.56	0.39	0.39	1.56	0.78
E. coli K-12 LA290 R64	3.13	6.25	3.13	0.78	0.78	1.56	1.56
E. coli W677	1.56	3.13	0.78	0.39	0.39	0.78	0.78
E. coli JR66/W677[d,e]	3.13	6.25	6.25	0.78	0.39	1.56	1.56
E. coli K-12 C600 R135[f]	3.13	3.13	1.56	0.78	6.25	12.5	1.56
E. coli JR225[f]	1.56	3.13	1.56	0.78	0.39	1.56	0.78
Klebsiella pneumoniae PCI602	3.13	3.13	1.56	0.78	0.78	3.13	1.56
K. pneumoniae 22#3038[d,e]	6.25	6.25	6.25	1.56	1.56	1.56	3.13
Shigella dysenteriae JS11910	6.25	6.25	6.25	1.56	1.56	3.13	3.13
S. flexneri 4b JS11811	6.25	6.25	6.25	1.56	1.56	3.13	3.13
S. sonnei JS11746	3.13	6.25	3.13	1.56	1.56	0.78	1.56
Salmonella typhi T-63	0.78	0.78	0.78	<0.20	0.39	0.39	0.39
S. enteritidis 1891	3.13	6.25	3.13	1.56	1.56	3.13	1.56
Proteus vulgaris OX19	1.56	1.56	1.56	0.39	<0.20	0.39	0.39
P. rettgeri GN311	0.78	1.56	0.78	0.39	0.39	0.78	0.39
P. rettgeri GN466	0.78	1.56	0.78	0.39	<0.20	0.78	0.39
P. inconstans Pv16[g]	3.13	3.13	1.56	0.78	0.78	1.56	0.78
P. inconstans 2991[g]	12.5	6.25	3.13	1.56	1.56	3.13	1.56
Serratia marcescens	6.25	6.25	1.56	0.78	0.78	1.56	1.56
Serratia sp. SOU	6.25	6.25	3.13	3.13	12.5	25	6.25
Serratia sp. 4	100	25	25	12.5	12.5	50	12.5
Pseudomonas aeruginosa A3	0.78	1.56	<0.20	0.39	0.39	1.56	0.20
P. aeruginosa No. 12	100	50	50	12.5	25	50	12.5
P. aeruginosa H9[e]	100	50	25	12.5	25	50	12.5
P. aeruginosa H11	>100	50	100	25	50	100	25
P. aeruginosa TI-13[e]	50	12.5	12.5	6.25	12.5	50	6.25
P. aeruginosa GN315[b]	50	25	12.5	6.25	50	50	6.25
P. aeruginosa 99[f]	>100	50	100	25	>100	100	25
P. aeruginosa B-13[e,e]	>100	100	50	50	>100	>100	25
P. aeruginosa 21-75[h]	>100	100	100	50	50	100	25
P. aeruginosa PSTI[f]	100	50	50	12.5	25	50	12.5
P. aeruginosa ROS134/PU21[f]	>100	100	>100	50	100	>100	50
P. aeruginosa K-Ps102[i]	100	25	25	12.5	25	50	6.25
P. maltophilia GN907[i]	>100	>100	>100	100	>100	100	100

Resistance: a) AAD(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.

* Concentrations are shown as the free bases.

derivative **22** in 92% yield. Treatment of **22** with $Ba(OH)_2 \cdot 8H_2O$ in an aqueous dioxane solution at 100°C overnight gave 1,2′,6′-tri-*N*-Boc-3-demethoxyistamycin B_0 (**23**) in 90% yield, mp 131~135°C, $[\alpha]_D^{20}$ +70° (*c* 0.3, chloroform).

Acylation of **23** with the *N*-hydroxysuccinimide ester of *N*-Boc-glycine, removal of the Boc groups with 90% trifluoroacetic acid, followed by column chromatography on Amberlite CG-50 (NH_4^+) gave 3-demethoxyistamycin B (**4**) as the dihydrate ($C_{16}H_{33}N_5O_4 \cdot 2H_2O$) in 49% yield, mp 88~92°C, (decomp.), $[\alpha]_D^{24}$ +147° (*c* 0.35, water), EI-MS: *m/z* 359 (M^+).

3-Demethoxy-2″-*N*-formimidoylistamycin B (**5**) was synthesized from **23** by acylation with the *N*-hydroxysuccinimide ester of *N*-benzyloxycarbonylglycine, catalytic hydrogenation with 5% Pd-C, treatment with ethylformimidate hydrochloride[8] in ethanol, followed by removal of the Boc groups with 90% trifluoroacetic acid. Purification by column chromatography on Amberlite IRA-400 (SO_4^{2-}) and carbon gave **5**, obtained as the disulfate tetrahydrate ($C_{17}H_{34}N_6O_4 \cdot 2H_2SO_4 \cdot 4H_2O$, 15% yield from **23**), mp 201~220°C (decomp.), $[\alpha]_D^{24}$ +89° (*c* 0.5, water), SIMS (glycerol matrix): *m/z* 583 [$(MH+2H_2SO_4)^+$], 485 [$(MH+H_2SO_4)^+$], 387 (MH^+).

The minimum inhibitory concentrations of compounds **1**~**5** were tested on a Mueller-Hinton agar medium and compared with those of **6** and **7**. As shown in Table 2, all these derivatives have good activity. Compounds **4** and **5** are more active than **6** and **7** against Gram-positive and -negative bacteria, but not pseudomonas strains. The strong antibacterial activity of **4** indicates a predominant role of the amino groups in the antibacterial action as has been observed in the case of hexadeoxykanamycin.[4]

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