Synthesis of 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-3′-deoxyribostamycin[1] (3′-Deoxybutirosin B)

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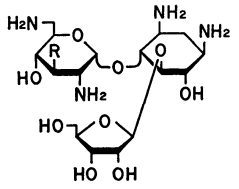
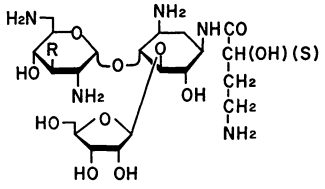
3′-Deoxybutirosin B (**13**) was prepared from ribostamycin by way of the following reaction sequences: 1) selective 3′-*O*-arylsulfonylation, 3′-iodination, and 3′-deoxygenation; 2) 1,6-cyclic carbamate formation,selective hydrolysis of the carbamate and acylation of the free 1-NH_2 group with an active ester of (*S*)-4-amino-2-hydroxybutyric acid. The structure of **13** was confirmed by comparison of its $\Delta[M]_{TACu}$ value with those of butirosin B and 4′-deoxybutirosin B.

As reported in a previous paper,[2] 3′-deoxyribostamycin exhibits antibacterial activity against various bacteria including those which produce kanamycin-neomycin phosphotransferase II[3] (P-ase II). 3′-Deoxyribostamycin, however, does not inhibit the growth of resistant organisms which produce kanamycin-neomycin phosphotransferase I[4] (P-ase I). On the other hand, butirosin B,[5] 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-ribostamycin, inhibits the growth of resistant organisms producing P-ase I but not those producing P-ase II.



Ribostamycin R : OH
3′-Deoxyribostamycin R : H

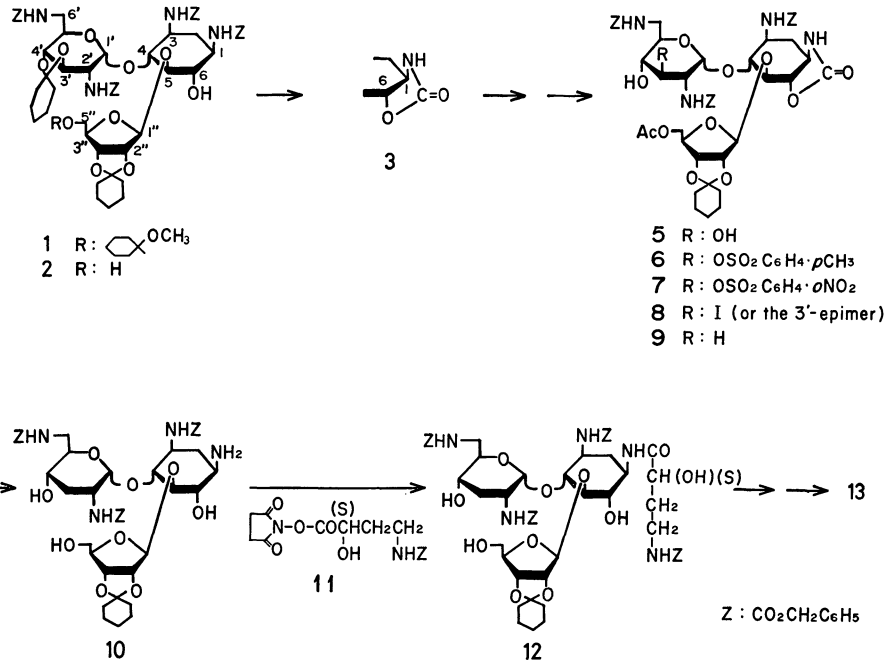


Butirosin B R : OH
3′-Deoxybutirosin B R : H

The titled compound, 3′-deoxybutirosin B is, therefore, expected to be active against both kinds of resistant bacteria.

This paper describes the synthesis of the titled compound starting from ribostamycin. This synthesis is an extension of the method[6] developed by us and involves the following reaction sequences: 1) removal of the 3′-hydroxyl group of ribostamycin by selective 3′-*O*-arylsulfonylation followed by iodination and hydrogenolysis of the iodine group in a similar fashion as reported in the synthesis[7] of tobramycin (3′-deoxykanamycin B) from kanamycin B; 2) coupling of (*S*)-4-amino-2-hydroxybutyryl group with the free 1-NH_2 group which is produced by way of 1,6-cyclic carbamate[8] formation followed by its selective cleavage.

Tetra-*N*-benzyloxycarbonyl-3′,4′:2″,3″-di-*O*-cyclohexylidene-5″-*O*-(1-methoxycyclohexyl) ribostamycin[9] (**1**) was treated with dilute acetic acid to split the acid-sensitive 5″-*O*-protecting group selectively. Treatment of the demethoxycyclohexyl derivative (**2**) with sodium hydride in *N*,*N*-dimethylformamide (DMF) gave the



1 R: OCH3
2 R: H

3

5 R : OH
6 R : OSO2C6H4·pCH3
7 R : OSO2C6H4·oNO2
8 R : I (or the 3′-epimer)
9 R : H

10

11

12

Z : CO2CH2C6H5

1,6-cyclic carbamate (**3**) as described in a previous paper.[8] Acetylation of **3** gave the 5″-*O*-acetyl derivative (**4**), from which the 3′,4′-*O*-cyclohexylidene group was selectively removed in a weakly acidic medium quantitatively.

Treatment of the partially decyclohexylidenated derivative (**5**) with *p*-toluenesulfonyl chloride or *o*-nitrobenzenesulfonyl chloride in pyridine at −37 °C successfully gave the selectively sulfonylated derivatives **6** and **7**, respectively.

Replacement of the 3′-*O*-tosyl or 3′-*O*-*o*-nitrobenzenesulfonyl group of **6** or **7** with iodide was carried out with 50% sodium iodide in DMF to give the 3′-iodo derivative (**8**). This iodination might be due to the participation by the 2′-acylamino group, as described in the preparation of tobramycin.[7] In the iodination, **7** reacted with sodium iodide more smoothly than **6**. Hydrogenation of **8** with Raney nickel in dioxane in the presence of triethylamine gave the 3′-deoxy derivative (**9**).

Selective opening of the 1,6-carbamate was successfully performed by use of a limited amount of barium hydroxide in dioxane, as reported in a previous paper,[8] to give **10**. To the free 1-NH_2 group, then, (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl group was introduced by using an active ester (**11**)[10] prepared from (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid, *N*-hydroxysuccinimide, and dicyclohexylcarbodiimide. Catalytic hydrogenolysis of the *N*-benzyloxycarbonyl groups of the amide (**12**) followed by removal of the 2″,3″-*O*-cyclohexylidene group with aqueous hydrochloric acid gave 3′-deoxybutirosin B (**13**). The compound had strong antibacterial activity[1] against various strains of both usual and resistant bacteria.

To confirm the structure of **13**, its $\Delta[M]_{TACu}$ value[11] was measured, as well as those of butirosin B and 4′-deoxybutirosin B,[12] to be +410°, −398°, and −528°, respectively. The facts that butirosin B and 4′-deoxybutirosin B had similar values in sign and magnitude and that **13** had a value of +800—900° larger than that of butirosin B or 4′-deoxybutirosin B show that **13** has no 3′-hydroxyl group, which renders **13** impossible to form a Cu-complex between 2′-NH_2 and 3′-OH groups, the contribution being estimated to be $\Delta[M]_{TACu} \approx -900°$.

Experimental

NMR spectra were recorded at 60 MHz with Varian A-60D spectrometer. Infrared spectra were determined in KBr discs with Hitachi 285 grating infrared spectrophotometer. Thin-layer chromatography (TLC) was carried out on Wakogel B-5 with sulfuric acid spray for detection unless otherwise stated. For column chromatography, silica gel (Wakogel C-200) was used.

Tetra-N-*benzyloxycarbonyl-3′,4′ : 2″,3″-di*-O-*cyclohexylideneribostamycin (2).* A solution of **1**[9] (4.50 g) in a mixture of 40% acetic acid–acetone (1 : 5, 92 ml) was heated at 37 °C for 7.5 h. The solution was poured into a mixture of chloroform (400 ml) and an aqueous saturated sodium hydrogencarbonate solution (200 ml) with stirring. The organic layer separated was washed with water, dried (Na_2SO_4), and concentrated to give a solid (4.50 g). The solid contained two components, **1** (TLC, R_f 0.84 with benzene–ethyl acetate=5 : 3) and **2** (R_f 0.35). The mixture was chromatographed over silica gel firstly with benzene–ethyl acetate (5 : 3) containing 0.5% triethylamine,* and **1** (1.11 g) was eluted. The developing solvent was then changed to benzene–ethyl acetate (1 : 1) containing 0.5% triethylamine to give **2**, 2.58 g (84%), mp 103—107 °C, $[\alpha]_D^{20}$ +23° (*c* 2, $CHCl_3$).

Found: C, 63.51; H, 6.47; N, 4.85%. Calcd for $C_{61}H_{74}N_4O_{18}$: C, 63.64; H, 6.48; N, 4.87%.

3,2′,6′-Tri-N-*benzyloxycarbonyl-3′,4′ : 2″,3″-di*-O-*cyclohexylideneribostamycin 1,6-Carbamate (3).* To an ice-cold solution of **2** (0.99 g) in dry DMF (11.5 ml), 50% oily sodium hydride (86 mg) was added and the mixture was stirred for 2 h in the cold under atmosphere of nitrogen. The mixture became clear after 30 min. The solution, after neutralization with acetic acid, was poured into a mixture of chloroform-saturated sodium chloride solution (1 : 1, 200 ml) with vigorous stirring. The organic layer separated was washed with water, dried (Na_2SO_4), and concentrated to give a pale yellow syrup. It was chromatographed over silica gel with benzene–ethyl acetate (1 : 1) containing 0.1% triethylamine. From the earlier and the later fractions, **2** (0.08 g) and **3** (0.62 g, 69%) were isolated, respectively. Compound **3**: mp 115—118 °C, $[\alpha]_D^{20}$ +26° (*c* 2, $CHCl_3$); IR: 1770 (cyclic carbamate[13]), 1715 (carbamate), 1520 (amide II) cm^{-1}.

Found: C, 62.07; H, 6.44; N, 5.50%. Calcd for $C_{54}H_{66}N_4O_{17}$: C, 62.18; H, 6.38; N, 5.37%.

5″-O-Acetyl-3,2′,6′-tri-N-*benzyloxycarbonyl-3′,4′ : 2″,3″-di*-O-*cyclohexylideneribostamycin 1,6-Carbamate (4).* Compound **3** was treated with acetic anhydride and pyridine in a usual manner to give **4** (98%), mp 110—113 °C, $[\alpha]_D^{20}$ +20° (*c* 1.5, $CHCl_3$); IR: 1770, 1730 (sh), 1715, 1520 cm^{-1}; NMR ($CDCl_3$) δ: 2.05 (3H, s, Ac).

Found: C, 61.72; H, 6.28; N, 4.92%. Calcd for $C_{56}H_{68}N_4O_{18}$: C, 61.98; H, 6.32; N, 5.16%.

5″-O-Acetyl-3,2′,6′-tri-N-*benzyloxycarbonyl-2″,3″*-O-*cyclohexylideneribostamycin 1,6-Carbamate (5).* A solution of **4** (2.96 g) in a mixture of acetone–60% acetic acid (1 : 1, 120 ml) was heated at 60 °C. After 1 h, the solution contained only **5** (TLC, R_f 0.15 with benzene–ethyl acetate=1 : 2), **4** (R_f 0.6) disappearing completely. The solution was concentrated *in vacuo* and the concentrate was extracted with chloroform. The organic layer was washed successively with aqueous sodium hydrogencarbonate, water, dried (Na_2SO_4), and concentrated to give a solid of **5** (2.75 g), which was purified by reprecipitation from chloroform–hexane, 2.61 g (95%), mp 109—112 °C, $[\alpha]_D^{20}$ +11° (*c* 2, $CHCl_3$); IR: 1760 (m), 1730 (sh), 1700, 1520 cm^{-1}; NMR ($CDCl_3$) δ: 2.03 (3H, s, Ac), 1.2—1.85 (≈11H).

Found: C, 59.59; H, 6.04; N, 5.33%. Calcd for $C_{50}H_{60}N_4O_{18}$: C, 59.75; H, 6.02; N, 5.57%.

5″-O-Acetyl-3,2′,6′-tri-N-*benzyloxycarbonyl-2″,3″*-O-*cyclohexylidene-3′*-O-*tosylribostamycin 1,6-Carbamate (6).* To a solution of **5** (1.77 g) in dry pyridine (38 ml), *p*-toluenesulfonyl chloride (1.91 g, 5 mol equivalents for **5**) was added and the solution was heated at 37 °C for 18 h and then additionally at 60 °C for 1 h. The solution showed, on TLC with benzene–ethyl acetate (1 : 1), a major spot (**6**, R_f 0.4) accompanied with other two minor spots of R_f 0.06 (**5**) and 0.6 (4′-*O*-tosyl isomer ?). Water (0.3 ml) was added and the solution was concentrated to give a reddish syrup. The chloroform solution of the syrup was washed successively with aqueous potassium hydrogensulfate, aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The resulting yellow syrup was chromatographed over silica gel with benzene–ethyl acetate (1 : 1) and from the fraction containing the major component, **6** was isolated as a colorless solid, 1.55 g (76%),

* Triethylamine was added in order to prevent removal of the cyclohexylidene groups during the chromatography.

mp 110—112 °C, $[\alpha]_D^{20}$ +13° (*c* 1.7, $CHCl_3$); IR: 1770 (cyclic carbamate), 1720 (carbamate), 1740 (sh, ester), 1520 (amide II); NMR ($CDCl_3$) δ: 2.05 (3H, s, Ac), 2.38 (3H, s, CH_3(Ts)).

Found: C, 59.30; H, 5.78; N, 4.72; S, 2.66%. Calcd for $C_{57}H_{66}N_4O_{20}S$: C, 59.06; H, 5.74; N, 4.83; S, 2.77%.

5″-O-Acetyl-3,2′,6′-tri-N-benzyloxycarbonyl-2″,3″-O-cyclohexylizene-3′-O-(o-nitrobenzenesulfonyl)ribostamycin 1,6-Carbamate (7). Compound **5** was treated with *o*-nitrobenzenesulfonyl chloride in a similar manner as described for **6** to give **7** (66%), mp 114—116 °C, $[\alpha]_D^{14}$ +6.5° (*c* 2.3, $CHCl_3$); NMR ($CDCl_3$) δ: 2.02 (3H, s, Ac).

Found: C, 56.25; H, 5.40; N, 5.75; S, 2.88%. Calcd for $C_{50}H_{63}N_5O_{22}S$: C, 56.51; H, 5.34; N, 5.88; S, 2.69%.

5″-O-Acetyl-3,2′,6′-tri-N-benzyloxycarbonyl-2″,3″-O-cyclohexylidene-3′-deoxy-3′-iodoribostamycin 1,6-Carbamate (8).

A. From 6: To a solution of **6** (0.58 g) in dry DMF (10 ml), sodium iodide (6 g, dried at 100 °C *in vacuo*) was added and the mixture was stirred at 100 °C for 10 h under the atmosphere of nitrogen. The reddish-brown solution, which soon solidified on cooling, was extracted with chloroform and the organic solution was concentrated with several additions of toluene. The resulting solid was extracted again with chloroform and the solution was washed successively with saturated sodium chloride solution, aqueous sodium thiosulfate, and water, dried (Na_2SO_4), and concentrated. The brown solid was chromatographed over silica gel with benzene–ethyl acetate (1: 1) to give a solid of **8**, 324 mg (58%), mp 108—110 °C, $[\alpha]_D^{15}$ +5° (*c* 3, $CHCl_3$); NMR ($CDCl_3$) δ: 2.1 (3H, s, Ac).

Found: C, 54.23; H, 5.49; N, 5.02; I, 11.05%. Calcd for $C_{50}H_{59}N_4O_{17}I$: C, 53.86; H, 5.34; N, 5.03; I, 11.38%.

B. From 7: Compound **7** was treated similarly as described above except that the reaction period and the developing solvent on column chromatography were changed to 1.5 h and benzene–ethyl acetate (2: 3). Yield of **8** was 65%.

5″-O-Acetyl-3,2′,6′-tri-N-benzyloxycarbonyl-2″,3″-O-cyclohexylidene-3′-deoxyribostamycin 1,6-Carbamate (9). To a solution of **8** (310 mg) in dioxane (9 ml), triethylamine (0.04 ml) was added, and the solution was hydrogenated with Raney nickel at 80 °C for 1 h under atmospheric pressure of hydrogen. After filtration, the solution was repeatedly hydrogenated with twice additions of fresh Raney nickel and triethylamine. Concentration of the solution gave a solid, which was chromatographed over silica gel with benzene–ethyl acetate (1: 2) to give **9**, 192 mg (70%), mp 97—99 °C, $[\alpha]_D^{13}$ +5° (*c* 1, $CHCl_3$).

Found: C, 60.35; H, 6.20; N, 5.52%. Calcd for $C_{50}H_{60}N_4O_{17}$: C, 60.72; H, 6.11; N, 5.66%.

3,2′,6′-Tri-N-benzyloxycarbonyl-2″,3″-O-cyclohexylidene-3′-deoxyribostamycin (10). To a solution of **9** (156 mg) in dioxane (3 ml), 0.1 M barium hydroxide solution (2.4 ml, 1.5 mol equivalents for **9**) was added and the mixture was stirred at 60 °C for 1 h. TLC (with benzene–methanol 4: 1) showed a single spot of **10** (R_f 0.5, ninhydrin positive) and none of **9** (R_f 0.8). After introduction of carbon dioxide followed by centrifugation, the solution was concentrated to dryness. The solid was extracted with dioxane and, after centrifugation, the supernatant layer was concentrated. The resulting solid (151 mg) still contained a trace of inorganic salt, however, it was chromatographically homogeneous and was used without purification for the next step. IR: 1700 cm^{-1} (carbamate).

3,2′,6′-Tri-N-benzyloxycarbonyl-1-N-[(S)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-2″,3″-O-cyclohexylidene-3′-deoxyribostamycin (12). To a solution of **10** (370 mg) and triethylamine (0.04 g) in THF (8 ml), the active ester[10] (**11**, 170 mg) was added and the solution was kept at room temperature overnight. The solution was concentrated and the residue was chromatographed over silica gel with chloroform–2-propanol (10: 1). The fractions containing the major component (**12**, R_f 0.42 on TLC with the above solvent system) were collected and concentrated to give a solid of **12**, 186 mg (40% based on **9**), mp 102—106 °C, $[\alpha]_D^{18}$ +2.5° (*c* 1, $CHCl_3$).

Found: C, 59.44; H, 6.21; N, 5.87%. Calcd for $C_{59}H_{73}N_5O_{19}\cdot 2H_2O$: C, 59.44; H, 6.51; N, 5.87%.

1-N-[(S)-4-Amino-2-hydroxybutyryl]-3′-deoxyribostamycin (3′-Deoxybutirosin B) (13). To a solution of **12** (178 mg) in dioxane (4.3 ml), water (1.4 ml) was added and the slight opalescent solution was hydrogenated with palladium black under atmospheric pressure of hydrogen. After 2 h, the solution showed, on TLC with a system of $CHCl_3$–CH_3OH–17% NH_4OH (2: 2: 1), two ninhydrin positive spots (R_f 0.4, major; and R_f 0.7, slight). The solution was filtered and the filtrate was concentrated to dryness. The residue was dissolved in a drop of 1 M aqueous hydrochloric acid and the solution was heated at 60 °C for 1 h. The solution then showed, on TLC with a system of $CHCl_3$–CH_3OH–17% NH_4OH (1: 4: 3) (detected by 0.2% ninhydrin in pyridine) a major spot at R_f 0.27. Addition of acetone gave precipitates, which were charged on a column of CM-Sephadex C-25 (NH_4 form) and, after washing the column with water, elution was effected with aqueous ammonia with linear increase in ammonia concentration (0.1—0.4 M). The eluate containing **13** was concentrated to give a solid, 35 mg (37%), $[\alpha]_D^{24}$ +27° (*c* 2, H_2O); IR: 1640, 1560 cm^{-1}.

Found: C, 43.44; H, 7.26; N, 11.45%. Calcd for $C_{21}H_{41}N_5O_{11}\cdot H_2CO_3$: C, 43.92; H, 7.20; N, 11.64%.

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