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Deoxysugar Synthesis. IV.[1] Deoxygenation of Aminoglycoside Antibiotics through Reduction of Their Dithiocarbonates

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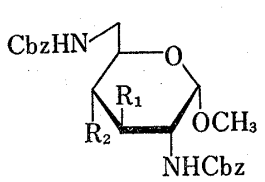
3-Dithiocarbonates (**1d**, **1e**, **3a**) and 3,4-bis analog (**1b**) derived from methyl 2,6-dibenzyloxycarbonylamino-2,6-dideoxy-α-D-glucopyranoside (**1a**) were converted into 3-deoxy compounds (**1c**, **1f**, **3b**) and 3-eno compound (**2**) respectively in good yield on treatment with tributylstannane. This reaction process was extended into the field of aminoglycoside antibiotics, giving 5-deoxykanamycin B (**8a**), 3′,4′,5-trideoxykanamycin B (**8c**), 3′,4′,5-trideoxy-3′-enokanamycin B (**8b**), 6-deoxybutirosin A (**9a**), and 3′-deoxybutirosin A (**9b**) whose antibacterial activities were discussed. Structures of these deoxysugars were confirmed by carbon-13 nuclear magnetic resonance spectrometry.

Keywords——aminoglycoside antibiotics; kanamycin; butirosin; deoxysugar; dithiocarbonate; tributylstannane; ^{13}C-NMR; deoxygenation

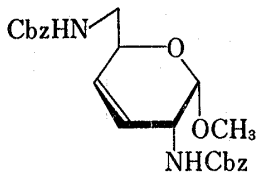
Various synthetic routes have been studied to replace sugar hydroxy groups by hydrogens. However, most of these deoxygenation reactions involved the S_N2 mechanism and ran into difficulty when the S_N2 processes are hindered. In 1975, Barton *et al.*[3] reported that thiobenzoates or S-methyl dithiocarbonates derived from secondary alcohols were reduced into the corresponding hydrocarbons on treatment with tributylstannane and they also suggested the particular applicability of this method in carbohydrate chemistry on the basis that this deoxygenation does not proceed through S_N2 reaction but radical one in character and, in addition, can be done under mild and neutral conditions. Accordingly, we have conducted such a study not only in hopes of increasing synthetic utility of this method in the field of aminoglycoside antibiotics, but also with expectation of new activities in their deoxygenated derivatives.

First, Barton's deoxygenation reaction was applied to a monosaccharide such as a protected 2,6-diamino-2,6-dideoxyglucopyranoside. This was on the basis that the aminosugar moiety attached to deoxystreptamine forms an essential activity center in aminoglycoside antibiotics such as kanamycin or neomycin; and the monosaccharide derivative may be useful as a model compound for the chemical modifications of more complex aminoglycoside antibiotics amenable to biological testing. Treatment of methyl 2,6-dibenzyloxycarbonylamino-2,6-dideoxy-α-D-glucopyranoside[4] (**1a**) with carbon disulfide and methyl iodide in an alkaline solution gave a 3,4-bis(S-methyl dithiocarbonate) **1b**. Following Barton's procedure, reaction of **1b** with tributylstannane was carried out in refluxing toluene, giving an 80% yield of a 3,4-dideoxy-3-eno compound **2** which was identical with the sample reported earlier.[1] Similar to tin hydride elimination of vicinal dihalides,[5] this process includes a stereospecific anti elimination and may be illustrated as an initially-generated radical at the 3 or 4 position converting into a double bond by anchimeric assistance of the leaving neighboring (methylthio)thiocarbonyloxy group.

1) Part III: T. Hayashi, N. Takeda, H. Saeki, and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **25**, 2134 (1977).
2) Location: *Hiromachi, Shinagawa-ku, Tokyo.*
3) D.H.R. Barton and S.W. McCombie, *J. C. S. Perkin* I, **1975**, 1574.
4) H. Saeki, N. Takeda, Y. Shimada, and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **24**, 724 (1976).
5) H.G. Kuivila, *Accounts of Chem. Res.*, **1**, 299 (1968) and references cited therein.



1a: $R_1=R_2=OH$
1b: $R_1=R_2=OCSSCH_3$
1c: $R_1=H$, $R_2=OH$
1d: $R_1=OCSSC_6H_5$, $R_2=OH$
1e: $R_1=OCSSC_6H_5$, $R_2=OCOCH_3$
1f: $R_1=H$, $R_2=OCOCH_3$



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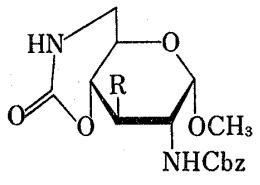
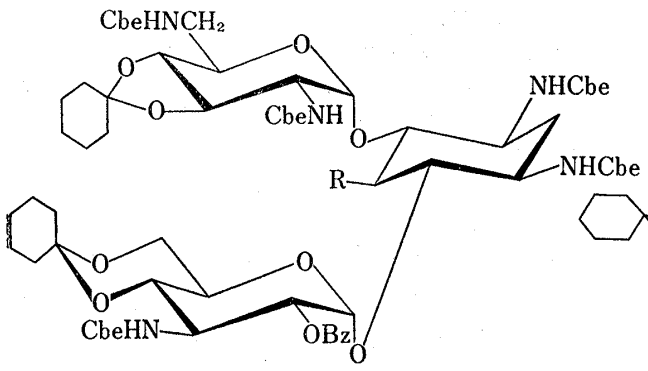


3a: $R=OCSSCH_3$
3b: $R=H$

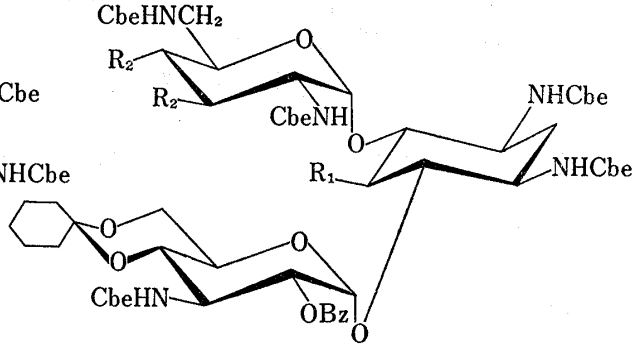
Cbz=$COOCH_2C_6H_5$

Chart 1

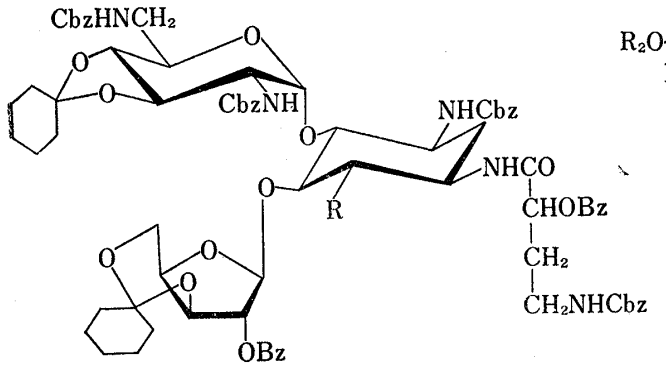
On the other hand, the 3,4-diol **1a** was treated with sodium hydride in dimethyl sulfoxide (DMSO) in place of sodium hydroxide, and successively with carbon disulfide and methyl iodide. Reaction proceeded with the removal of one benzyloxy group, giving a 3-O-[(methylthio)thiocarbonyl]-4,6-carbamate **3a** in good yield. Tin hydride reaction of **3a** also afforded



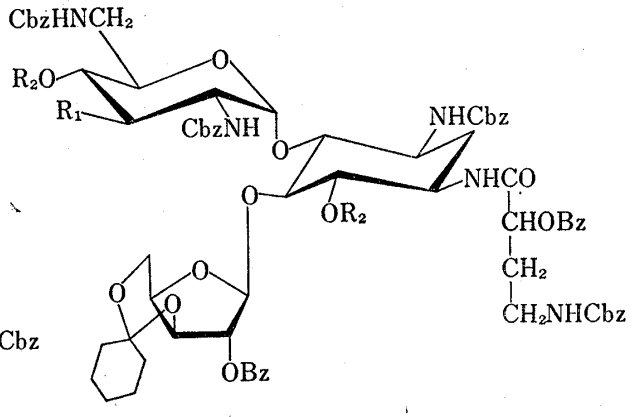
4a: R=OH
4b: $R=OCSSCH_3$
4c: R=H



5a: $R_1=R_2=OH$
5b: $R_1=R_2=OCSSCH_3$
5c: $R_1=H$, R_2=double bond



6a: R=OH
6b: $R=OCSSCH_3$
6c: R=H
6d: $R=OSO_2CH_3$



7a: $R_1=OH$, $R_2=H$
7b: $R_1=OCSSC_6H_5$, $R_2=H$
7c: $R_1=OCSSC_6H_5$, $R_2=COCH_3$
7d: $R_1=H$, $R_2=COCH_3$

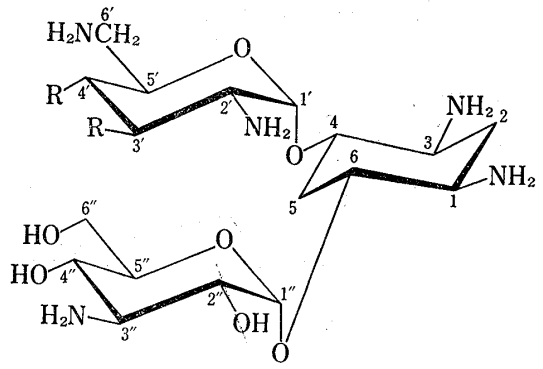
Cbe=$COOC_2H_5$, Cbz=$COOCH_2C_6H_5$, Bz=COC_6H_5

Chart 2

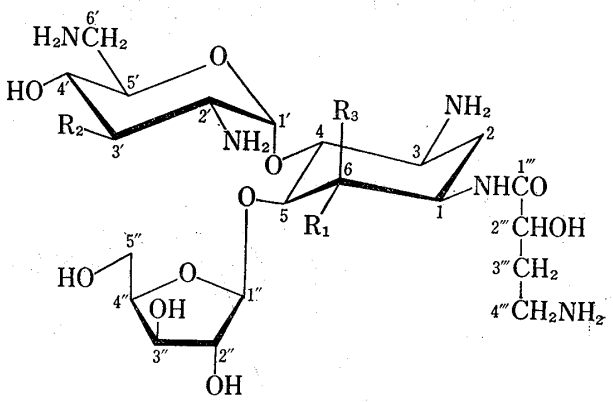
a 3-deoxy compound **3b** in 77% yield. The same compound was synthesized by treatment of the known 3-deoxy derivative of **1a** (**1c**) with methyl sulfinylcarbanion and was identified.

Reaction of the 3,4-diol **1a** with (phenylthio)thiocarbonyl chloride in pyridine proceeded selectively, giving a 3-(S-phenyl dithiocarbonate) **1d** in 70% yield, whose acetylation formed its 4-acetate **1e**, mp 139—141°. Tin hydride reaction of **1d** and **1e** also afforded the 3-deoxy compound **1c** and the 4-O-acetyl-3-deoxy compound **1f** respectively, in good yield.

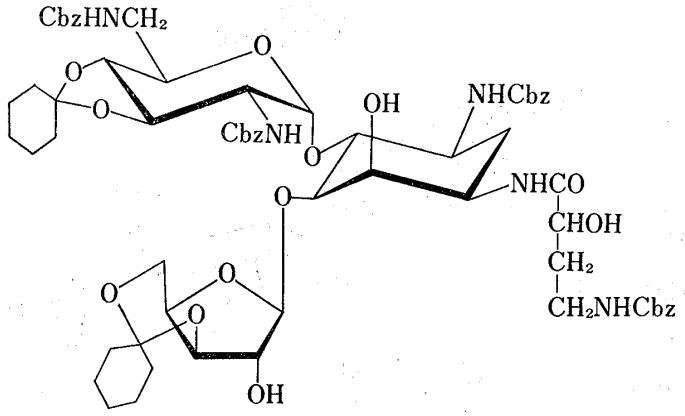
Based on these facts, Barton's deoxygenation process was extended into the field of aminoglycoside antibiotics such as kanamycin B or butirosin A as follows. Xanthation of penta-N-ethoxycarbonyl-2″-O-benzoyl-3′,4′: 4″,6″-di-O-cyclohexylidenekanamycin B[6] (**4a**) followed by treatment with methyl iodide gave a 5-(S-methyl dithiocarbonate) **4b** (76% yield) whose reaction with tributylstannane afforded a 5-deoxy derivative **4c** (78% yield). On the other hand, analogous treatment of penta-N-ethoxycarbonyl-2″-O-benzoyl-4″,6″-O-cyclohexylidenekanamycin B[6] (**5a**) gave a 3′,4′,5-tris-(S-methyl dithiocarbonate) **5b** in 44% yield. Tin hydride reaction of **5b** also afforded a 3′,4′,5-trideoxy-3′-eno compound **5c** in 40% yield. Further, analogous xanthation of tetra-N-benzyloxycarbonyl-2″,2‴-di-O-benzoyl-3′,4′: 3″,5″-di-O-cyclohexylidenebutirosin A[7] (**6a**) gave the dithiocarbonate **6b** (43% yield) which was



8a : R=OH
8b : R=double bond
8c : R=H



9a : R_1=R_3=H, R_2=OH
9b : R_1=OH, R_2=R_3=H
9c : R_1=H, R_2=R_3=OH



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Cbz=$COOCH_2C_6H_5$

Chart 3

6) S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, **45**, 3624 (1972); Y. Takagi, T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiotics*, **7**, 405 (1973).
7) H. Saeki, T. Hayashi, Y. Shimada, N. Takeda, and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **25**, 2089 (1977).

also converted into a 6-deoxy compound **6c** in 80% yield on treatment with tributylstannane. Treatment of tetra-N-benzyloxycarbonyl-2″,2‴-O-benzoyl-3″,5″-O-cyclohexylidenebutirosin A[7] (**7a**) with (phenylthio)thiocarbonyl chloride in pyridine yielded a 3′-(S-phenyl dithiocarbonate) **7b** (22% yield) whose acetylation formed a 4′,6-diacetate **7c**. Tin hydride reaction of **7c** also afforded a 3′-deoxy compound **7d** in 82% yield. On conventional deblocking in the usual manner, these deoxy compounds, **4c**, **5c**, **6c**, and **7c**, were converted into 5-deoxykanamycin B (**8a**), 3′,4′,5-trideoxy-3′-enokanamycin B (**8b**), 6-deoxybutirosin A (**9a**), and 3′-deoxybutirosin A[8] (**9b**), respectively. Hydrogenation of **8b** over Adams' catalyst afforded 3′,4′,5-trideoxykanamycin B (**8c**).

In connextion with this study, mesylation of the afore-mentioned butirosin derivative **6a** in pyridine gave a 6-mesylate **6d** which gave a butirosin analog **10** with an isomeric hydroxy group at the 6-position on treatment with sodium acetate in aqueous methyl cellosolve. Deblocking of the latter compound **10** afforded 6-epibutirosin A (**9c**).

Carbon-13 Nuclear Magnetic Resonance Spectra

Much work on ^{13}C–NMR spectral analysis has been recently carried out in the field of aminoglycoside antibiotics[9] and this technique presents us with important and necessary information complementary to other physical methods in the elucidation of the polyfunctional

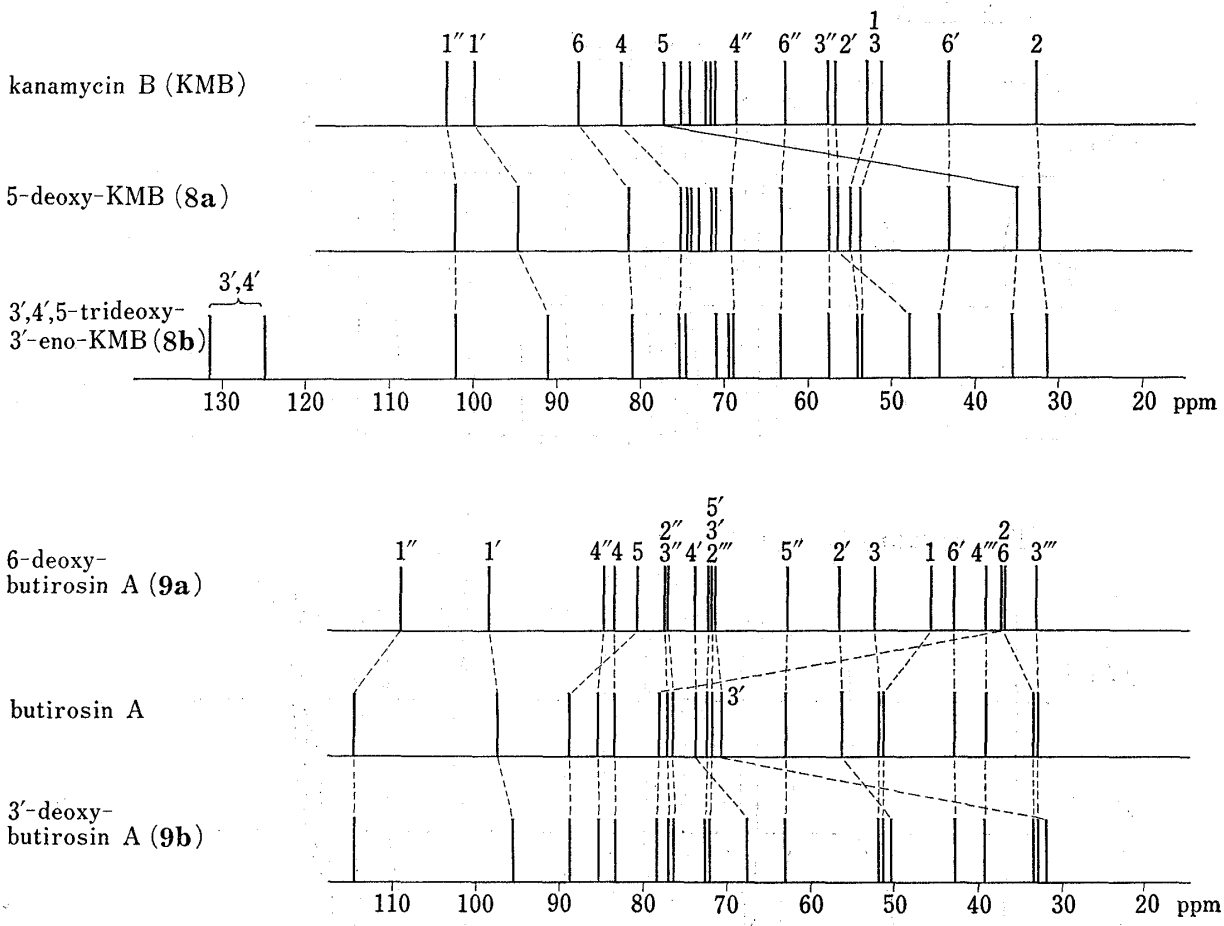

Fig. 1. Correlation of ^{13}C-NMR Spectra at pD 7

8) *Cf.* T. Okutani, T. Asako, K. Yoshioka, K. Hiraga, and M. Kida, *J. Am. Chem. Soc.*, **99**, 1287 (1977); I. Watanabe, A. Ejima, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Japan*, **50**, 487 (1977).

9) J.B. Morton, R.C. Long, P.J.L. Daniels, R.W. Tkach, and J.H. Goldstein, *J. Am. Chem. Soc.*, **95**, 7464 (1973); T.L. Nagabhushan, P.J.L. Daniels, R.S. Taret, and J.B. Morton, *J. Org. Chem.*, **40**, 2835 (1975); D.H. Davies, G. Greeves, A.K. Mallams, J.B. Morton, and R.W. Tkach, *J. Chem. Soc., Perkin I*, **1975**, 814.

structures of these compounds. In our present work, ^{13}C–NMR analysis was also undertaken in order to confirm positions of the deoxygenated carbons in these synthesized deoxy derivatives.

Proton noise decoupled ^{13}C–NMR spectra of deoxykanamycins and butirosins and their parent compounds were all taken in the sulfate form at the pD range between 7.0 and 7.5 and listed in Table I and also illustrated in Figure 1. In the spectrum of 5-deoxykanamycin B (**8a**, 5-deoxy-KMB), C-2 in the deoxystreptamine moiety resonates at 32.2 ppm, compared with that of kanamycin B (KMB) at 32.6 ppm. Subsequently, another peak resonating at 34.9 ppm in the alkane region can be assigned to C-5. This fact is also supported by up-field shifts[10] (6.0 and 7.1 ppm) observed on the neighbouring carbons, C-6 and C-4. Further, the signal at 76.9 ppm in the spectrum of kanamycin B disappears, verifying its assignment to C-5 which was deduced before by Koch *et al.*[11] It can be noted that C-1′ shows a large shift (4.2 ppm) with deoxygenation at C-5 as compared with C-1″ (0.9 ppm). This might be accounted for in terms of conformational change between the deoxystreptamine and the 2,6-diamino-2,6-dideoxyglucopyranoside ring which may arise from the existence of interaction between the C-5 hydroxy and C-2′ amino groups.

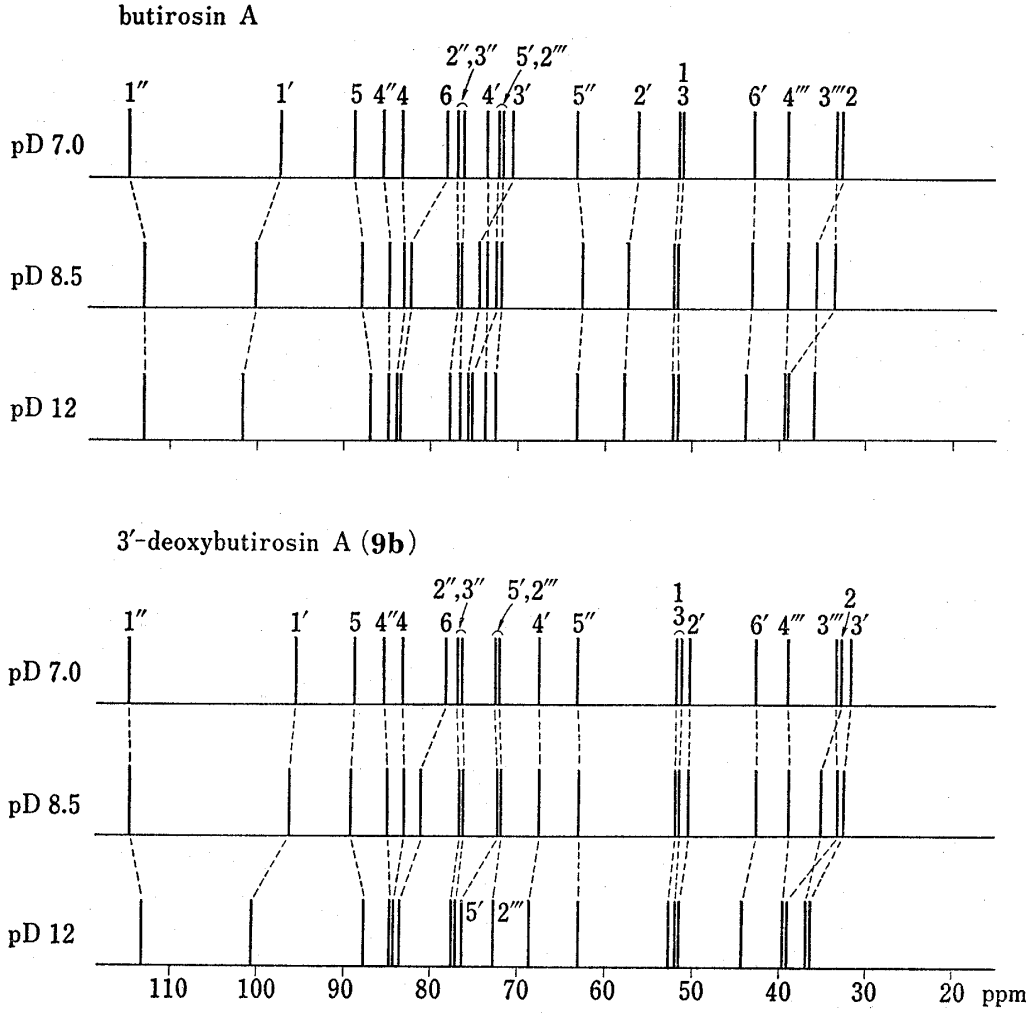

Fig. 2. Correlation of the Spectra of Butirosin A and 3′-Deoxybutirosin A (**9b**) at Different pDs

10) G.C. Levy and G.L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, 1972, p. 38.
11) K.F. Koch, J.A. Rhoades, E.W. Hageman, and E. Wenkert, *J. Am. Chem. Soc.*, **96**, 3300 (1974).

Table I. ^{13}C-NMR Chemical Shifts of Aminoglycosides at pD 7

Carbon	Kanamycin B (KMB)	5-Deoxy-KMB (**8a**)	3′,4′,5-Trideoxy-3′-eno-KMB (**8b**)	Butirosin A	6-Deoxybutirosin A (**9a**)	3′-Deoxybutirosin A (**9b**)
1′	98.6	94.4	92.1	97.3	98.4	95.6
2′	56.5	56.2	47.9	56.3	56.6	50.5
3′	(72.0)	(72.9)	131.5[a]	70.7	71.6	31.9
4′	(71.6)	(71.3)	124.8[a]	73.7	73.8	67.7
5′	(73.7)	(73.8)	(69.6)	72.2[b]	72.0[b]	72.7[b]
6′	43.0	43.0	44.3	43.0	43.0	42.8
1	52.6[c]	54.1[c]	54.1[c]	51.7[c]	45.7	51.4[c]
2	32.6	32.2	31.5	32.9	37.2	33.0
3	51.1[c]	53.5[c]	53.6[c]	51.3[c]	52.3	51.8[c]
4	82.1	75.0	75.2	83.3	83.3	83.3
5	76.9	34.9	35.7	88.7	80.7	88.8
6	87.1	81.1	80.9	78.1	37.2	78.4
1″	102.8	101.9	102.1	114.5	108.9	114.6
2″	(71.0)	(70.9)	(70.9)	77.0[d]	77.2[d]	77.0[d]
3″	57.5	57.3	57.5	76.4[d]	77.1[d]	76.4[d]
4″	68.4	69.0	(69.0)	85.5	84.6	85.5
5″	(75.2)	(74.1)	(74.6)	63.2	62.9	63.3
6″	62.5	63.0	63.2	—	—	—
1‴	—	—	—	178.2	177.3	178.2
2‴	—	—	—	71.8[b]	71.9[b]	72.2[b]
3‴	—	—	—	33.4	33.4	33.5
4‴	—	—	—	39.2	39.2	39.2

Tentative assignments are shown in parenthesis; a, b, c, and d may be reversed within the vertical columns respectively.

Table II. ^{13}C–NMR Chemical Shifts of Butirosin A and Its 3′-Deoxy Analog (**9b**) at pD 8.5 and pD 12

Carbon	Butirosin A		3′-Deoxybutirosin A	
	pD 8.5	pD 12	pD 8.5	pD 12
1′	100.4	102.0	96.0	100.8
2′	57.9	58.4	50.8	51.9
3′	75.0	76.2	32.8	36.5
4′	74.3	74.3	67.8	68.9
5′	73.1	76.2	72.2	76.5
6′	43.8	44.5	43.0	44.3
1	52.6[a]	52.9[a]	52.0[a]	52.1[a]
2	36.4	36.6	35.4	37.0
3	52.3[a]	52.2[a]	52.0[a]	52.8[a]
4	83.5	84.4	83.4	83.7
5	88.1	87.3	89.4	87.6
6	83.1	84.1	81.3	84.3
1″	113.4	113.4	114.6	113.3
2″	77.3[b]	78.4[b]	76.9[b]	77.7[b]
3″	77.3[b]	77.1[b]	76.9[b]	77.7[b]
4″	85.0	85.1	85.2	84.3
5″	63.4	63.6	63.3	63.1
1‴	178.3	180.7	178.1	179.9
2‴	72.5	73.1	72.2	72.8
3‴	34.1	39.8	33.5	39.3
4‴	39.5	39.8	39.2	39.6

a and b may be reversed within the vertical columns.

The spectrum of 3′,4′,5-trideoxy-3′-enokanamycin B (**8b**) shows C-3′, C-4′ and C-5 at 131.5, 124.8 and 35.7 ppm respectively, reflecting its structure. The C-2′ resonance is shifted up-field by *ca.* 8 ppm owing to the existence of the neighboring double bond.

The ^{13}C–NMR spectra of butirosin and its derivatives have not been completely assigned; however, the following observation may be possible on the basis of general rules of chemical shift[10] and analyses of the related compounds hitherto examined.[12] In the spectrum of 6-deoxybutirosin A (**9a**), C-6 shifts to 37.2 ppm, while C-6 of butirosin resonates at 78.1 ppm. In addition, the adjacent carbons, C-1 and C-5, also shift higher in the field. One interesting point is that, different from 5-deoxykanamycin B (**8a**), a large shift of the anomeric carbon of the xylose ring, C-1″, was observed with 6-deoxygenation.

In the spectrum of 3′-deoxybutirosin A (**9b**), two carbons, C-2′ and C-4′, adjacent to the deoxygenated site shift up-field and resonate at 50.5 and 67.7 ppm, respectively (shifting values: 5.8 and 6.0 ppm) and C-3′ appears at 31.9 ppm.

Table III. Minimal Inhibitory Concentrations (mcg/ml, Heart Infusion Agar) of Aminoglycoside Antibiotics (Sulfate Form)

Test organisms	5-Deoxy-KMB (**8a**)	KMB	3′-Eno-5-Deoxy-KMB (**8b**)	3′,4′5-Trideoxy-KMB (**8c**)	DKB
Staphylococcus aureus 209P	≤0.1	≤0.1	0.2	≤0.1	≤0.1
Staphylococcus aureus 109	25	25	0.8	0.4	0.4
Escherichia coli NIHJ	1.5	1.5	3.1	1.5	0.8
Escherichia coli 665[a]	>200	>200	25	12.5	3.1
Escherichia coli 676	200	>200	12.5	3.1	1.5
Klebsiella 806	1.5	1.5	6.2	1.5	0.8
Klebsiella 867	—	—	25	12.5	1.5
Proteus vulgaris 025	0.8	0.8	3.1	1.5	0.8
Proteus mirabilis 1306	>200	>200	50	12.5	6.2
Pseudomonas aeruginosa 1001	25	25	6.2	1.5	0.8
Pseudomonas aeruginosa 1055[a]	200	>200	12.5	3.1	0.8
Pseudomonas aeruginosa 1080	200	200	12.5	1.5	0.8
Pseudomonas aeruginosa 1255[a]	>200	>200	25	6.2	3.1

Test organisms	6-Deoxy-butirosin A (**9a**)	6-Epi-butirosin A (**9c**)	3′-Deoxy-butirosin A (**9b**)	3′,4′-Dideoxy-butirosin A	Butirosin[b]
Staphylococcus aureus 209P	1.5	12.5	0.4	≤0.1	≤0.1
Staphylococcus aureus 109	100	>200	12.5	—	12.5
Escherichiacoli	3.1	100	1.5	0.4	0.8
Escherichia coli 665[a]	>200	>200	3.1	3.1	200
Escherichia coli 676	>200	>200	1.5	—	50
Klebsiella 806	3.1	100	1.5	0.8	1.5
Klebsiella 867	6.2	200	1.5	0.8	1.5
Proteus vulgaris 025	50	200	0.8	0.8	0.8
Proteus mirabilis 1306	200	>200	12.5	50	50
Pseudomonas aeruginosa 1001	200	>200	3.1	3.1	6.2
Pseudomonas aeruginosa 1055[a]	>200	>200	3.1	6.2	100
Pseudomonas aeruginosa 1080	>200	>200	12.5	—	25
Pseudomonas aeruginosa 1255[a]	>200	>200	3.1	—	>200

a) resistant strains proved to be inactivators of 3′-O-phosphorylation type. See S. Sugawara, S. Inaba, M. Madate, and H. Saeki, *Sankyo Kenkyusho Nempo*, **25**, 56 (1973).
b) Butirosin complex was used.

12) P.W.K. Woo and R.D. Westland, *Carbohydrate Res.*, **31**, 27 (1973); S. Inouye, M. Kojima, and T. Niida, *J. Antibiotics*, **26**, 717 (1973).

As shown in Table II and Figure 2, the pH change in the butirosin series led to the assignment of the relevant carbons β to the amino function.[13] In the spectrum of 3′-deoxybutirosin A (**9b**), C-2 and C-3‴ both shift lower in the field with an increase of pD, exhibiting almost the same shifting pattern as that of butirosin.[14] The additional signal in the alkane region, which was assigned earlier as C-3′, also shifted within pD 7.5—12 with a shifting value of 4.6 ppm, providing further proof that deoxygenation took place at the C-3′ position β to the amino function.

Antibacterial Activity

Minimum inhibitory concentration (MIC) assays were conducted in comparison with those of the parent compounds as illustrated in Table III. 5-Deoxykanamycin B (**8a**) exhibited almost the same activity as kanamycin B (KMB). 3′,4′,5-Trideoxykanamycin B (**8c**) is slightly less active than dideoxykanamycin B (DKB), and its 3′-eno analog (**8b**) showed a further loss in activity. On the other hand, it was observed that, different from kanamycin series, 6-deoxybutirosin A (**9a**) and 6-epibutirosin A (**9c**) exhibited a serious loss in their activities. This fact suggests that the existence and orientation of a C-6 hydroxy function in butirosin play an irreplaceable role in its autibacterial activity. 3′-Deoxybutirosin A[8] (**9b**) shows activities similar to but slightly weaker than 3′,4′-dideoxybutirosin A[15] against butirosin sensitive and resistant strains.

Experimental

Melting points are not corrected. Infrared absorption spectra (IR) were recorded on a JASCO A-2 spectrometer (Japan Spectroscopic Co., Ltd.) and proton magnetic resonance spectra (^{1}H–NMR) on a Varian A-60 or a Hitachi-Perkin-Elmer R-24 spectrometer, using, unless otherwise specified, TMS as the internal standard. Carbon magnetic resonance spectra (^{13}C–NMR) were measured for solutions in D_2O (0.1—0.15 M) on a Varian XL 100A-15 spectrometer at 25.2 MHz. ^{13}C Chemical shifts are given in ppm downfield from DSS as an external reference ($\delta^{TMS}=\delta^{DSS}-2.9$ ppm) and Fourier transform to the frequency domain was accomplished with a Varian 620L-100 computer. Optical rotations were recorded on a Perkin-Elmer 241 automatic polarimeter in 10 cm tube. Thin-layer chromatography (TLC) was performed on TLC-plates, silica gel F_{254} precoated, layer thickness 0.25 mm (E. Merck) and spots were visualized by UV-irradiation or by spraying with vanadic acid–sulfuric acid followed by heating. Columns for ordinary chromatography were prepared with Wakogel C-200 (WAKO Pure Chemical Industries, Ltd.). Plates for preparative TLC were provided with Silica gel $60F_{254}$ (E. Merck). Amounts of absorbant used and developing solvents are shown in parenthesis.

Methyl 2,6-Dibenzyloxycarbonylamino-2,6-dideoxy-3,4-di-O-[(methylthio)thiocarbonyl]-α-D-glucopyranoside (1b)——To a solution of methyl 2,6-dibenzyloxycarbonylamino-2,6-dideoxy-α-D-glucopyranoside[4] (**1a**, 2 g) and CS_2 (5 ml) in DMSO (10 ml) was added dropwise 5 N NaOH (5 ml) at 15° under N_2 atmosphere. After stirring for 20 min, CH_3I (10 ml) was added portionwise to the resulting red solution and stirring was continued for 45 min. The solvent was evaporated *in vacuo* and the residue was extracted with AcOEt. The extract was washed with brine, dried and evaporated, giving 2.92 g of oil whose column chromatography (60 g, hexane–acetone, 2: 1, v/v) afforded 2.7 g (97%) of **1b** as oil. IR ν_{max}^{KBr} cm^{-1}: 3450 (br.), 1730, 1520, 1220, 1060. NMR (60 MHz, $CDCl_3$) δ: 7.28 (10H, s), 5.20 (2H, br. s, 3-H and 4-H), 5.05 (4H, s, $-CH_2C_6H_5$), 3.32 (3H, s), 2.48 and 2.40 (3H each, s, $-SCH_3$). *Anal.* Calcd. for $C_{27}H_{32}N_2O_8S_4$: C, 50.86; H, 4.78; N, 4.49; S, 20.19. Found: C, 50.62; H, 5.00; N, 4.37; S, 20.00.

Methyl 2,6-Dibenzyloxycarbonylamino-2,3,4,6-tetradeoxy-3-eno-α-D-glucopyranoside (2)——A solution of tributylstannane (2.7 g) in toluene (10 ml) was added to a refluxing solution of **1b** (1.3 g) in toluene (7 ml) over a period of 1 hr. Then, the mixture was refluxed for another 1 hr and, after cooling, the solvent was evaporated *in vacuo*. The residue was purified by chromatography (20 g, benzene–AcOEt, 3: 1, v/v) and gave **2** (690 mg, 80%) as amorphous powder, mp 147° (from MeOH). The analytical sample was obtained by further purification by preparative TLC and was identified with the authentic sample[1] by spectrometry.

13) G. Kotowycz and R.V. Lemieux, *Chem. Rev.*, **73**, 669 (1973).
14) The assignment of each carbon at different pD can be followed by outstanding difference of signal intensities due to a longer relaxation time of C-2 than C-3‴, which is expected by the number of hydrogen atoms at vicinal positions.
15) H. Saeki, Y. Shimada, Y. Ohashi, M. Tajima, S. Sugawara, and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1145 (1974).

IR ν_{max}^{KBr} cm^{-1}: 3320, 1690, 1540, 1300, 1270, 1240, 1040, 1010. NMR (60 MHz, $CDCl_3$) δ: 7.35 (10H, s), 5.68 (2H, br. **s**), 5.14 (4H, s), 4.82 (1H, d, J=4 Hz), 3.40 (3H, s). MS *m/e*: 426 (M^+, Calcd. for $C_{23}H_{26}N_2O_6$), 276 (M^+—$NHCOOCH_2C_6H_5$), 216 (276—CH_3OCHO). *Anal.* Calcd. for $C_{23}H_{26}N_2O_6$: C, 64.77; H, 6.15; N, 6.57. Found: C, 64.34; H, 5.99; N, 6.49.

Methyl 6-Amino-2-benzyloxycarbonylamino-2,6-dideoxy-3-O-[(methylthio)thiocarbonyl]-α-D-glucopyranoside 4,6-Carbamate (3a)——To an ice-cold and stirred solution of **1a** (2 g) in DMSO (5 ml) was added through a dropping funnel 2.5 mol equivalents of methyl sulfinylcarbanion in DMSO prepared from NaH (500 mg, 50% oil dispersion) and DMSO (8 ml). The mixture was stirred for 20 min and, after addition of CS_2 (2 ml), was further stirred for 15 min, CH_3I (5 ml) was added. After stirring was continued for 30 min at room temperature, work-up of the mixture in the usual manner followed by chromatography (40 g, hexane–acetone, 2:1, v/v) and recrystallization from benzene–hexane afforded **3a** (1.2 g, 64%), mp 110—112°, powder. IR ν_{max}^{KBr} cm^{-1}: 3400 (br.), 1720 (br.), 1510, 1220, 1100, 1050. NMR (60 MHz, $CDCl_3$) δ: 7.36 (5H, s), 5.12 (2H, br. s), 4.82 (1H, d, J=4 Hz), 3.45 (3H, s), 2.52 (3H, s). *Anal.* Calcd. for $C_{18}H_{22}N_2O_7S_2$: C, 48.86; H, 4.97; N, 6.33; S, 14.50. Found: C, 48.58; H, 4.68; N, 5.98; S, 14.04.

Methyl 6-Amino-2-benzyloxycarbonylamino-2,3,6-trideoxy-α-D-glucopyranoside 4,6-Carbamate (3b)——i) To a refluxing solution of **3a** (300 mg) in toluene (3 ml) was added dropwise tributylstannane (600 mg) in toluene (2 ml). After 4 hr refluxing, a solution of tributylstannane (200 mg) in 0.5 ml of toluene was added and reflux was continued for 1 hr. The solvent was evaporated *in vacuo* and the residue was washed with *n*-hexane. Column chromatography (15 g, benzene–AcOEt, 3:1, v/v) gave **3b** (180 mg, 79%), mp 234—235°, prisms (from MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350, 1710, 1680, 1660, 1280, 1100, 1060, 1020, 970. NMR (60 MHz, $CDCl_3$) δ: 7.38 (3H, s), 5.30 (3H, s), 4.86 (1H, d, J=3.5 Hz), 3.30 (3H, s), 2.25 (2H, br.). *Anal.* Calcd. for $C_{16}H_{20}N_2O_6$: C, 57.13; H, 5.99; N, 8.33. Found: C, 56.94; H, 6.21; N, 8.44.

ii) To an ice-cold solution of methyl 2,6-dibenzyloxycarbonylamino-2,3,6-trideoxy-α-D-glucopyranoside[1] (**1c**, 60 mg) in DMSO (1 ml) was added a methyl sulfinylcarbanion solution prepared by heating a mixture of NaH (20 mg, 50% oil suspension) and DMSO (1 ml) and stirring was continued for 1.5 hr at room temperature. Work-up as described above gave **3b** (18.3 mg, 40%) which was identified with the sample obtained as above.

(Phenylthio)thiocarbonyl Chloride——To an ice-cold and stirred solution of thiophenol (8.76 g) in 4 N NaOH (20 ml) was added dropwise a solution of thiocarbonyl dichloride (9.15 g) in $CHCl_3$ (20 ml) over a 20 min period and the mixture was stirred for 3 hr with cooling. Then, the mixture was diluted with ice-water and extracted with $CHCl_3$. The extract was washed with water, dried and evaporated *in vacuo* and the residue was distilled to give (phenylthio)thiocarbonyl chloride (7.44 g, 49.5%), bp 95—96°/2 mmHg, pale yellow oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 1480, 1440, 1110, 1080, 760, 740. NMR (60 MHz, $CDCl_3$) δ: 7.52 (5H, s).

Methyl 2,6-Dibenzyloxycarbonylamino-2,6-dideoxy-3-O-[(phenylthio)thiocarbonyl]-α-D-glucopyranoside (1d) and Its Acetate (1e)——To an ice cold solution of **1a** (500 mg) in 10 ml of pyridine was added portionwise (phenylthio)thiocarbonyl chloride (620 mg) with stirring and the mixture was allowed to stand overnight. The resulting colored mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed with water and successively with 2 N HCl and water, dried and evaporated *in vacuo*. The residue was dissolved in $CHCl_3$, treated with activated charcoal, charged on a silica gel column (30 g) and eluted with $CHCl_3$. Thus, **1d** (465 mg, 70%), yellow amorphous powder, was obtained along with 30 mg of a 3,4-bis(dithiocarbonate) as the fast-mobile component. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1710, 1510, 1220 (br.), 1040, 1020. NMR (60 MHz, $CDCl_3$) δ: 7.28 (15H, s), 5.08 (4H, s), 4.62 (1H, d, J=4 Hz), 3.22 (3H, s). *Anal.* Calcd. for $C_{30}H_{32}N_2O_8S_2 \cdot 1/2CHCl_3$: C, 54.48; H, 4.87; N, 4.17; S, 9.54. Found: C, 54.74; H, 5.00; N, 3.89; S, 9.55.

Acetylation of **1d** with Ac_2O–pyridine in the usual manner gave **1e**, mp 139—141°, needles, in 73% yield. IR ν_{max}^{KBr} cm^{-1}: 3300, 1750, 1700, 1540, 1230, 1060, 1040, 1020, 910. NMR (60 MHz, $CDCl_3$) δ: 7.40 (15H, s), 5.12 (4H, s), 4.72 (1H, d, J=4 Hz), 3.30 (3H, s), 2.02 (3H, s). *Anal.* Calcd. for $C_{32}H_{34}N_2O_9S_2$: C, 58.70; H, 5.23; N, 4.28; S, 9.79. Found: C, 58.82; H, 5.25; N, 4.20; S, 9.90.

Methyl 2,6-Dibenzyloxycarbonylamino-2,3,6-trideoxy-α-D-glucopyranoside (1c) and Its Acetate (1f)——(i) To a boiling solution of **1e** (250 mg) in toluene (5 ml) was added dropwise a solution of tributylstannane (500 mg) in toluene (5 ml) over a 15 min period and the mixture was refluxed for 1 hr. Work-up as described above and purification of the product by chromatography gave **1f** (144.6 mg, 77%). The analytical sample was obtained by further recrystallization from hexane–EtOH as needles, mp 163.5—164.5° (118.8 mg, 64%). IR ν_{max}^{KBr} cm^{-1}: 3350, 1740, 1710, 1690, 1535, 1280, 1240, 1055, 1020. NMR (60 MHz, $CDCl_3$) δ: 7.40 (10H, s), 5.14 (4H, s), 4.62 (1H, d, J=4 Hz), 3.34 (3H, s), 2.04 (3H, s). *Anal.* Calcd. for $C_{25}H_{30}N_2O_8$: C, 61.72; H, 6.22; N, 5.76. Found: C, 61.30; H, 6.03; N, 5.60.

To a cooled solution of **1f** (92.4 mg) in MeOH (5 ml) was added 2 N methanolic $NaOCH_3$ (0.01 ml) and the mixture was stirred for 1 hr. Then, the mixture was neutralized with resin (IR-120, H^+ form) and evaporated *in vacuo*. Recrystallization of the residue from hexane–EtOH gave **1c** (81.95 mg, 97%), mp 167—168°, needles, which was identified with the authentic sample[4] by mixed mp and spectrometry.

ii) To a boiling solution of **1d** (150 mg) in toluene (6 ml) was added a solution of tributylstannane (400 mg) in toluene (5 ml). After reflux for 4 hr, the mixture was worked-up as described above to give a syrupy product whose preparative TLC (benzene–AcOEt, 3:1, v/v) afforded **1c** (70 mg, 64%).

Penta-N-ethoxycarbonyl-2″-O-benzoyl-3′,4′: 4″,6″-di-O-cyclohexylidene-5-deoxykanamycin B (4c)——To a solution of penta-N-ethoxycarbonyl-2″-O-benzoyl-3′,4′: 4″,6″-di-O-cyclohexylidenekanamycin B[6] (**4a**, 972 mg) and CS_2 (1.4 ml) in DMSO (4 ml) was added 5 N NaOH (0.8 ml) with ice-cooling and stirring and the mixture was stirred for 30 min with cooling. Then, CH_3I (2.5 ml) was added dropwise and stirring was continued for 1 hr. Work-up in the usual manner[16] gave **4b** (796 mg, 76%) as a powder, mp 158—160°. NMR (60 MHz, $CDCl_3$) δ: 8.15—7.65 (5H, m), 2.68 (3H, s), 1.60 (20H, m). *Anal.* Calcd. for $C_{54}H_{79}N_5O_{21}S_2$: C, 54.12; H, 6.65; N, 5.84; S, 5.35. Found: C, 53.81; H, 6.58; N, 5.55; S, 5.40.

A solution of tributylstannane (600 mg) in toluene (2 ml) was added over a 30 min period to the refluxing solution of **4b** (548 mg) in toluene (12 ml) under N_2. After 1.5 hr refluxing, tributylstannane (200 mg) was added and reflux was continued for 1 hr. Evaporation of the solvent[16] gave **4c** (390 mg, 78%) as a powder. The analytical sample was obtained by reprecipitation from $CHCl_3$–hexane, mp 163°, powder. NMR (60 MHz, $CDCl_3$) δ: 8.6—7.5 (5H, m), 1.6 (20H, m). *Anal.* Calcd. for $C_{52}H_{77}N_5O_{20}$: C, 56.26; H, 7.12; N, 6.31. Found: C, 56.59; H, 6.91; N, 6.12.

Penta-N-ethoxycarbonyl-2″-O-benzoyl-4″,6″-O-cyclohexylidene-3′,4′,5-trideoxy-3′-enokanamycin B (5c)——To an ice-cold solution of penta-N-ethoxycarbonyl-2″-O-benzoyl-4″,6″-O-cyclohexylidenekanamycin B[6] (**5a**, 3.65 g) and CS_2 (9 ml) in DMSO (20 ml) was added dropwise 5 N NaOH (5.4 ml) and the mixture was stirred for 20 min. Then, after addition of CH_3I (20 ml) with cooling, stirring was continued for 30 min at room temperature. Work-up as described above[16] gave **5b** (2.06 g, 44%) as a powder, mp 155—157°. NMR (60 MHz, $CDCl_3$) δ: 8.15 (2H, m), 7.6 (3H, m), 2.64, 2.54 and 2.45 (3H, each, s), 1.5 (10H, m). *Anal.* Calcd. for $C_{52}H_{75}N_5O_{21}S_6 \cdot H_2O$: C, 47.45; H, 5.85; N, 5.32; S, 14.60. Found: C, 47.61; H, 5.75; N, 5.30; S, 14.89.

To a refluxing solution of **5b** (1.84 g) in toluene (50 ml) was added tributylstannane (2 g in 3 ml of toluene) over 1 hr period as described earlier. The reaction progress was monitored by TLC and 2 g more of the reagent was added during 4 hr refluxing. Work-up as described before[16] afforded **5c** (540 mg, 40%) as a powder, mp 198—200°. NMR (60 MHz, $CDCl_3$) δ: 8.15 (2H, m), 7.55 (3H, m), 5.68 (2H, br. s), 1.60 (10H, br.). *Anal.* Calcd. for $C_{46}H_{67}N_5O_{18} \cdot 2H_2O$: C, 54.49; H, 7.00; N, 6.91. Found: C, 54.88; H, 6.75; N, 6.64.

Tetra-N-benzyloxycarbonyl-2″,2‴-di-O-benzoyl-3′,4′: 3″,5″-di-O-cyclohexylidene-6-deoxybutirosin A (6c)——To an ice-cold and stirred solution of tetra-N-benzyloxycarbonyl-2″,2‴-O-benzoyl-3′,4′: 3″,5″-di-O-cyclohexylidenebutirosin A[7] (**6a**, 5 g) and CS_2 (5 ml) in DMSO (20 ml) was added dropwise 5 N NaOH (4 ml). After stirring for 20 min, CH_3I (12 ml) was added and stirring was continued for 30 min at room temperature. Work-up as described above[16] gave **6b** (2.3 g, 43.3%) as a powder (triturated in ether), mp 118—125°. NMR (60 MHz, $CDCl_3$) δ: 8.1 (4H, m), 7.6 (6H, m), 7.35 (20H, br. s), 2.1 (3H, s), 1.6 (20H, m). *Anal.* Calcd. for $C_{81}H_{91}N_5O_{22}S_2 \cdot H_2O$: C, 61.64; H, 5.90; N, 4.44; S, 4.06. Found: C, 61.55; H, 5.87; N, 4.54; S, 4.84.

Tributylstannane (5 g in 3 ml of toluene) was added portionwise to a refluxing solution of **6b** (2.5 g) in toluene (40 ml) as described above. After refluxing for 2 hr, work-up of the mixture[16] afforded **6c** (1.85 g, 80%). The analytical sample was obtained by preparative TLC and reprecipitation from $CHCl_3$–hexane, mp 104—106°, powder. NMR (60 MHz, $CDCl_3$) δ: 8.5 (4H, m), 7.5 (6H, m), 7.3 (20H, br. s), 1.5 (20H, m). *Anal.* Calcd. for $C_{79}H_{89}N_5O_{21}$: C, 65.70; H, 6.17; N, 4.85. Found: C, 65.40; H, 6.12; N, 4.83.

Tetra-N-benzyloxycarbonyl-4′,6-di-O-acetyl-2″,2‴-di-O-benzoyl-3″,5″-O-cyclohexylidene-3′-deoxybutirosin A (7d)——A mixture of tetra-N-benzyloxycarbonyl-2″,2‴-di-O-benzoyl-3″,5″-O-cyclohexylidenebutirosin A[7] (**7a**, 10.5 g), (phenylthio)thiocarbonyl chloride (9 g) and pyridine (100 ml) was allowed to stand overnight at room temperature. The mixture was poured into ice water and extracted with $CHCl_3$. Usual work-up[16] followed by treatment with activated charcoal gave **7b** (2.53 g, 22%) as a powder. The analytical sample was obtained by preparative TLC and reprecipitation from $CHCl_3$–hexane, mp 111—113°, powder. NMR (60 MHz, $CDCl_3$) δ: 8.0 (4H, m), 7.5 (6H, m), 7.32 (5H, s), 7.25 (20H, s), 1.5 (10H, br.). *Anal.* Calcd. for $C_{80}H_{85}N_5O_{22}S_2$: C, 62.70; H, 5.55; N, 4.57; S, 4.18. Found: C, 62.31; H, 5.53; N, 4.60; S, 3.90.

Acetylation of **7b** in pyridine in the usual manner gave **7c**, mp 110—112°, in quantitative yield. NMR (60 MHz, $CDCl_3$) δ: 8.05 (4H, m), 7.55 (6H, m), 7.32 (5H, s), 7.25 (20H, s), 2.12 (3H, s), 1.83 (3H, s). *Anal.* Calcd. for $C_{84}H_{89}N_5O_{24}S_2 \cdot H_2O$: C, 61.80; H, 5.58; N, 4.29; S, 3.92. Found: C, 61.76; H, 5.43; N, 4.38; S, 3.58.

To a refluxing solution of **7c** (1.35 g) in toluene (40 ml), tributylstannane (2 g in 3 ml of toluene) was added as described before. After 1 hr reflux, 500 mg of the reagent was added and reflux was continued for 1 hr. Work-up as usual[16] gave **7d** (1 g, 82%) as a powder, mp 107—108°. NMR (60 MHz, $CDCl_3$) δ: 8.6 (4H, m), 7.55 (6H, m), 7.3 (20H, s), 2.0 (3H, s), 1.86 (3H, s), 1.5 (10H, br.). *Anal.* Calcd. for $C_{77}H_{85}N_5O_{23} \cdot H_2O$: C, 63.07; H, 5.94; N, 4.78. Found: C, 63.06; H, 5.82; N, 4.70.

5-Deoxykanamycin B (8a)——A solution of **4c** (450 mg) and 2 N methanolic $NaOCH_3$ (0.1 ml) in 20 ml of MeOH was stirred for 1 hr at room temperature, then was neutralized with Amberlite IR-120 (H^+ form). Work-up as usual gave a desbenzoyl derivative of **4c**, mp 185—192°, a powder, quantitatively. NMR (60 MHz, $CDCl_3$) δ: 1.55 (20H, m), 1.2 (15H, t, J=7 Hz). *Anal.* Calcd. for $C_{45}H_{73}N_5O_{19} \cdot H_2O$: C, 53.73; H, 7.66; N, 6.97. Found: C, 53.41; H, 7.31; N, 6.75.

16) The product was purified by silica gel chromatography (MeOH–$CHCl_3$, 1—2: 100, v/v) and reprecipitation from hexane–$CHCl_3$.

A mixture of the desbenzoyl derivative (387 mg) obtained as above, acetic acid (3.2 ml) and water (1.8 ml) was heated for 10 min on a steam bath. Evaporation of the solvent *in vacuo* followed by work-up as usual gave 5-deoxy-penta-N-ethoxycarbonylkanamycin B (320 mg, 90%), a powder (triturated with MeOH). NMR (60 MHz, $CDCl_3$) δ: 4.7 (1H, m), 3.85 (10H, q, J=7 Hz), 0.98 (15H, t, J=7 Hz). *Anal.* Calcd. for $C_{33}H_{57}N_5O_{19}\cdot H_2O$: C, 46.86; H, 6.98; N, 8.28. Found: C, 47.31; H, 6.95; N, 8.03.

A mixture of the N-protected 5-deoxykanamycin B (270 mg) obtained as above, $Ba(OH)_2\cdot 8H_2O$ (2.25 g), dioxane (8 ml) and water (10 ml) was heated on a steam bath for 5 hr, then was saturated with CO_2. After filtration, the mixture was concentrated *in vacuo* and charged on a column of CG-50 (NH_4^+ form). The column was washed with water and eluted with increase in concentration of ammonia (0.15—0.6 N). Fractions were monitored by TLC and major fractions containing **8a** were collected. After complete removal of ammonia, the solution was saturated with CO_2 and freeze-dried, giving **8a** (54 mg) as films, mp 210—218°, $[\alpha]_D^{21}$ +100.5° (c=1.04, water) in a carbonate form. *Rf* values (Toyo Roshi 51A) (BuOH–AcOH–H_2O, 4: 3: 1, v/v): 0.19 (kanamycin B: 0.23); (BuOH–pyridine–H_2O–AcOH, 6: 4: 3: 1, v/v): Rf_{KMB}=1.16. NMR (60 MHz, D_2O[17]) δ: 5.1 (2H, br.), 2.3—2.1 (1H, m), 1.7—1.2 (3H, m). Elementary analysis was carried out with a sulfate of **8a**. *Anal.* Calcd. for $C_{18}H_{37}N_5O_9\cdot 5/2H_2SO_4$: C, 30.34; H, 5.89; N, 9.83; S, 11.23. Found: C, 30.28; H, 6.66; N, 9.42; S, 11.56.

3′,4′,5-Trideoxy-3′-enokanamycin B (8b)——A mixture of **5c** (1.15 g), AcOH (6 ml) and water (4 ml) was heated for 10 min on a steam bath. Evaporation of the mixture *in vacuo* and recrystallization of the residue (1.12 g) from MeOH gave a descyclohexylidene compound of **5c** (750 mg), mp 247—248°. NMR (60 MHz, C_5D_5N) δ: 8.35 (2H, m), 7.40 (3H, m), 5.85 (2H, br. s). *Anal.* Calcd. for $C_{40}H_{59}N_5O_{18}\cdot H_2O$: C, 52.46; H, 6.66; N, 7.65. Found: C, 52.93; H, 6.54; N, 7.64.

A mixture of the product (1.09 g) obtained as above, $Ba(OH)_2\cdot 8H_2O$ (7.5 g), dioxane (7 ml) and water (9 ml) was refluxed for 5 hr. Work-up as described for **8a** gave **8b** (350 mg), mp 258—264° as a sulfate form. NMR (60 MHz, D_2O[17]) δ: 5.95 (2H, br. s, 3′,4′-H), 5.35 (1H, br., anomeric H), 5.20 (1H, d, J=3 Hz, anomeric H), 2.4—2.2 (1H, m), 1.8—1.4 (3H, m). *Anal.* Calcd. for $C_{18}H_{35}N_5O_7\cdot 5/2H_2SO_4$: C, 31.86; H, 5.90; N, 10.34, S, 11.80. Found: C, 31.74; H, 5.76; N, 10.16; S, 12.06.

3′,4′,5-Trideoxykanamycin B (8c)——An aqueous solution of **8b** (30 mg) was adjusted to pH 1—2 with 2 N HCl and shaked with preactivated PtO_2 (20 mg) under H_2 atmosphere (45 lb/inch) for 4 hr at room temperature. Catalyst was filtered off and the filtrate was neutralized with Amberlite IR-45 (OH^- form) and evaporated *in vacuo*. The residue was dissolved in water and saturated with CO_2 and freeze-dried to give **8c** (30 mg), mp 230—234° (dec.). NMR (60 MHz, D_2O[17]) δ: 5.50 (1H, d, J=3 Hz), 5.32 (1H, d, J=3 Hz), 2.3—1.7 (8H, m).

6-Deoxybutirosin A (9a)——A mixture of **6c** (1.8 g), 2 N $NaOCH_3$ (0.5 ml) and MeOH (10 ml) was stirred overnight at room temperature. After neutralization with AcOH, the mixture was worked-up as usual to give a desacylated compound quantitatively. The analytical sample was obtained by reprecipitation from $CHCl_3$–hexane, mp 107—109°. NMR (60 MHz, $CDCl_3$) δ: 7.40 (20H, br. s), 1.55 (20H, m). *Anal.* Calcd. for $C_{65}H_{81}N_5O_{19}\cdot H_2O$: C, 62.25; H, 6.62; N, 5.58. Found: C, 61.81; H, 6.55; N, 5.43.

A solution of the desacylated compound (1 g) obtained as above in 80% aqueous AcOH (16 ml) was heated on a steam bath for 40 min. The mixture was evaporated *in vacuo* and the residue was recrystalized from EtOH to give 6-deoxy-N-protected butirosin (800 mg, 91%), mp 243—245°. *Anal.* Calcd. for $C_{53}H_{65}N_5O_{19}$: C, 59.16; H, 6.05; N, 6.51. Found: C, 58.70; H, 6.07; N, 6.83.

A solution of the 6-deoxy compound (780 mg) in 80% aqueous EtOH (100 ml) was refluxed with cyclohexene (4 ml) and 10% Pd-C (1 g) for 1 hr, when the evolution of CO_2 ceased. Then, the mixture was filtered and evaporated *in vacuo*. The residue (310 mg) was charged onto a column of CG-50 (NH_4^+ form, 30 ml) and eluted with graduated concentrations of ammonia (0.15—0.6 N). Fractions containing **9a** were collected and freeze-dried to give **9a** as a carbonate form (130 mg), mp 172—175°. *Anal.* Calcd. for $C_{21}H_{41}N_5O_{11}\cdot 2H_2CO_3\cdot 2H_2O$: C, 39.48; H, 7.00; N, 10.00. Found: C, 39.37; H, 6.60; N, 9.64.

3′-Deoxybutirosin A (9b)——As described above, a solution of **7d** (1 g) in MeOH (20 ml) was treated with 2 N methanolic $NaOCH_3$ (0.2 ml) and the resulting desacylated compound (377 mg, 47%) was hydrolysed with aqueous AcOH. The N-protected butirosin (345 mg) thus obtained was refluxed in 80% aqueous EtOH (10 ml) with cyclohexene (1 ml) and 10% Pd-C (300 mg) for 1.5 hr. Purification of the product was analogously performed over CG-50 (NH_4^+ form, 25 ml) to give 100 mg of **9b**, mp 270°, in a sulfate form. *Anal.* Calcd. for $C_{21}H_{41}N_5O_{11}\cdot 2H_2CO_3\cdot 2H_2O$: C, 39.48; H, 7.00; N, 10.00. Found: C, 39.63; H, 6.62; N, 9.65.

Tetra-N-benzyloxycarbonyl-3′,4′: 3″,5″-di-O-cyclohexylidene-6-epibutirosin A (10)——Methanesulfonyl chloride (2 g) was added dropwise to a solution of **6a** (2 g) in pyridine (40 ml) with stirring and ice-cooling. The stirring was continued for 4 hr at room temperature and the mixture was worked-up as usual to give a 6-sulfonate (**6d**) (1.8 g, 85%). The analytical sample was obtained by reprecipitation from $CHCl_3$–hexane, mp 113—118°. NMR (60 MHz, $CDCl_3$) δ: 8.1 (4H, m), 7.6 (6H, m), 7.35 (20H, s), 3.10 (3H, s), 1.6 (20H, br.). *Anal.* Calcd. for $C_{80}H_{91}N_5O_{24}S\cdot H_2O$: C, 61.74; H, 5.98; N, 4.50; S, 2.06. Found: C, 61.31; H, 5.89; N, 4.53; S, 2.21.

17) DSS as an external reference was used.

The mesylate **6d** (500 mg) and sodium acetate (400 mg) was dissolved in 95% aqueous methyl cellosolve (10 ml), and the solution was heated under reflux for 13 hr. Extraction with $CHCl_3$ and usual work-up[16] gave **10** (360 mg, 88%) as a powder, mp 118—120°. NMR (60 MHz, $CDCl_3$) δ: 7.30 (20H, s), 1.6 (20H, br.). *Anal.* Calcd. for $C_{65}H_{81}N_5O_{20}\cdot H_2O$: C, 61.47; H, 6.54; N, 5.51. Found: C, 61.51; H, 6.27; N, 5.51.

6-Epibutirosin A (9c)——A solution of **10** (2.1 g) in 75% aqueous AcOH (36 ml) was heated on a steam bath for 30 min. The solvent was removed *in vacuo* and the residue was purified by column chromatography (MeOH–$CHCl_3$, 1: 25, v/v), giving a descyclohexylidene compound (930 mg, 51%). The product (467 mg) obtained as above was dissolved in 80% aqueous EtOH (10 ml) and the mixture was heated with cyclohexene (1.5 ml) and 10% Pd-C (400 mg) under reflux for 1 hr. After filtration and evaporation *in vacuo*, the residue (167 mg) was charged on a column of CG-50 (NH_4^+ form, 30 ml) and eluted with graduated concentrations of ammonia (0.15—0.6 N), giving **9c** (118 mg). An analytical sample was obtained in a carbonate form, mp 180—182°, $[\alpha]_D^{20}$ +19.67° (c=1.5, water). *Rf* value (TLC, MeOH–conc. NH_4OH, 1: 1, v/v): 0.29 (*Rf* for butirosin: 0.23). NMR (60 MHz, D_2O[17]) δ: 5.9 (1H, d, J=4 Hz), 5.25 (1H, br. s), 2.3—1.8 (4H, m). *Anal.* Calcd. for $C_{21}H_{41}N_5O_{12}\cdot 2H_2CO_3\cdot 2H_2O$: C, 38.60; H, 6.85; N, 9.79. Found: C, 37.14; H, 6.33; N, 9.50.