Synthesis of 1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]-3′,4′-dideoxyneamine[1)]

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The titled compound (**5**) has been prepared from 3′,4′-dideoxyneamine or ribostamycin *via* 1,6-cyclic carbamate intermediates (**2** and **12**). 3′,4′-Dideoxygenation, which is the key step in the synthesis from ribostamycin, was accomplished by the hydrogenation of 3′,4′-unsaturated derivative which was prepared from the corresponding 3′,4′-di-*O*-mesyl derivative by the action of sodium iodide and zinc dust in DMF. The title compound was found to have an improved antibacterial spectrum broader than 3′,4′-dideoxyneamine.

As described in the preceding paper,[2)] 3′,4′-dideoxygenation of butirosin B was found to give a compound having a broader antibacterial spectrum than butirosin B. The recognition of the remarkable effects of combination of 3′,4′-dideoxygenation and N–1 acylation with (*S*)-4-amino-2-hydroxybutyryl group led us to the synthesis of N–1 acylated derivative of 3′,4′-dideoxyneamine. Since neamine forms a large portion of kanamycin B and ribostamycin, and appears to play an essential role in the antibacterial action of these antibiotics, we were interested in studying the above-mentioned modification of neamine. The present paper deals with the synthesis of 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-3′,4′-dideoxyneamine (**5**).

The selective acylation of C-1-amino group of 2-deoxystreptamine moiety is generally difficult, however, in the present synthesis, the N-1-acylation has been achieved through a cyclic carbamate intermediate by a method[3)] similar to that described for butirosin synthesis.[2)]

When tetra-*N*-benzyloxycarbonyl-3′,4′-dideoxyneamine (**1**), which has been prepared from 3′,4′-dideoxyneamine,[4)] was treated with sodium hydride in DMF, the cyclic 1,6-carbamate (**2**) was obtained in 62% yield. Selective hydrolysis of the cyclic carbamate group with a limited amount of barium hydroxide in aqueous dioxane yielded 3,2′,6′-tri-*N*-benzyloxycarbonyl 3′,4′-dideoxyneamine (**3**). Acylation of the C-1-amino group with (*S*)-2-hydroxy-4-phthalimidobutyric acid[5)] (HPBA) in the presence of *N*-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide (DCC) gave the N-1-acyl derivative (**4**), which, on deblocking, led to the title compound (**5**).

An alternative synthesis of the title compound (**5**) has been achieved by a route from ribostamycin. Methanolysis of tetra-*N*-benzyloxycarbonylribostamycin[6)] (**6**) gave tetra-*N*-benzyloxycarbonylneamine (**7**) in high yield. Direct benzyloxycarbonylation of neamine has also been attempted, however, in this case, separation of the product from inorganic material was unusually troublesome.

Selective cyclohexylidenation of **7** has been accomplished in a manner similar to that described in the synthesis of 3′,4′-dideoxyneamine,[4)] affording **8**. Mesylation gave the 3′,4′-di-*O*-mesyl derivative (**9**), which, on treatment with sodium iodide and zinc dust in DMF by the procedure previously reported,[2,7)] furnished the 3′,4′-unsaturated derivative (**10**) in 76% yield.

Decyclohexylidenation of **10** by acid hydrolysis gave **11**, which was then treated with sodium hydride in DMF to give the cyclic carbamate **12**. The carbamate was selectively hydrolyzed by alkaline treatment to give **13**. Finally, N-1-acylation of **13** with HPBA, NHS and DCC followed by hydrogenation and deblocking afforded **5**, which was identical with the specimen prepared by the foregoing method.

Retention of configuration at C-2 of the side chain in **5** was confirmed by acid hydrolysis,[8)] which gave (*S*)-4-amino-2-hydroxybutyric acid.

The N-1-acylated derivative (**5**) of 3′,4′-dideoxyneamine showed enhanced antibacterial activity broader than 3′,4′-dideoxyneamine, exhibiting activity against various strains of sensitive and resistant bacteria[1)] as expected.

Experimental

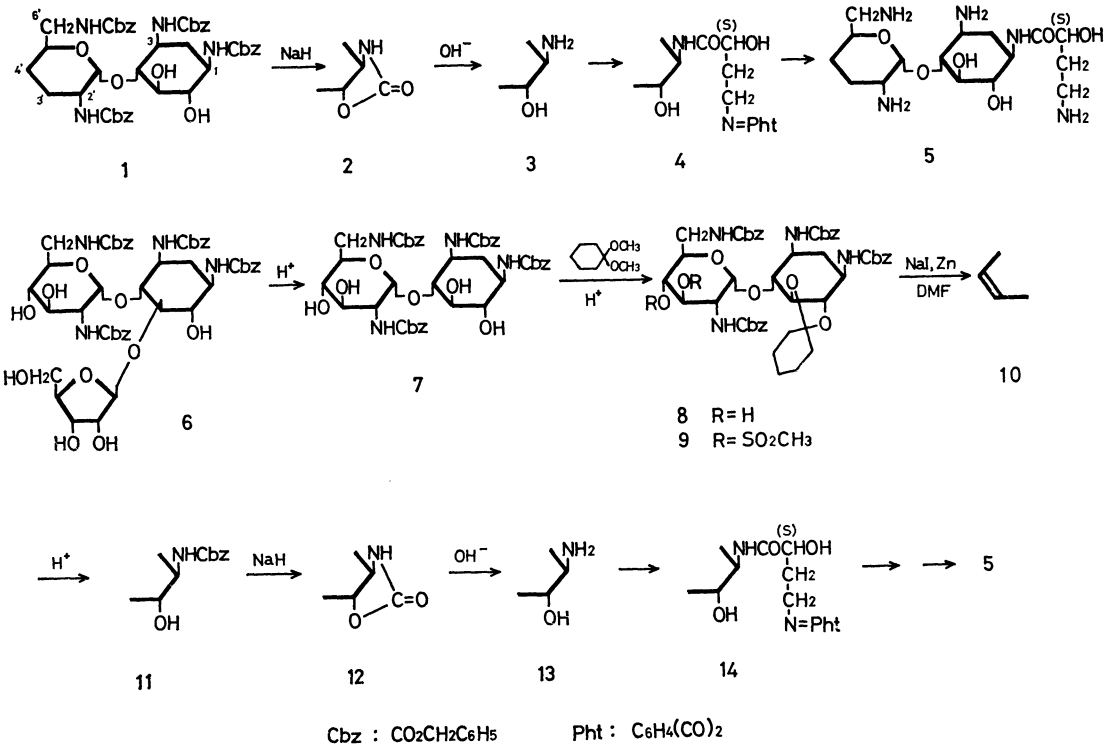
1,3,2′,6′-Tetra-N-*benzyloxycarbonyl-3′,4′-dideoxyneamine (**1**).* Prepared in the usual manner,[9)] $[\alpha]_D^{15}$ +45° (*c* 2, $CHCl_3$).

3,2′,6′-Tri-N-*benzyloxycarbonyl-3′,4′-dideoxyneamine 1,6-Carbamate (**2**).* A solution of **1** (1.34 g) in DMF was ice-cooled and, after replacement of the atmosphere with nitrogen, 50% oily NaH (235 mg) was added and the mixture was stirred for 30 min. The clear solution was allowed to stand overnight at ~5 °C. On tlc with $CHCl_3$–EtOH (20 : 1), the solution showed a single spot (R_f 0.25) except **1** (R_f 0.33). After neutralizing the solution with AcOH, the solution was poured into a mixture of $CHCl_3$–H_2O with stirring. The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated to give a yellow syrup. The syrup was chromatographed on a short column of silica gel with $CHCl_3$–EtOH (20 : 1) to give a solid, which was reprecipitated from $CHCl_3$–*n*-hexane, 683 mg (62%), $[\alpha]_D^{25}$ +58° (*c* 1.9, $CHCl_3$); IR (KBr): 1770, 1710, 1535 cm^{-1}.

Found: C, 61.92; H, 5.99; N, 7.67%. Calcd for $C_{37}H_{42}N_4O_{11}$: C, 61.83; H, 5.89; N, 7.80%.

3,2′,6′-Tri-N-*benzyloxycarbonyl-3′,4′-dideoxyneamine (**3**).* To a solution of **2** (620 mg) in dioxane (12 ml), an aqueous $Ba(OH)_2$ solution (5 ml, which contained 150 mg of $Ba(OH)_2 \cdot 8H_2O$) was added and the mixture was stirred at 60 °C for 1 hr. Another aliquot (5 ml) of $Ba(OH)_2$ solution was added and the solution was stirred for further 1 hr. On tlc with C_6H_6–EtOH (10 : 1), the mixture showed spots of R_f 0.06 (**3**, major), 0.16 (slight), 0.25 (slight) and 1 (**2**, slight). Carbon dioxide was introduced and after filtration, the solution was evaporated to give a solid (590 mg). The solid showed no peak near 1760 cm^{-1}. Since purification of the solid was tedious, the crude solid was used for the next step without purification.

3,2′,6′-Tri-N-*benzyloxycarbonyl-3′,4′-dideoxy-1*-N-[(S)*-2-hydroxy-*



4-phthalimidobutyryl)neamine (4). To an ice-cold solution of HPBA (136 mg) and NHS (69 mg) in THF (4 ml), DCC (120 mg) was added and the mixture was stirred for 1 hr in the cold. A suspension of well dried **3** (crude 290 mg) and triethylamine (30 mg) in dioxane (3 ml) was added and the mixture was stirred at room temperatures overnight. On tlc with $CHCl_3$–EtOH (20 : 1), the mixture showed a spot of **4** at R_f 0.33. After filtration, the solution was evaporated and the residue was chromatographed on a column of silica gel with $CHCl_3$–EtOH (20 : 1) to give a solid, 240 mg (62%), which was recrystallized from MeOH, mp 228—230 °C, $[\alpha]_D^{22}$ +32° (*c* 1.5, $CHCl_3$); IR (KBr): 1705, 1690, 1655, 1535 cm^{-1}.

Found: C, 61.34; H, 5.93; N, 7.39%. Calcd for $C_{48}H_{53}N_5O_{14}\cdot H_2O$: C, 61.20; H, 5.89; N, 7.43%.

1-N-[(S)-4-Amino-2-hydroxybutyryl]-3′,4′-dideoxyneamine (5). *a) From 4:* To a solution of **4** (111 mg) in dioxane-80% aqueous EtOH (1 : 1, 2 ml), 80% hydrazine hydrate (80 mg) was added and the mixture was heated at 60 °C for 2 hr. On tlc with EtAc–MeOH (1 : 1), the solution showed a ninhydrin-positive spot (R_f 0.13). The solution was evaporated and the residue was dissolved in 50% aqueous dioxane (2 ml). After addition of a drop of AcOH, the solution was hydrogenated with Pd black at 40—45 °C overnight. The mixture was filtered, evaporated, and the residue was chromatographed on a column of CM-Sephadex C-25 (NH_4 form, 10 ml) with H_2O and then with aqueous ammonia of 0.2—0.5 M. At about 0.4 M ammonia, **5** was eluted; a solid, 25 mg (53%), $[\alpha]_D^{22}$ +38° (*c* 0.85, H_2O); IR (KBr): 1650, 1560 cm^{-1}; $R_{f\ 3',4'\text{-dideoxyneamine}}$ 0.47 (ppc with 1-BuOH–pyridine–H_2O–AcOH (6 : 4 : 3 : 1)).

Found: C, 46.92; H, 8.52; N, 17.24%. Calcd for $C_{16}H_{33}N_5O_6\cdot H_2O$: C, 46.93; H, 8.62; N, 17.10%.

b) From 14: To a solution of **14** (101 mg) in dioxane–80% aqueous EtOH (1 : 1, 3 ml), 80% hydrazine hydrate (96 mg) was added and the solution was heated at 60 °C for 2 hr. On tlc with $CHCl_3$–MeOH–17% NH_3 (2 : 1 : 1), the solution showed a single spot (R_f 0.2) except **14** (R_f 0.7). The solution was evaporated and the residue was dissolved in 50% aqueous dioxane (3 ml). After addition of a drop of AcOH, the solution was hydrogenated with Pd black and then the product was purified similarly as described above to give a solid, 40 mg (93%), $[\alpha]_D^{20}$ +36° (*c* 1.2, H_2O). The IR and NMR spectra were identical with those of the specimen prepared from **4**.

1,3,2′,6′-Tetra-N-benzyloxycarbonylneamine (7). A solution of **6** (28.4 g) in 0.4 M methanolic hydrogen chloride (300 ml) was allowed to stand at room temperature. After 10 min, a solid began to precipitate. After overnight standing, the upper clear layer showed, on tlc with $CHCl_3$–EtOH (10 : 1), a spot of R_f 0.1 (**6** and methyl riboside; both gave the same R_f value) and the precipitate showed a spot of R_f 0.4 (**7**). The mixture was filtered and the solid was washed with hot MeOH to give a solid (19.24 g). From the methanol solution, another crop of the solid (2.43 g) was obtained. Total yield 21.7 g (88%). The solid was reprecipitated from hot dioxane–H_2O, $[\alpha]_D^{20}$ +44° (*c* 0.8, DMF); IR (KBr): 1705, 1695, 1535; 730, 695 cm^{-1}.

Found: C, 61.19; H, 5.87; N, 6.39%. Calcd for $C_{44}H_{50}N_4O_{14}$: C, 61.53; H, 5.87; N, 6.52%.

1,3,2′,6′-Tetra-N-benzyloxycarbonyl-5,6-O-cyclohexylideneneamine (8). To a solution of **7** (10 g) in DMF (50 °C, 100 ml), *p*-toluenesulfonic acid (390 mg) and 1,1-dimethoxycyclohexane (12 ml) were added and the solution was heated at 55 °C (bath temperature) under stirring *in vacuo* (25 Torr) for 1 hr. Another aliquots of *p*-toluenesulfonic acid (195 mg) and 1,1-dimethoxycyclohexane (11 ml) were added and the solution was again treated as above for 1.5 hr. After overnight standing at room temperatures, the solution showed, on tlc with $CHCl_3$–EtOH (25 : 1), spots of R_f 0.8 (dicycolhexylidene derivative), 0.3 (**8**, major) and 0.15 (slight, 3′,4′-*O*-cyclohexylidene isomer). The solution was poured into $NaHCO_3$ solution to give a precipitate (12.35 g). The solid was then treated with hot benzene to remove the benzene-soluble products (4.98 g; R_f 0.8 and 0.3 (slight)). The benzene-insoluble product (R_f 0.3 and 0.15 (slight)) was reprecipitat-

ed from $CHCl_3$–ether to give a solid, 5.72 g (52%), mp 185.5—187 °C, $[\alpha]_D^{20}$ +5.3° (*c* 0.9, $CHCl_3$); IR (KBr): 1705, 1535 cm^{-1}.

Found: C, 64.22; H, 6.29; N, 5.74%. Calcd for $C_{50}H_{58}N_4O_{14}$: C, 63.95; H, 6.23; N, 5.97%.

NMR (in DMSO-d_6): τ 8.5 (10H, broad, cyclohexylidene), 2.57 (20H s, $CH_2C_6H_5$).

1,3,2',6'-Tetra-N-*benzyloxycarbonyl-5,6*-O-*cyclohexylidene-3',4'-di*-O-*mesylneamine* (**9**). A sample of **8** was treated similarly as described in the preparation of compound **9** in a preceding paper;[2] yield 98%, $[\alpha]_D^{20}$ +11° (*c* 0.9, $CHCl_3$), R_f 0.35 (tlc with $CHCl_3$–EtOH (50 : 1)); IR (KBr): 1350, 1175 cm^{-1} (ν SO_2).

Found: C, 57.34; H, 5.76; N, 4.93; S, 5.89%. Calcd for $C_{52}H_{62}N_4O_{18}S_2$: C, 57.03; H, 5.71; N, 5.12; S, 5.85%.

NMR (in $CDCl_3$): τ 7.20 and 6.92 (each 3H s, SO_2CH_3).

1,3,2',6'-Tetra-N-*benzyloxycarbonyl-5,6*-O-*cyclohexylidene-3',4'-dideoxy-3'-enoneamine* (**10**). To a solution of **9** (1.60 g) in dry DMF (30 ml), sodium iodide (17 g) and zinc dust (7.8 g) were added and the mixture was stirred at 95—96 °C (oil bath temperature) for 1.5 hr. On tlc with $CHCl_3$–EtOH (50 : 1), the mixture showed spots at R_f 0.25 (**10**, major) and 0 (trace). The solution was then treated with $CHCl_3$ as described for compound **10** in the preceding paper[2] and the resulting solid was chromatographed on silica gel and $CHCl_3$–EtOH (50 : 1) to give a solid, 1.00 g (76%), which was recrystallized from EtOH, mp 198—199.5 °C, $[\alpha]_D^{20}$ −23° (*c* 0.8, $CHCl_3$); IR (KBr): 1705, 1525 cm^{-1} (peaks at 1350 and 1175 cm^{-1} in **9** had disappeared).

Found: C, 66.38; H, 6.29; N, 6.14%. Calcd for $C_{50}H_{56}N_4O_{12}$: C, 66.36; H, 6.24; N, 6.19%.

NMR (in $CDCl_3$): τ 4.43 (2H incomplete s, H-3',4').

1,3,2',6'-Tetra-N-*benzyloxycarbonyl-3',4'-dideoxy-3'-enoneamine* (**11**). To a solution of **10** (903 mg) in $CHCl_3$ (15 ml). *p*-toluenesulfonic acid hydrate (21 mg) in MeOH (7 ml) was added and the solution was heated at 50 °C for 1.5 hr. After triethylamine (0.03 ml) was added, the solution was evaporated to give a solid, which was washed throughly with H_2O; yield 810 mg (98%). The solid was recrystallized from EtOH, mp 234—238 °C, $[\alpha]_D^{20}$ −26° (*c* 0.8, dioxane).

Found: C, 64.02; H, 5.92; N, 6.77%. Calcd for $C_{44}H_{48}N_4O_{12}$: C, 64.07; H, 5.87; N, 6.79%.

NMR (in DMSO-d_6): τ 4.31 (2H incomplete s, H-3',4'), 2.5—3.0 (4H, disappeared on deuteration, $NHCO_2$-).

3,2',6'-Tri-N-*benzyloxycarbonyl-3',4'-dideoxy-3'-enoneamine 1,6-Carbamate* (**12**). The solution of **11** (917 mg) in DMF (10 ml) was treated with 50% NaH (167 mg) as described for the preparation of **2**. The reaction completed in 2 hr. The crude product (880 mg) was chromatographed on a column of silica gel with $CHCl_3$–EtOH (15 : 1) to give a solid, 577 mg (73%), which was reprecipitated from $CHCl_3$–*n*-hexane, $[\alpha]_D^{20}$ −19° (*c* 1, dioxane); R_f 0.35 (tlc with $CHCl_3$–EtOH (15 : 1); **11**: R_f 0.40); IR (KBr): 1770, 1700, 1525 cm^{-1}.

Found: C, 62.03; H, 5.82; N, 7.88%. Calcd for $C_{37}H_{40}N_4O_{11}$: C, 62.00; H, 5.63; N, 7.82%.

NMR (in $CDCl_3$): τ 4.43 (2H incomplete s, H-3',4').

3,2',6'-Tri-N-*benzyloxycarbonyl-3',4'-dideoxy-3'-enoneamine* (**13**). To a solution of **12** (361 mg) in aqueous dioxane (12 : 13, 12 ml), anhydrous Na_2CO_3 (447 mg) was added and the mixture was heated at 80 °C for 2 hr. The solution was cooled and then filtered from precipitants. The filtrate was evaporated to give a solid, which was extracted with chloroform. The solution was evaporated to give a solid, 287 mg (83%), which was reprecipitated from $CHCl_3$–*n*-hexane, $[\alpha]_D^{20}$ −19° (*c* 1.1, $CHCl_3$); R_f 0.5 (tlc with $CHCl_3$–MeOH–17% NH_3 (2 : 1 : 1); **12**: R_f 0.65); IR (KBr): 1700, 1535 cm^{-1}.

Found: C, 62.31; H, 6.08; N, 7.65%. Calcd for $C_{36}H_{42}N_4O_{10}$: C, 62.60; H, 6.13; N, 8.11%.

NMR (in $CDCl_3$): τ 4.33 (2H broad s, H-3', 4').

3,2',6'-Tri-N-*benzyloxycarbonyl-3',4'-dideoxy-3'-eno-1*-N-[*(S)-2-hydroxy-4-phthalimidobutyryl*]*neamine* (**14**). The active ester prepared from HPBA (138 mg), NHS (66 mg) and DCC (184 mg) in THF was treated with **13** (227 mg) in dioxane containing triethylamine (38 mg) in a manner as described for the preparation of **4**. The crude product (550 mg) was dissolved in $CHCl_3$ and the solution was washed with water. The product was chromatographed on a column of silica gel with $CHCl_3$–EtOH (15 : 1) to give a solid, 155 mg (51%), which was recrystallized from EtOH, $[\alpha]_D^{20}$ −32° (*c* 1.3, dioxane); R_f 0.25 (tlc with $CHCl_3$–EtOH (15 : 1); **13**: R_f 0); IR (KBr): 1780 (5-membered imide), 1710, 1640, 1540 cm^{-1}.

Found: C, 62.76; H, 5.65; N, 7.33%. Calcd for $C_{48}H_{51}N_5O_{14}$: C, 62.53; H, 5.58; N, 7.60%.

NMR (in DMSO-d_6): τ 4.37 (2H, H-3',4'), 2.08 (4H s, $(CO)_2C_6H_4$).

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