Studies on Aminosugars. XXXVI. Syntheses of 3′,4′-Dideoxy- and 3′,4′,5″-Trideoxyribostamycin[1]

Daishiro IKEDA,* Toshihisa SUZUKI,* Tsutomu TSUCHIYA,* Sumio UMEZAWA,* and Hamao UMEZAWA**

*Department of Applied Chemistry, Faculty of Engineering, Keio University, Hiyoshi, Yokohama 223

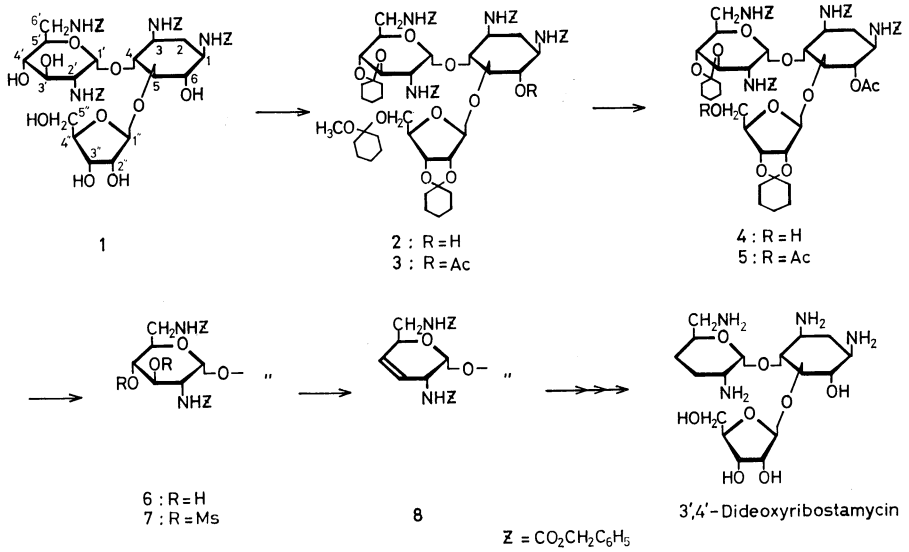
**Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo 141

(Received July 9, 1973)

3′,4′-Dideoxyribostamycin and 3′,4′,5″-trideoxyribostamycin have been prepared from ribostamycin *via* 3′,4′-unsaturation and 5″-iodo-3′,4′-unsaturation intermediates, respectively.

As described in previous papers, the 3′-deoxy[2,3] and 3′,4′-dideoxy[4,5] derivatives of kanamycins and neamine have been found to exhibit strong antibiotic activity against resistant bacteria carrying R factor and *Pseudomonas aeruginsoa*. The syntheses of these deoxy compounds were designed on the basis of mechanism of resistance[6] in resistant bacteria and as an extension of these works, we have now synthesized deoxyderivatives of another aminoglycoside antibiotic, ribostamycin,[7] which undergoes 3′-phosphorylation by the same enzymes,[8] kanamycin phosphotransferases I and II of resistant bacteria.

In order to protect the four amino groups, ribostamycin were treated with benzyl chloroformate in aqueous methanol in the presence of sodium carbonate to give tetra-*N*-benzyloxycarbonylribostamycin (**1**). The compound **1** was allowed to react with 1,1-dimethoxycyclohexane in dimethyl formamide (DMF) in the presence of catalytic amount of *p*-toluenesulfonic acid at 50 °C under diminished pressure. As described by Bissett, Evans, and Parrish,[9] the reaction under diminished pressure effectively removed the methanol which was liberated and the reaction was completed within 2 hr to give a tricyclohexylidene derivative (**2**), in a yield of 60%, in which one of the cyclohexylidene groups presented in a 1-methoxycyclohexyl form. The presence of the hemiketal group was confirmed by the observation of methoxyl group at τ 6.83 in the NMR spectrum. Acetylation of **2** gave the 6-*O*-acetyl derivative (**3**). Selective removal of the 5″-*O*-protecting group was achieved by treatment with acetone–40% acetic acid (4: 1) at room temperature to give a di-*O*-cyclohexylidene derivative (**4**) in a yield of 82%. Further acetylation gave the 5″,6-di-*O*-acetyl derivative (**5**). The cyclohexylidene group attached to the *trans* hydroxyl groups at 3′ and 4′ was selectively removed



1) A part of this paper was read by S. Umezawa at Symposium of New Natural Product Syntheses, XXIIIrd International Congress of Pure and Applied Chemistry at Boston, U.S.A., July 28, 1971; XXIIIrd ICPAC, Vol. 2, 173, Butterworths, London. Short communication: S, Umezawa. T. Tsucniya, D. Ikeda, and H. Umezawa, *J. Antibiot.* (Tokyo), **25**, 613 (1972).

2) S. Umezawa, T. Tsuchiya, R. Muto, Y. Nishimura, and H. Umezawa, *ibid.*, **24**, 274 (1971); S. Umezawa, Y. Nishimura, H. Hineno, K. Watanabe, S. Koike, T. Tsuchiya, and H. Umezawa, This Bulletin, **45**, 2847 (1972).

3) Y. Takagi, T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiot.* (Tokyo), **26**, in press.

4) H. Umezawa, S. Umezawa, T. Tsuchiya, and Y. Okazaki, *ibid.*, **24**, 485 (1971); S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, This Bulletin, **45**, 3624 (1972).

5) S. Umezawa, T. Tsuchiya, T. Jikihara, and H. Umezawa, *J. Antibiot.* (Tokyo), **24**, 711 (1971); This Bulletin, in press.

6) H. Umezawa, Progress in Antimicrobial and Anticancer Chemotherapy, **2**, 567, University of Tokyo Press, 1970.

7) E. Akita, T. Tsuruoka, N. Ezaki, and T. Niida, *J. Antibiot.* (Tokyo), **23**, 173 (1970).

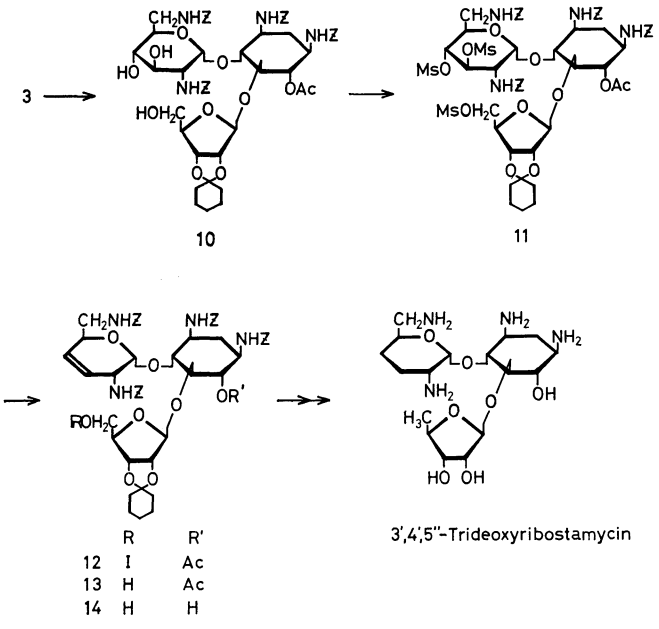
8) M. Yagisawa, H. Yamamoto, H. Naganawa, S. Kondo, T. Takeuchi, and H. Umezawa, *ibid.*, **25**, 748 (1972).

9) F. H. Bissett, M. E. Evans, and F. W. Parrish, *Carbohyd. Res.*, **5**, 184 (1967).

by treatment with hot acetone–60% acetic acid (1:1) to give the di-*O*-acetyl-mono-*O*-cyclohexylidene derivative (**6**) in a yield of 94%. Mesylation of **6** gave the 3′,4′-di-*O*-mesyl derivative (**7**). Subsequent 3′,4′-unsaturation was carried out by sodium iodide-zinc dust method reported by Tipson and Cohen[10] and Horton *et al.*[11] to give the 3′-eno derivative (**8**), which was obtained in a fairly good yield as expected from our works[12] on 3,4-unsaturated sugars. Treatment of **8** with methanolic ammonia gave the deacetylated derivative (**9**), which was then catalytically hydrogenated and decyclohexylidenated to give 3′,4′-dideoxyribostamycin in a yield of 54% from **8**.

On the other hand, the above-mentioned compound **3** was led to 3′,4′,5″-trideoxyribostamycin. Treatment of **3** with hot acetone–aqueous acetic acid gave the mono-*O*-cyclohexylidene derivative (**10**), which was then mesylated to give 3′,4′,5″-tri-*O*-mesyl derivative (**11**). Treatment of **11** with sodium iodide and zinc dust in DMF afforded the unsaturated iodo derivative **12**, namely, 6-*O*-acetyl-tetra-*N*-benzyloxycarbonyl-2″, 3″-*O*-cyclohexylidene-3′,4′-dideoxy-3′-eno-5″-iodoribostamycin (**12**) in a yield of 73%. Reduction of the iodo compound with Raney nickel and hydrogen in the presence of triethylamine gave the unsaturated 5″-deoxy derivative (**13**). Removal of the acetyl group by treatment with methanolic ammonia gave **14**, which was then catalytically hydrogenated with palladium black and hydrolyzed with acid to give the final product, 3′,4′,5″-trideoxyribostamycin.

The semisynthetic 3′,4′-dideoxyribostamycin showed[1] antibacterial activity similar to the parent ribostamycin against most of bacteria and, moreover, activity against *E. coli* carrying R factor of kanamycin phosphotransferase II and *Pseudomonas aeruginosa* resistant to ribostamycin. On the other hand, 3′,4′,5″-trideoxyribostamycin was found[1] to be less active than the parent antibiotic, indicating the involvement of the 5″-hydroxyl in antibacterial activity.



Experimental

Thin layer chromatography (tlc) was carried out on silica gel and the spots were visualized with sulfuric acid. Paper chromatography was performed on Toyo-Roshi paper No. 50 and the spots were detected with 0.5% ninhydrin in pyridine.

1,2′,3,6′-Tetra-N-*benzyloxycarbonylribostamycin (1).* To a mixture of ribostamycin sulfate (10 g, as ribostamycin·1.3 H_2SO_4) and anhydrous sodium carbonate (9 g) in 75% aqueous methanol (300 ml), benzyl chloroformate (15 g) was added under stirring at 5 °C and stirring was continued for 1 hr at that temperature. On tlc with benzene–methanol (4:1), a spot (R_f 0.48) appeared. The reaction mixture was evaporated to dryness. The residue was extracted with hot acetone (100 ml×3). Evaporation of the solution gave a solid, 15.3 g.

Found: C, 58.29; H, 5.80; N, 5.37%. Calcd for $C_{44}H_{58}N_4O_{18}\cdot H_2O$: C, 58.33; H, 5.99; N, 5.55%.

1,2′,3,6′-Tetra-N-*benzyloxycarbonyl-3′,4′;2″,3″-di*-O-*cyclohexylidene-5″*-O-*(1-methoxycyclohexyl)ribostamycin (2).* To a solution of **1** (2.76 g) and anhydrous *p*-toluenesulfonic acid (100 mg) in dry DMF (40 ml), 1,1-dimethoxycyclohexane (5.5 ml) was added and the solution was heated at 50 °C for 2 hr under reduced pressure (30—35 Torr). On tlc with benzene–diisopropyl ether (5:1) a spot (**2**, R_f 0.49) accompanied by spots of R_f 0.90 and 0.59, appeared, but the spot for the starting material (R_f 0) was still strong. Therefore another 1,1-dimethoxycyclohexane (6 ml) and anhydrous *p*-toluenesulfonic acid (15 mg) were added and the solution was again heated at 50 °C under the reduced pressure for 2 hr. After addition of sodium hydrogen carbonate solution, the mixture was evaporated to dryness and the residue was extracted with acetone. Evaporation of the acetone solution gave a solid, which was chromatographed on a column of silica gel (240 g) with benzene–ethyl acetate (7:2) containing triethylamine (0.5%). The portion containing **2** was evaporated to give a residue, which was reprecipitated from benzene–*n*-hexane yielding 1.93 g (60%), mp 92—94 °C, $[\alpha]_D^{17}$ +16.2° (*c* 2, $CHCl_3$).

Found: C, 64.39; H, 6.80; N, 4.58%. Calcd for $C_{68}H_{86}N_4O_{19}$: C, 64.65; H, 6.86; N, 4.43%.

NMR (in $CDCl_3$): τ 8.8—8.1 (30H broadened signals, cyclohexylidene protons), 6.83 (3H, s, $OC\underline{H}_3$), 2.65 (20H, $CO_2CH_2C_6\underline{H}_5$).

6-O-Acetyl-1,2′,3,6′-tetra-N-*benzyloxycarbonyl-3′,4′;2″,3″-di*-O-*cyclohexylidene-5″*-O-*(1-methoxycyclohexyl)ribostamycin (3).* To a solution of **2** (1.65 g) in dry pyridine (33 ml), acetic anhydride (1.96 g) was added and the solution was allowed to stand overnight at 37 °C. On tlc with benzene–ethyl acetate (7:2) a spot (R_f 0.42) appeared. After addition of a small amount of methanol followed by standing for 1 hr, the solution was evaporated and the residue was dissolved in chloroform. The solution was washed with saturated sodium hydrogen carbonate solution and water, dried over sodium sulfate and evaporated to give a colorless solid. Reprecipitation from benzene–*n*-hexane yielded 1.60 g (95%), mp 95—97 °C, $[\alpha]_D^{17}$ +8.3° (*c* 1, $CHCl_3$).

Found: C, 64.39; H, 6.86; N, 4.43%. Calcd for $C_{70}H_{88}N_4O_{20}$: C, 64.40; H, 6.80; N, 4.29%.

NMR (in $CDCl_3$): τ 8.8—8.2 (30H broadened signals), 7.94 (3H, s, OAc), 6.85 (3H, s, $OC\underline{H}_3$), 2.65 and 2.63 (20H in total, $CO_2CH_2C_6\underline{H}_5$).

10) R. S. Tipson and A. Cohen, *ibid.*, **1**, 338 (1965).
11) E. Albano, D. Horton, and T. Tsuchiya, *ibid.*, **2**, 349 (1966).
12) S. Umezawa, Y. Okazaki, and T. Tsuchiya, This Bulletin, **45**, 3619 (1972).

6-O-Acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-3',4';2'',3''-di-O-cyclohexylideneribostamycin (4). A solution of **3** (450 mg) in acetone–40% acetic acid (4:1, 5 ml) was allowed to stand at room temperature for 3 hr. On tlc with benzene–ethyl acetate (5:2) a spot (**4**, R_f 0.22) accompanied by a minor spot (**10**, R_f 0) appeared. The solution was poured into a mixture of chloroform and saturated sodium hydrogen carbonate solution under stirring. The chloroform solution separated was washed with water, dried over sodium sulfate and evaporated. The syrup (430 mg) was chromatographed on a column of silica gel (22 g) with the same solvent system. The portion containing **4** was evaporated to give a solid (330 mg, 82%), mp 109—111 °C, $[\alpha]_D^{17}$ +18° (*c* 2, $CHCl_3$).

Found: C, 63.62; H, 6.32; N, 4.88%. Calcd for $C_{63}H_{76}N_4O_{19}$: C, 63.41; H, 6.42; N, 4.70%.

NMR (in $CDCl_3$): τ 8.8—8.2 (20H broadened signals, cyclohexylidene protons), 7.97 (3H, s, OAc), 2.72 and 2.68 (5H and 15H, s, respectively, $CO_2CH_2C_6\underline{H}_5$).

5'',6-Di-O-acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-3',4';2'',3''-di-O-cyclohexylideneribostamycin (5). To a solution of **4** (200 mg) in pyridine (4 ml), acetic anhydride (27.4 mg) was added and the solution was allowed to stand at room temperature overnight. On tlc with benzene–ethyl acetate (5:2) a spot (R_f 0.37) appeared. The solution was poured into ice water with stirring. The resulting precipitate was filtered, washed with water and dried to give a solid, 200 mg (97%), which was reprecipitated from benzene–*n*-hexane, mp 95—98 °C, $[\alpha]_D^{17}$ +22° (*c* 0.67, $CHCl_3$).

Found: C, 63.06; H, 6.16; N, 4.64%. Calcd for $C_{65}H_{78}N_4O_{20}$: C, 63.19; H, 6.37; N, 4.54%.

NMR (in $CDCl_3$): τ 8.8—8.2 (20H broadened signals) 7.99 and 7.97 (both 3H, s, OAc), 2.69 and 2.66 (5H and 15H, s, respectively, $CO_2CH_2C_6\underline{H}_5$).

5'',6-Di-O-acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylideneribostamycin (6). A solution of **5** (200 mg) in acetone–60% acetic acid (1:1, 7 ml) was heated at 50 °C for 1 hr. A single product (R_f 0.14 on tlc with benzene–ethyl acetate (3:2)) appeared. The solution was evaporated with toluene to give a solid, which was dissolved in chloroform. The solution was washed with saturated sodium hydrogen carbonate solution and water, dried over sodium sulfate and evaporated to give a solid. Reprecipitation from benzene–*n*-hexane yielded 175 mg (94%), mp 102—105 °C, $[\alpha]_D^{17}$ +7.7° (*c* 1.8, $CHCl_3$).

Found: C, 61.22; H, 5.95; N, 4.89%. Calcd for $C_{59}H_{70}N_4O_{20}$: C, 61.34; H, 6.11; N, 4.85%.

NMR (in $CDCl_3$): τ 8.9—8.2 (10H broadened signals, cyclohexylidene protons), 8.00 (6H, s, OAc), 2.73 and 2.70 (5H and 15H, s, respectively, $CO_2CH_2C_6\underline{H}_5$).

5'',6-Di-O-acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylidene-3',4'-di-O-mesylribostamycin (7). To a solution of **6** (280 mg) in pyridine (5 ml), methanesulfonyl chloride (110 mg) was added and the solution was allowed to stand at room temperature overnight. After addition of a small amount of water, the solution was evaporated and the residue was dissolved in chloroform. The solution was washed successively with saturated potassium hydrogen sulfate solution, saturated sodium hydrogen carbonate solution and water, dried over sodium sulfate and evaporated to give a glassy solid, which was reprecipitated from benzene–*n*-hexane to give a solid, 279 mg (88%), mp 109—114 °C, $[\alpha]_D^{17}$ −4.7° (*c* 1.1, $CHCl_3$).

Found: C, 56.16; H, 5.61; N, 4.35; S, 4.58%. Calcd for $C_{61}H_{74}N_4O_{24}S_2$: C, 55.86; H, 5.69; N, 4.27; S, 4.89%.

NMR (in $CDCl_3$): τ 8.8—8.2 (10H), 7.93 (6H, s, OAc), 7.18 and 6.92 (both 3H, s, SO_2CH_3), 2.65, 2.62 and 2.59 (5H, 5H and 10H, s, respectively, $CO_2CH_2C_6\underline{H}_5$).

5'',6-Di-O-acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylidene-3',4'-dideoxy-3'-eno-ribostamycin (8). To a solution of **7** (270 mg) in dry DMF (6 ml), anhydrous sodium iodide (3.0 g) and zinc dust (1.5 g) were added and the mixture was heated in an oil bath (91 °C) for 1 hr under vigorous stirring. Chloroform (30 ml) was added and the reaction mixture was filtered to remove the insoluble matters, which were washed with chloroform. The filtrate and the washings combined were washed successively with saturated sodium chloride solution, sodium thiosulfate solution and with water, dried over sodium sulfate and evaporated to give a yellow syrup (214 mg). On tlc with benzene–ethyl acetate (5:2), the syrup showed two spots of R_f 0.34 (**8**) and 0 (minor). The syrup was chromatographed on a column of silica gel (6 g) with benzene–ethyl acetate (5:2). The eulate containing **8** was evaporated to give a colorless solid, 156 mg (70%), mp 82—85 °C, $[\alpha]_D^{20}$ −30° (*c* 2, $CHCl_3$).

Found: C, 63.44; H, 6.02; N, 4.83%. Calcd for $C_{59}H_{68}N_4O_{18}$: C, 63.20; H, 6.11; N, 5.00%.

NMR (in $CDCl_3$): τ 8.8—8.2 (10H), 8.00 (6H, s, OAc), 4.42 (2H slightly broadened singlet, H-3',4'), 2.75, 2.71, 2.69, and 2.67 (each 5H, s, $CO_2CH_2C_6\underline{H}_5$).

1,2',3,6'-Tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylidene-3',4'-dideoxy-3'-eno-ribostamycin (9). A solution of **8** (130 mg) in 10% ammonia in methanol (3 ml) was allowed to stand at room temperature overnight. The solution was evaporated and the residue was dissolved in benzene-ethyl acetate (1:1). The solution was passed through a short column of silica gel and the eluate containing **8** was evaporated to give a solid, 118 mg (98%), mp 89—93 °C, $[\alpha]_D^{21}$ −10° (*c* 2, $CHCl_3$).

Found: C, 64.05; H, 6.31; N, 5.24%. Calcd for $C_{55}H_{64}N_4O_{16}$: C, 63.69; H, 6.22; N, 5.40%.

NMR (in $CDCl_3$): τ 8.8—8.2 (10H), 4.45 (2H, slightly broadened singlet, H-3',4'), 2.75 and 2.72 (5H and 15H, s, respectively, $CO_2CH_2C_6\underline{H}_5$).

3',4'-Dideoxyribostamycin. A solution of **9** (50 mg) in aqueous dioxane (1:1, 2 ml) was hydrogenated with palladium black and hydrogen (50 psi) at 40 °C for 18 hr. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in 1M hydrochloric acid (0.5 ml) and the solution was heated at 60 °C for 1 hr. Addition of acetone gave a solid. The solid was charged on a column of CM Sephadex C-25 (NH_4^+) and, after washing the column with water (80 ml), it was developed with 0.01—0.4M ammonia with gradual increase in concentration. The product was eluted at the concentration of 0.3M ammonia. The portion was evaporated to give a solid, 11 mg (52%), mp 155—156 °C (decomp.), $[\alpha]_D^{20}$ +35° (*c* 1, H_2O). On paper chromatography with *n*-butanol–pyridine–water–acetic acid (6:4:3:1), it gave $R_{f\ \text{ribostamycin}}$ 1.50.

Found: C, 46.10; H, 8.38; N, 12.53%. Calcd for $C_{17}H_{34}N_4O_8\cdot H_2O$: C, 46.35; H, 8.24; N, 12.72%.

NMR (in D_2O): τ 9.0—7.7 (6H, m, H-2,3',4'), 4.65 (1H slightly broadened doublet, $J\sim1$ Hz, H-1''), 4.52 (1H, m, H-1').

On hydrolysis with 1M hydrochloric acid at 60 °C overnight, the product (R_f 0.40) gave 3',4'-dideoxyneamine[5] (R_f 0.56 on tlc with chloroform–methanol–17% ammonia (1:4:3)).

6-O-Acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylideneribostamycin (10). Compound **3** (966 mg) was treated in a similar manner as described in the preparation of **6** from **5**. The solid obtained was reprecipitated from benzene–*n*-hexane yielding 808 mg (98%), mp 104—107 °C, $[\alpha]_D^{14}$ +1.3° (*c* 0.78, $CHCl_3$).

Found: C, 61.34; H, 5.80; N, 5.18%. Calcd for $C_{57}H_{68}N_4O_{19}$: C, 61.50; H, 6.16; N, 5.03%.

NMR (in $CDCl_3$): τ 8.9—8.2 (10H), 8.00 (3H, s, OAc), 2.73, 2.70 and 2.69 (5H, 10H and 5H, s, respectively, $CO_2CH_2C_6\underline{H}_5$).

6-O-Acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylidene-3',4',5''-tri-O-methanesulfonylribostamycin (11). Compound **10** (737 mg) was treated with methanesulfonyl chloride (465 mg) in pyridine (15 ml) in a similar manner as described in the preparation of **7** from **6**. The solid obtained was chromatographed on a short column of silica gel with benzene–ethyl acetate (2: 1). The eluate containing **11** was evaporated to give a solid, 840 mg (95%), mp 112—114 °C, $[\alpha]_D^{17}$ −8.8° (*c* 2.6, $CHCl_3$).

Found: C, 53.74; H, 5.60; N, 4.29; S, 7.31%. Calcd for $C_{60}H_{74}N_4O_{25}S_3$: C, 53.48; H, 5.54; N, 4.16; S, 7.14%.

NMR (in $CDCl_3$): τ 8.8—8.2 (10H), 7.97 (3H, s, OAc), 7.18, 6.98 and 6.96 (3H, s, each, $SO_2C\underline{H}_3$), 2.71, 2.70, 2.67 and 2.66 (5H, s, each, $CO_2CH_2C_6\underline{H}_5$).

6-O-Acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylidene-3',4',5''-trideoxy-3'-eno-5''-iodoribostamycin (12). To a solution of **11** (544 mg) in dry DMF (11 ml), anhydrous sodium iodide (5.45 g) and zinc dust (2.73 g) were added and the mixture was heated in an oil bath (90 °C) for 1 hr under vigorous stirring. Chloroform (50 ml) was added and the reaction mixture was filtered from the precipitates, which were washed with chloroform. The filtrate and washings combined were washed successively with saturated sodium chloride solution, sodium thiosulfate solution and with water, dried over sodium sulfate and evaporated. On tlc with benzene–ethyl acetate (5: 2), the slightly yellowish syrup showed two spots of R_f 0.61 (**12**) and 0.47 (**11**, minor). The syrup (500 mg) was chromatographed on a column of silica gel (25 g) with benzene–ethyl acetate (3: 1). The eluate containing **12** was evaporated to give a colorless solid, 350 mg (73%), mp 88—90 °C, $[\alpha]_D^{18}$ −30° (*c* 2, $CHCl_3$).

Found: C, 57.47; H, 5.38; N, 4.73; I, 11.08%. Calcd for $C_{57}H_{65}N_4O_{16}I$: C, 57.57; H, 5.51; N, 4.71; I, 10.68%.

NMR (in $CDCl_3$): τ 8.8—8.2 (10H), 8.00 (3H, s, OAc), 4.35 (2H slightly broadened singlet, H-3',4'), 2.71, 2.68, 2.67 and 2.63 (each 5H, s, $CO_2CH_2C_6\underline{H}_5$).

6-O-Acetyl-1,2',3,6'-tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylidene-3',4',5''-trideoxy-3'-eno-ribostamycin (13). A solution of **12** (240 mg) in dioxane (3 ml) was hydrogenated with Raney nickel and hydrogen (50 psi) in the presence of triethylamine (0.1 ml) for 15 hr at 40 °C. On tlc with benzene–ethyl acetate (5: 2), the reaction mixture showed three spots of R_f 0.44 (**13**), 0.25 (de-OAc derivative) and 0.61 (**12**). After filtration, the solution was treated again with a fresh Raney nickel and hydrogen in the presence of triethylamine for 15 hr at 40 °C. The reaction mixture was filtered and the filtrate was evaporated to give a brown solid (171 mg). The solid was chromatographed on a column of silica gel (10 g) with benzene–ethyl acetate (3: 1). From the earlier eluate, **13** was obtained as a colorless solid, which was reprecipitated from benzene–*n*-hexane, yielding 91 mg (43%), mp 90—93 °C, $[\alpha]_D^{14}$ −29.8° (*c* 1.3, $CHCl_3$).

Found: C, 64.67; H, 6.17; N, 5.22%. Calcd for $C_{57}H_{66}N_4O_{16}$: C, 64.39; H, 6.26; N, 5.27%.

NMR (in $CDCl_3$): τ 8.82 (3H, d, J=6.5 Hz, $C\underline{H}_3$–CH(4'')), 8.8—8.2 (10H), 7.98 (3H, s, OAc), 4.38 (2H, s, H-3',4'), 2.70, 2.66, 2.64 and 2.61 (each 5H, s, $CO_2CH_2C_6\underline{H}_5$).

From the late eluate, the de-*O*-acetylated product (**14**) was obtained in a yield of 57 mg (28%).

1,2',3,6'-Tetra-N-benzyloxycarbonyl-2'',3''-O-cyclohexylidene-3',4',5''-trideoxy-3'-eno-ribostamycin (14). Compound **13** was treated similarly as described in the preparation of **9** from **8**. Yield 89%, mp 87—90 °C, $[\alpha]_D^{14}$ −26.6° (*c* 1.2, $CHCl_3$).

Found: C, 64.79; H, 6.14; N, 5.57%. Calcd for $C_{55}H_{64}N_4O_{15}$: C, 64.69; H, 6.32; N, 5.49%.

NMR (in $CDCl_3$): τ 8.78 (3H, d, J=6.5 Hz, CH_3–C(4'')), 8.8—8.2 (10H), 4.41 (2H, s, H-3',4'), 2.71 and 2.67 (5H and 15H, s, respectively, $CO_2CH_2C_6\underline{H}_5$).

3',4',5''-Trideoxyribostamycin. A solution of **14** (120 mg) in aqueous dioxane (1: 1, 4 ml) was hydrogenated with palladium black and hydrogen (50 psi) at 35—40 °C for 15 hr. When water was added, the reaction mixture became turbid indicating that the hydrogenation was incomplete. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was again treated as above with a fresh palladium black and hydrogen for 20 hr. The reaction mixture was filtered and the filtrate was evaporated to give a solid, which was dissolved in 1M hydrochloric acid (1 ml) and the solution was heated at 50 °C for 1 hr. After cooling, acetone was added to give a solid. The solid was charged on a column of Amberlite CG-50 (NH_4^+) and, after washing the column with water, it was developed with 0.1—0.4M ammonia with gradual increase in concentration. The product was emerged at the concentration of 0.3M ammonia. The eluate was evaporated to give a solid, 20 mg (40%), $[\alpha]_D^{14}$ +56° (*c* 0.7, H_2O). On paper chromatography with *n*-butanol–pyridine–water acetic acid (6:4:3:1), it gave $R_{f\ \text{ribostamycin}}$ 1.6.

Found: C, 48.32; H, 8.48; N, 13.11%. Calcd for $C_{17}H_{34}N_4O_7\cdot H_2O$: C, 48.10; H, 8.55; N, 13.21%.

NMR (in D_2O): τ 9.0—7.7 (6H, m), 8.65 (3H, d, J= 6.5 Hz, $CH_3C\underline{H}$), 4.75 (1H slightly broadened singlet, H-1''), 4.62 (1H, m, H-1').

The authors wish to thank Mr. Saburo Nakada for the elemental analysis and to Mr. Hiroshi Miyamoto for the technical assistance.