Synthesis of Butirosin B and Its 3′,4′-Dideoxy Derivative[1]

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The total synthesis of butirosin B (**5**) is described. Its 3′,4′-dideoxy derivative (**11**) has been synthesized, a cyclic carbamate derivative (**2**) being used as the key compound. The dideoxy derivative (**11**) was prepared by hydrogenation of a 3′,4′-unsaturated derivative (**10**) obtained by the reaction of a di-*O*-mesyl derivative (**9**) with sodium iodide and zinc dust. The dideoxy derivative (**11**) was found to have significant activity against resistant bacteria producing phosphotransferase I.

Butirosins[2] and the related antibiotic, ribostamycin[3] show an interesting contrast. Chemically, butirosin B is an acylated derivative of ribostamycin with (*S*)-4-amino-2-hydroxybutyryl side chain attached to the C–1 amino group of the 2-deoxystreptamine moiety. A comparison of the antibacterial spectrum of butirosin B and that of ribostamycin showed that butirosin has a broader spectrum, inhibiting kanamycin-resistant bacteria which produce kanamycin phosphotarnsferase I.[4] The present paper deals with the syntheses of butirosin B and its 3′,4′-dideoxy derivative from ribostamycin. Since ribostamycin has been synthesized,[5] the present synthesis constitutes the total synthesis of butirosin B.

As the starting material we used tetra-*N*-benzyloxycarbonyl-3′,4′;2″,3″-di-*O*-cyclohexylidene-5″-*O*-(1-methoxycyclohexyl)ribostamycin (**1**), an intermediate in the synthesis of 3′,4′-dideoxyribostamycin.[6] The derivative (**1**) has one hydroxyl group adjacent to the 1-*N*-benzyloxycarbonyl group, other hydroxy and amino groups being protected. When sodium hydride was added in a solution of **1** in DMF at room temperatures, smooth reaction occurred to give tri-*N*-benzyloxycarbonyl-3′,4′;2″,3″-di-*O*-cyclohexylidene-5″-*O*-(1-methoxycyclohexyl)ribostamycin 1,6-carbamate (**2**) in 78% yield. The IR spectrum showed a sharp peak at 1770 cm^{-1}, indicating the presence of a cyclic carbamate.[7] The structure of (**2**) was also confirmed by its NMR spectrum. A method was given for simultaneous blocking of adjacent amino and hydroxyl groups in a trans-diequatorial configuration by carbamate-ring formation, the above reaction being used as an alternative method. Formation of cyclic carbamate can be viewed as an anchimeric attack of the C–6 alkoxide-ion on the urethane carbonyl at C–1.

Treatment of the carbamate (**2**) with a limited amount of barium hydroxide in aqueous dioxane selectively cleaved the carbamate ring, to give an aminol (**3**) in 70% yield. The aminol was then condensed with (*S*)-2-hydroxy-4-phthalimidobutyric acid[8] (HPBA) by the active ester method to give the acylated product (**4**), which was treated successively with hydrazine in 80% ethanol to remove the phthaloyl group, with palladium black and hydrogen to remove the benzyloxycarbonyl groups and with 1M hydrochloric acid to remove the cyclohexylidene groups, giving butirosin B (**5**). The R_f values on ppc and tlc, the optical rotation, the IR and NMR spectra and the antibacterial activity[1] were identical with those of the natural specimen of butirosin B.

We undertook to prepare a modification of butirosin B. Butirosin A is active against kanamycin-resistant bacteria producing phosphotransferase I but inactivated by bacteria producing phosphotransferase II[3] (*e.g.*, *E. coli* JR 66/W677) by phosphorylation of the 3′-hydroxyl group. Synthesis of 3′,4′-dideoxybutirosin B was therefore undertaken.

Compound **4** was taken as the starting material and the 5″-protecting group was selectively removed by controlled acid-hydrolysis to give **6**. After acetylation, the tri-*O*-acetyl derivative (**7**) was further treated with acetic acid to give the diol (**8**). Mesylation then gave the 3′,4′-di-*O*-mesyl derivative (**9**). Similar treatment of **9** with sodium iodide and zinc dust in DMF to that for the preparation of 3′,4′-dideoxykanamycin B (under stricter conditions) gave 3′,4′-dideoxy-3′-eno derivative (**10**) in yield of 73%.[9] Hydrogenation of the double bond followed by the removal of the protecting groups gave 3′,4′-dideoxybutirosin B (**11**). In order to confirm the structure, **11** was partially hydrolyzed to give 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)-3′,4′-dideoxyneamine.[1]

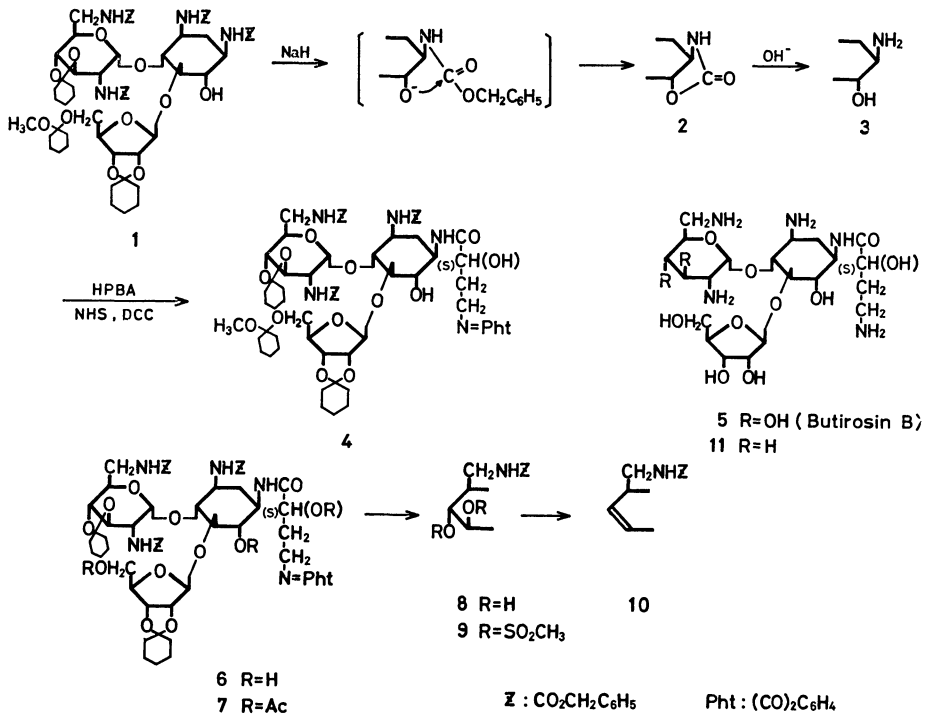
3′,4′-Dideoxybutirosin B was found to be active[1] against kanamycin-resistant bacteria producing phosphotransferase II as expected.

Experimental

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

*3,2′,6′-Tri-*N*-benzyloxycarbonyl-3′,4′; 2″,3″-di-*O*-cyclohexylidene-5″-*O*-(1-methoxycyclohexyl)ribostamycin 1,6-Carbamate (2).* 50% Oily sodium hydride (97 mg) was added to an ice-cold solution of **1** (1.30 g) in dry DMF (15 ml), after displacement of the air in the reaction vessel with nitrogen, and the mixture was stirred at room temperature for 2 hr. On tlc with benzene–ethyl acetate (3 : 2), the solution showed a single spot at R_f 0.40 (**1**: 0.87). The pale-yellow solution was neutralized with acetic acid, evaporated, and the residue was poured into a mixture of chloroform and water with stirring. The organic layer separated was washed with water, dried with Na_2SO_4 and evaporated. The resulting syrup was chromatographed on a short column of silica gel with benzene–ethyl acetate–triethylamine (60 : 30 : 0.5) to give a solid, 930 mg (78%), $[\alpha]_D^{25}$ +14.4° (*c* 2, $CHCl_3$); IR(KBr): 1770, 1720, 1530 cm^{-1}.

Found: C, 63.55; H, 7.03; N, 4.68%. Calcd for $C_{61}H_{78}$-



N_4O_{18}: C, 63.42; H, 6.80; N, 4.85%.

NMR (in $CDCl_3$): τ 8.8—8.2 (30H, cyclohexylidene), 6.82 (3H s, OCH_3), ~2.67 (15H, $CH_2C_6H_5$).

3,2′,6′-Tri-N-benzyloxycarbonyl-3′,4′; 2″,3″-di-O-cyclohexylidene-5″-O-(1-methoxycyclohexyl)ribostamycin (3). A barium hydroxide solution (2.5 ml, containing 68 mg of barium hydroxide octahydrate) was added to a solution of **2** (500 mg) in dioxane (2 ml), and the mixture was stirred at 98 °C for 40 min. The solution became neutral. On tlc with benzene-methanol (7 : 1), the solution gave two spots of R_f 0.11 (**3**) and R_f 0.75 (**2**) in almost equal strength. Another aliquot (2.5 ml) of barium hydroxide solution was added and the solution was treated as above. The solution became weakly alkaline and, on tlc, gave three spots of R_f 0.11 (major), 0.75 (slight) and 0.87 (slight). After introducing carbon dioxide, the solution was evaporated and the residue was extracted with chroloform. The solution was washed with water, dried with Na_2SO_4 and evaporated. The residue (485 mg) was chromatographed on a column of silica gel with benzene-ethyl acetate (3 : 1) and then with benzene-methanol-triethylamine (70 : 10 : 0.5) to give a solid, 342 mg (70%), $[\alpha]_D^{27}$ +16.7° (*c* 2, $CHCl_3$); weakly ninhydrine positive; IR (KBr): 1720, 1530 cm^{-1}.

Found: C, 63.69; H, 7.28; N, 4.90%. Calcd for $C_{60}H_{80}N_4O_{17}$: C, 63.81; H, 7.41; N, 4.96%.

3,2′,6′-Tri-N-benzyloxycarbonyl-3′,4′;2″,3″-di-O-cyclohexylidene-1-N-((S)-2-hydroxy-4-phthalimidobutyryl)-5″-O-(1-methoxycyclohexyl)ribostamycin (4). To an ice-cold solution of HPBA (62 mg, 1.3 mol eq.) and *N*-hydroxysuccinimide (NG) (27 mg, 1.3 mol eq.) in THF (2 ml) was added dicyclohexylcarbodiimide (54 mg, 1.3 mol eq.) and the mixture was stirred for 1 hr in the cold. A solution of **3** (200 mg, 1 mol eq.) and triethylamine (30 mg) in THF (2 ml) was added to the resulting suspension containing dicyclohexylurea, and the mixture was stirred at room temperature overnight. On tlc with benzene-ethyl acetate (1 : 1), the mixture showed three spots of R_f 0.7, 0.4 (**4**) and 0. After filtration, the solution was evaporated and the residue was chromatographed on a column of silica gel with benzene-ethyl acetate-triethylamine (60 : 40 : 0.5) to give a solid, 161 mg (67%), $[\alpha]_D^{27}$ +9.9° (*c* 2, $CHCl_3$); IR (KBr): 1710, 1655, 1530 cm^{-1}.

Found: C, 63.58; H, 6.56; N, 5.04%. Calcd for $C_{72}H_{89}N_5O_{21}$: C, 63.56; H, 6.59; N, 5.15%.

NMR (in $CDCl_3$): τ 9.0—7.5 (~36H, cyclohexylidene, $(CH_2)_2$, two OH (?)), 6.82 (3H s, OCH_3), 2.73—2.6 (15H, $CH_2C_6H_5$), 2.28 (4H, $(CO)_2C_6H_4$).

1-N-((S-4-Amino-2-hydroxybutyryl)ribostamycin (Butirosin B) (5). 80% Hydrazine hydrate (3 g) was added to a suspension of **4** (198 mg) in 80% aqueous ethanol (4.5 ml), and the mixture was heated at 60 °C for 2 hr. On tlc with ethyl acetate-methanol (1 : 1), the clear solution showed a ninhydrin-positive spot (R_f 0.15). The solution was evaporated and the residue was extracted with chloroform. The solution was evaporated and the residue was extracted with dioxane. The solution was filtered and to the filtrate (2 ml) were added water (1 ml) and a few drops of acetic acid and the mixture was hydrogenated with palladium black at 40—45 °C overnight. The reaction mixture was filtered, evaporated, and the residue was dissolved in 1M hydrochloric acid (1 ml). The solution was heated at 55° C for 1 hr. Addition of acetone gave a solid, which was chromatographed on a column of CM-Sephadex C-25 (NH_4^+ form, 20 ml) with water (100 ml) and aqueous ammonia of 0.05—0.5 M (linear increase). Butirosin B was eluted with *ca.* 0.4 M ammonia. Evaporation of the solution gave a soild, 51 mg (63%), $[\alpha]_D^{30}$ +34° (*c* 2, H_2O) [lit,[2] +33° (*c* 1.5, H_2O)]; ppc, $R_{f_{ribostamycin}}$ 0.53 (1-butanol-pyridine-water-acetic acid (6 : 4 : 3 : 1)], the value being identical with that of natural butirosin B; IR (KBr): 1640, 1560 cm^{-1}.

Found: C, 43.65; H, 7.49; N, 12.08%. Calcd for $C_{21}H_{41}N_5O_{12}\cdot H_2O$: C, 43.97; H, 7.56; N, 12.21%.

NMR (in D_2O): τ 8.3—7.7 (5H), 4.6 (2H m, H-1′, 1″).

3,2′,6′-Tri-N-benzyloxycarbonyl-3′,4′; 2″,3″-di-O-cyclohexylidene-1-N-((S)-2-hydroxy-4-phthalimidobutyryl)ribostamycin (6). A solution of **4** (440 mg) in 40% acetic acid–acetone (1 : 9, 10 ml) was allowed to stand at room temperature for 2 hr. 40% Acetic acid (0.6 ml) was added and the solution was allowed to stand for 6 hr. On tlc with benzene-ethyl

acetate (1 : 2), the solution showed three spots at R_f 0.72 (**4**, slight), 0.43 (**6**, major) and 0 (slight). The latest one increased with prolonged reaction. The solution was poured into a mixture of chloroform and sodium hydrogen carbonate solution with stirring. The organic layer was washed with water, dried with Na_2SO_4 and evaporated. The residue (410 mg) was chromatographed on a column of silica gel with benzene–ethyl acetate (1 : 2). After complete elution of **4** (100 mg), the eluant was changed to chloroform-methanol (27 : 1, **6** is scarcely soluble in the first solvent system, but soluble in this system). A solid (290 mg, 71%) obtained was reprecipitated from chloroform–*n*-hexane, $[\alpha]_D^{28}$ +16° (*c* 2, $CHCl_3$).

Found: C, 62.34; H, 6.33; N, 5.55%. Calcd for $C_{65}H_{77}N_5O_{20}$: C, 65.54; H, 6.22; N, 5.61%.

NMR (in $CDCl_3$): τ 8.9—7.5 (~26H), 4.9 (6H, $CH_2C_6H_5$), ~2.7 (15H two peaks, $CH_2C_6H_5$), 2.25 (9H incomplete d, $(CO)_2C_6H_4$).

*1-N-((S)-2-Acetoxy-4-phthalimidobutyryl)-6,5″-di-O-acetyl-3,2′, 6′-tri-N-benzyloxycarbonyl-3′,4′;2″,3″-di-O-cyclohexylideneribostamycin (**7**).* A sample (320 mg) of **6** was treated with acetic anhydride and pyridine in the usual manner to give a solid, 311 mg (88%, reprecipitated from benzene–*n*-hexane), $[\alpha]_D^{25}$ +9° (*c* 1.7, $CHCl_3$).

Found: C, 62.22; H, 6.27; N, 4.99%. Calcd for $C_{71}H_{83}N_5O_{23}$: C, 62.04; H, 6.09; N, 5.10%.

NMR (in $CDCl_3$): τ 7.94, 7.85 and 7.78 (each 3H s, Ac).

*1-N-((S)-2-Acetoxy-4-phthalimidobutyryl)-6,5″-di-O-acetyl-3,2′, 6′-tri-N-benzyloxycarbonyl-2″,3″-O-cyclohexylideneribostamycin (**8**).* A solution of **7** (300 mg) in 60% acetic acid–acetone (4 ml+5 ml) was allowed to stand at 60 °C for 75 min. On tlc with benzene–ethyl acetate (1 : 3), the solution showed a single spot of R_f 0.42. The solution was evaporated and the residue was shaken with a mixture of chloroform and saturated sodium hydrogen carbonate solution. From the organic layer, a solid of **8** was obtained, 252 mg (90%, reprecipitated from chloroform–*n*-hexane), $[\alpha]_D^{25}$ +1.5° (*c* 1.7, $CHCl_3$).

Found: C, 60.69; H, 6.11; N, 5.15%. Calcd for $C_{65}H_{75}N_5O_{23}$: C, 60.32; H, 5.84; N, 5.41%.

NMR (in $CDCl_3$): τ 8.8—8.3 (10H, cyclohexylidene), 8.2—7.6 (4H), 7.97, 7.87 and 7.82 (each 3H s, Ac), 4.9 (6H, $CH_2C_6H_5$), 2.70 and 2.65 (5H s, 10H s, respectively, $CH_2C_6H_5$), 2.20 (4H incomplete d).

*1-N-((S)-2-Acetoxy-4-phthalimidobutyryl)-6,5″-di-O-acetyl-3,2′, 6′-tri-N-benzyloxycarbonyl-2″,3″-O-cyclohexylidene-3′,4′-di-O-mesylribostamycin (**9**).* Mesyl chloride (45 mg) was added to a solution of **8** (240 mg) in pyridine (5 ml), and the solution was allowed to stand for 37 °C overnight. On tlc with benzene–ethyl acetate (1 : 1), the solution showed two spots at R_f 0.18 (monomesyl derivative) and 0.39 (**9**). Mesyl chloride (20 mg) was added and the solution was heated at 60 °C for 2 hr. After addition of a drop of water, the solution was evaporated and the residue was dissolved in chloroform. The solution was washed successively with potassium hydrogen sulfate solution, sodium hydrogen carbonate solution and water, dried over Na_2SO_4, and evaporated to give a syrup, which was reprecipitated from chloroform–*n*-hexane to give a solid of **9**, 258 mg (98%), $[\alpha]_D^{20}$ −9° (*c* 2, $CHCl_3$); IR 1175 cm^{-1}.

Found: C, 55.82; H, 5.63; N, 4.79; S, 4.27%. Calcd for $C_{67}H_{79}N_5O_{27}S_2$: C, 55.84; H, 5.49; N, 4.83; S, 4.42%.

NMR (in $CDCl_3$): τ 7.93, 7.86 and 7.77 (each 3H s, Ac), 7.23 and 6.96 (each 3H s, SO_2CH_3).

*1-N-((S)-2-Acetoxy-4-phthalimidobutyryl)-6,5″-di-O-acetyl-3,2′, 6′-tri-N-benzyloxycarbonyl-2″,3″-O-cyclohexylidene-3′,4′-dideoxy-3′-enoribostamycin (**10**).* Sodium iodide (2.1 g; dried at 110 °C for 2 hr), zinc dust (1.0 g; dried at 110 °C for 2 hr) and molecular sieves (100 mg; Union Carbide Co., Grade 3A, dried at 260 °C for 3 hr) were added to a solution of **9** (200 mg) in DMF (4 ml; dried over CaH_2 and distilled in vacuo just before use), and the mixture was stirred at 89.5—90 °C (oil bath temperature) for 1 hr. The temperature was maintained strictly in the above range with a thermostabilizer. Temperature fluctuation caused a decrease in the tlc with benzene–ethyl acetate (1 : 1), the mixture showed a spot at R_f 0.48 (**10**) and trace spots at R_f 0.56 (**9**) and 0. Chloroform (20 ml) was added and the mixture was filtered with the aid of chloroform (20 ml). The filtrate was washed successively with saturated sodium chloride solution, sodium thiosulfate solution and water, dried over Na_2SO_4 and evaporated. The syrup was chromatographed on a short column of silica gel with benzene–ethyl acetate (1 : 1) to give a solid of **10**, 128 mg (73%), which was reprecipitated from chloroform–*n*-hexane, mp 99—102 °C, $[\alpha]_D^{23}$ −24.5° (*c* 2, $CHCl_3$).

Found: C, 61.77; H, 5.71; N, 5.56%. Calcd for $C_{65}H_{73}N_5O_{21}$: C, 61.94; H, 5.84; N, 5.56%.

NMR (in $CDCl_3$): τ 7.99, 7.90 and 7.83 (each 3H s, Ac), 4.38 (2H slightly broadened s, H-3′,4′).

*1-N-((S)-4-Amino-2-hydroxybutyryl)-3′,4′-dideoxyribostamycin (3′,4′-Dideoxybutirosin B) (**11**).* A sample of **10** was treated as in the preparation of butirosin B from **4**, giving **11**, yield 80%, $[\alpha]_D^{16}$ +25° (*c* 1.8, H_2O), $R_{f\,\text{butirosin B}}$ 1.73 (ppc with 1-butanol–pyridine–water–acetic acid (6:4:3:1)), R_f 0.24 (tlc with chloroform–methanol–17% ammonia (1 : 4 : 3)).

Found: C, 46.74; H, 7.70; N, 13.13%. Calcd for $C_{21}H_{41}N_5O_{10}\cdot H_2O$: C, 46.57; H, 8.00; N, 12.93%.

NMR (in D_2O): τ 9.0—7.5 (~10H), 4.6 (2H m, H-1′,1″).

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