Aminoglycoside Antibiotics. 1. Regiospecific Partial Syntheses of Ribostamycin and Butirosin B

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Received January 26, 1978

A regiospecific partial synthesis of ribostamycin from neamine was developed. It involved the use of carbobenzyloxy groups and derived cyclic carbamate groups to protect all functional groups except for the 3′- and 5-hydroxyl groups, followed by selective protection of the 3′-hydroxyl group by tosylation or tetrahydropyranyl ether formation. Condensation with protected ribosyl chloride followed by deblocking then gave ribostamyin. A related approach was utilized for the synthesis of butirosin B. In this case the side chain, which was protected as a cyclic carbamate, could be deprotected by alkaline hydrolysis without cleavage of the side chain.

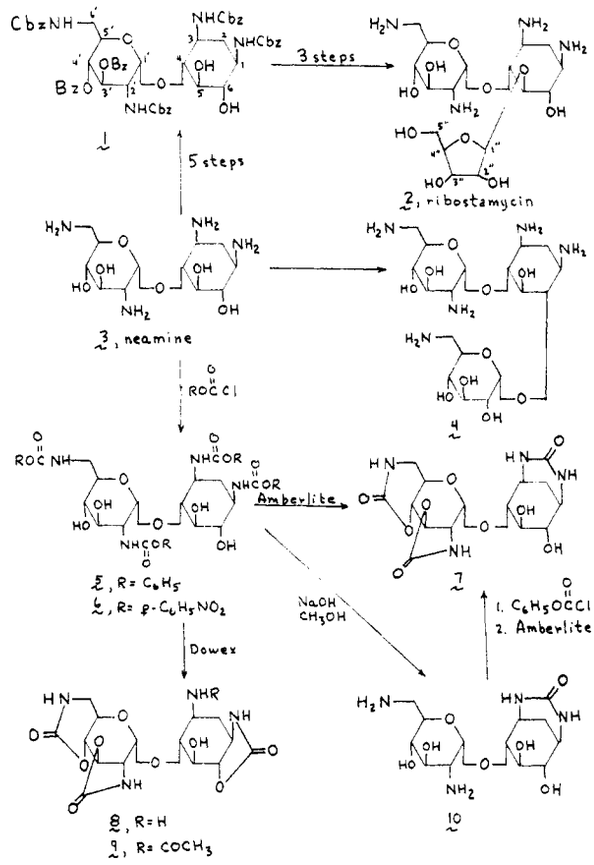
In recent years the preparation of semisynthetic aminoglycoside antibiotics has become increasingly important. Advantages shown by new compounds such as amikacin[1] and 3,4-dideoxykanamycin B[2] over their parent antibiotics, especially in their activities against resistant strains of bacteria, have stimulated research in this area. These analogues and certain others are the result of chemical modification of intact antibiotics. An alternative approach to the development of new aminoglycosides is to begin with a portion of the structure, for example the neamine or paromamine molecule, and add another sugar by glycosidic linkage. This approach has the advantage of affording new combinations of the constituent parts of aminoglycosides not accessible by modification of the intact antibiotics. Its main disadvantage lies in problems of regiospecificity and anomeric specificity in forming the glycosidic bond with the new sugar.

Our general objective in aminoglycoside synthesis is to develop regioselective methods for compounds containing the neamine unit. This unit is important because it occurs in the structures of a number of significant antibiotics including kanamycin B, neomycin, ribostamycin, and the butirosins.[3] Other investigators have addressed themselves to this problem with varying degrees of success. Substitution at O-6 of neamine was obtained in the synthesis of kanamycin B[4] and in two different syntheses of 6-*O*-(β-D-ribofuranosyl)neamine.[5,6] The synthesis of tobramycin from nebramine (3′-deoxyneamine)[7] and the syntheses of kanamycin C from paromamine were closely related problems.[8] Two syntheses of 6-*O*-(β-D-ribofuranosyl)paromamine from paromamine were reported.[9,10] The synthesis of ribostamycin from neamine provided an example of 5-O-glycosidation.[11]

The regiospecific synthesis of ribostamycin from neamine became our first specific objective. Ito and co-workers had already accomplished this goal,[11] but we felt that their method was difficult and possibly not general. They utilized intermediate **1**, which had been developed by Umezawa and coworkers for the synthesis of kanamycin B[4] (**4**, Scheme I). Its preparation involved a difficult separation of isomeric monoketals. Furthermore, this intermediate has both 5- and 6-hydroxyl groups free. Ito reported selective glycosidation on the 5-hydroxyl group with tri-*O*-D-ribosyl chloride. However, Umezawa used the same intermediate for 6-O-glycosidation in the kanamycin B synthesis. A variety of other neamine derivatives with both 5- and 6-hyroxyl groups unprotected also gave preferential 6-O-glycosidation.[6,12] For this reason we decided to prepare a neamine derivative whose 5-hydroxyl group was the only unprotected function. Then there would be no doubt about the regiospecificity.

Inspection of the neamine structure revealed that every hydroxyl group, except the 5-hydroxyl group, was near enough to an amino group that a cyclic carbamate derivative could be formed by utilizing these functional groups. Thus, we visualized the tris(cyclic carbamate) **8** in which only the 5-hydroxyl group and possibly the 3-amino group would be free. The cyclic carbamate group had been first described in carbohydrate chemistry by Miyai and Gross.[13] Umezawa prepared the bis(cyclic carbamate) of 2-deoxystreptamine.[14]

Scheme I



One method for cyclic carbamate synthesis is to prepare the phenylcarbamate derivative of the amino group and then treat the compound with base[14] (Scheme I). This treatment results in the displacement of phenoxide by a proximate hydroxyl group with formation of the cyclic derivative. We readily prepared the tetraphenylcarbamate and tetra-*p*-nitrophenylcarbamate derivatives (**5** and **6**) of neamine. Treatment of either derivative with the weakly basic resin Amberlite IR-45 (OH^- form) resulted in the nearly quantitative formation of a bis(cyclic carbamate) derivative (**7**) which also had a cyclic urea function. However, when the strongly basic resin Dowex 1 × 2 (OH^- form) was used the desired tris(cyclic carbamate) **8** was formed in 60% yield. In the course of this reaction, the 3-amino group became deprotected. It was readily reprotected as its acetyl derivative (e.g., **9**).

The presence of a cyclic urea group in compound **7** was es-

0022-3263/78/1943-3327$01.00/0

tablished as follows. It lacked the infrared absorption at 5.63 μm characteristic of the N^1, O^6-five-membered cyclic carbamate group, but it had absorption at 5.71 μm characteristic of a six-membered cyclic urea. Acetylation did not occur with acetic anhydride in dry methanol, but a di-O-acetylated product was obtained with acetic anhydride in pyridine. This result shows that **7** had two free hydroxyl groups, but no free amino group. Finally, the preparation of a known[15] $N,^1N^3$-cyclic urea derivative (**10**) from tetra-*N*-phenoxycarbonylneamine and methanolic sodium hydroxide was repeated and the product was converted into **7** by preparation of the 2′,6′-di-*N*-phenoxycarbonyl derivative followed by treatment with Amberlite IR-45 in the OH^- form.

Tri-*O*-acetylribosyl chloride and tri-*O*-benzoylribosyl chloride were prepared by known procedures[16,17] and their coupling with intermediates **7** and **9** was attempted. Unfortunately, both of these intermediates were nearly insoluble in solvents such as benzene and dichloromethane which favor Koenigs–Knorr couplings with five-membered ring sugar halides. They were soluble in *N,N*-dimethylformamide (DMF), but the ribosyl chlorides decomposed without coupling in this solvent. A variety of promoters, including Drierite, mercuric cyanide, mercuric chloride, and silver triflate, were used, but without success.

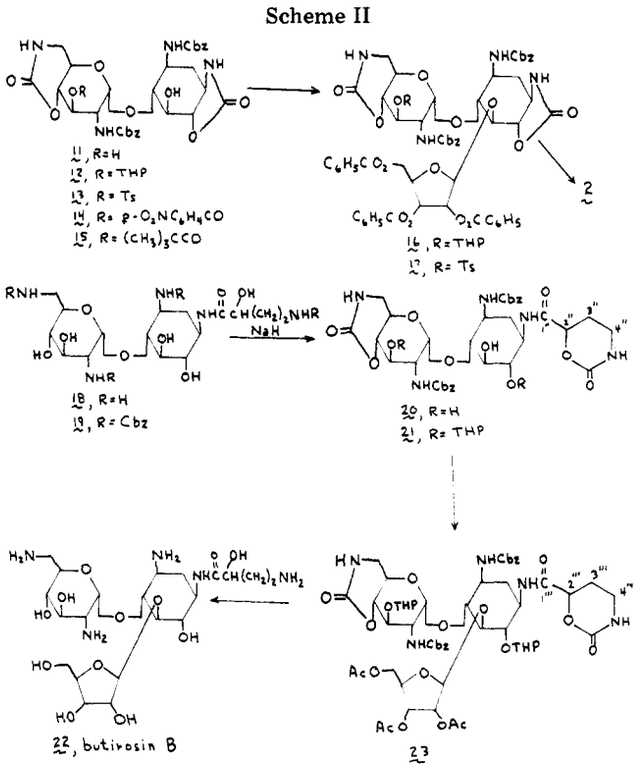
This failure led us to look for more soluble cyclic carbamate derivatives of neamine. The bis(cyclic carbamate) **11** was readily prepared by treating tetra-*N*-carbobenzyloxyneamine with sodium hydride in DMF (the carbobenzyloxy derivatives do not react with Amberlite or Dowex resins). Under the conditions utilized, the third cyclic carbamate group does not form. The product (**11**) had both the 3′- and 5′-hydroxyl groups free, which required that we find a selective method for protecting the former hydroxyl group. Among a variety of derivatives prepared from **11** we found that the tetrahydropyranyl ether (THP) and the *p*-toluenesulfonate (Ts) formed exclusively and in high yield on the 3′-hydroxyl group. Other derivatives including *p*-nitrobenzoate and pivalate esters **14** and **15** were formed less selectively and they were poorly soluble in cholorform. Both the THP derivative (**12**) and the tosylate (**13**) had good solubility in chloroform, which allowed them to be used in Koenigs–Knorr condensations under optimum conditions.

Treatment of either **12** or **13** with tri-*O*-benzoylribosyl chloride in chloroform in the presence of mercuric cyanide and Drierite gave the glycosides **16** or **17** in approximately 50% yields (Scheme II). Hydrolysis of the benzoyl and cyclic carbamate groups with barium hydroxide, followed by catalytic hydrogenolysis, then gave the desired ribostamycin (**2**). It showed one spot on thin-layer chromatography and it had an R_f value identical with that of authentic ribostamycin in two solvent systems. The specific optical rotations of the two samples were nearly identical (Experimental Section) and their infrared spectra were superimposable.

Although ribostamycin is almost as potent as butirosin in antibacterial activity, butirosin is active against more strains of bacteria because its (*S*)-4-amino-2-hydroxybutyryl side chain inhibits inactivation by bacterial enzymes.[18] The commercial butirosin is a mixture of butirosin A and butirosin B. These two components differ in the nature of the pentose at O-5. Butirosin A has D-xylose, whereas butirosin B has D-ribose at this position.[19] Our second goal was the synthesis of butirosin analogues in which new sugars replaced the xylose or ribose unit. Again, the best way to develop intermediates and methodology for these analogues appeared to be the prior synthesis of a parent compound, butirosin B (**22**), since we could readily confirm the regiospecificity and anomeric purity of the product.

The hydrolysis of butirosin to 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]neamine (**18**) had been reported previously.[20]

Scheme II



We chose the latter compound as our starting material.[21] It was converted into its tetra-*N*-carbobenzyloxy derivative **19** and then treated with sodium hydride in DMF to give the bis(cyclic carbamate) **20.** This product has only its 3′-, 5-, and 6-hydroxyl groups free. There was some doubt as to whether we could selectively block the 3′- and 6-hydroxyl groups, but this operation was done in high yield by way of the THP derivative **(21).** Coupling of **21** with tri-*O*-acetylribosyl chloride gave the glycoside **(23)** in 40% yield and deblocking by alkaline hydrolysis and hydrogenolysis afforded butirosin B **(22).** No hydrolysis of the side chain occurred under the conditions used for cyclic carbamate hydrolysis. The structure and purity of this butirosin B were confirmed by its R_f value on TLC, which was identical with that of authentic material in two solvent systems, and the comparison of the specific optical rotations and infrared spectra of these two samples (Experimental Section).

In summary, we have developed synthetic procedures and intermediates for the regiospecific syntheses of ribostamycin and butirosin B. These procedures do not require any difficult separations of isomers and anomers. The use of these intermediates for the synthesis of novel ribostamycin and butirosin analogues is in progress.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were determined on a Beckman IR-33 spectrophotometer as KBr pellets. Nuclear magnetic resonance spectra were recorded on a Varian EM-360 and T-60 spectrometers using Me_4Si or sodium 2,2-dimethyl-4-silapentane-5-sulfonate as the standard. Optical rotations were taken on a Carl Zeiss OLD4 automatic polarimeter under the indicated conditions. Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Purdue University and Chemalytics, Inc., Tempe, Arizona.

Tetra-*N*-phenoxycarbonylneamine (5). An ice-cooled solution of neamine hydrochloride (0.51 g, 1.08 mmol) and sodium bicarbonate (1.68 g, 20 mmol) in 22 mL of water was treated dropwise over 10 min with a solution of phenyl chloroformate (2.5 g, 15.5 mmol) in 36 mL of acetone. The mixture was stirred 30 min more and neutralized with 10% hydrochloric acid. The resulting precipitate was washed with ether and water and vacuum dried to give 0.62 g (72%) of **5** as a white solid: mp 266 °C dec; $[\alpha]^{22}_{546}$ +53.8° (*c* 1.0, DMF); IR 1720 and 1705

(NHCO I), 1525 cm^{-1} (NHCO II); NMR (Me_2SO-d_6) δ 6.9–7.95 (phenyl). In subsequent experiments the product was obtained in yields up to 85% by partially concentrating the reaction mixture and adding more water.

Anal. Calcd for $C_{40}H_{42}N_4O_{14}$: C, 59.85; H, 5.24; N, 6.98. Found: C, 59.66; H, 5.33; N, 6.92.

Tetra-*N*-*p*-nitrophenoxycarbonylneamine (6). An ice-cooled solution of neamine hydrochloride (0.365 g, 0.810 mmol) and sodium bicarbonate (0.70 g, excess) in 6 mL of water was treated over 10 min with a solution of *p*-nitrophenoxycarbonyl chloride (9.8 g, 3.64 mmol) in 10 mL of acetone. The precipitated product was washed with ether and water and vacuum dried to give 0.65 g (95%) of **6** as white powder; mp 210 °C dec; $[\alpha]_{546}^{22}$ +48.95° (*c* 1.24, DMF); IR 1715 (NHCO I), 1515 (NHCO II), 1340 cm^{-1} (NO_2): NMR (Me_2SO-d_6) δ 7.45 (d, J = 4 Hz, aromatic) and 8.15–8.40 (d, J = 4 Hz, aromatic).

Anal. Calcd for $C_{40}H_{38}N_8N_8O_{22}\cdot 2H_2O$: C, 47.15; H, 4.13; N, 11.00. Found: C, 47.17; H, 4.26; N, 10.74.

2′,3′:4′,6′-*N*,*O*-Carbonyl-1,3-*N*,*N*-carbonylneamine (7). From Tetra-*N*-phenoxycarbonylneamine (5) or Tetra-*N*-*p*-nitrophenoxycarbonylneamine (6). A solution of **5** (0.25 g, 0.25 mmol) or an equivalent amount of **6** in 20 mL of freshly distilled *N*,*N*-dimethylformamide (DMF) was stirred with 6.0 g of DMF-washed Amberlite IR-45 (OH^-) at 25 °C for 18 h, filtered through a pad of diatomaceous earth, and concentrated under reduced pressure. Treatment of the residue with ether gave 1.20 g (98%) as white powder: mp >250 °C dec; $[\alpha]_{546}^{22}$ +66.4° (*c* 1.0, DMF); IR 1770 (carbamate, five-membered), 1760 (ureide), 1720 (carbamate, six-membered), 1550 and 1535 cm^{-1} (NHCO II of ureide).

Anal. Calcd for $C_{15}H_{20}N_4O_9\cdot 2H_2O$: C, 41.28; H, 5.50; N, 12.84. Found: C, 41.43; H, 5.56; N, 12.84.

From 1,3-*N*,*N*-Carbonylneamine (10). An ice-cooled solution of **10** (0.55 g, 0.91 mmol) and sodium bicarbonate (0.35 g, 4.16 mmol) in 6 mL of water was treated with phenyl chloroformate (0.61 g, 3.9 mmol) in 9 mL of acetone. After 1.5 h the mixture was neutralized with hydrochloric acid and concentrated under reduced pressure. A DMF extract of the residue was concentrated and diluted with ether to give a solid. Recrystallization from DMF–ether gave 0.17 g of 1,3-*N*,*N*-carbonyl-2′,6′-di-*N*-phenoxycarbonylneamine as a white powder: mp 236–238 °C dec; IR 1720, 1710 (NHCO I), and 1520 cm^{-1} (NHCO II). Recrystallization of a small portion of this product from DMF–ether gave an analytical sample of mp 252–254 °C dec.

Anal. Calcd for $C_{27}H_{32}N_4O_{11}$: C, 55.10; H, 5.44; N, 9.52. Found: C, 54.81; H, 5.70; N, 9.23.

A solution of the above compound (0.10 g) in 10 mL of DMF was stirred with 3.0 g of DMF-washed Amberlite IR-45 resin for 15 h at 25 °C. The mixture was filtered through diatomaceous earth, concentrated under reduced pressure, and diluted with ether to give 0.049 g of **7** as a white solid identical in infrared spectrum and R_f value on TLC (acetone–DMF on silica gel) with the sample of **7** prepared as described above.

1,6:2′,3′:4′,6′-*N*,*O*-Carbonylneamine (8). A solution of tetra-*N*-phenoxycarbonylneamine (**5**; 0.90 g, 1.12 mmol) or an equivalent amount of **6** in 25 mL of freshly distilled DMF was stirred with 10.0 g of Dowex 1 × 2 resin (OH^-) at 25 °C for 20 h. The mixture was filtered, the resin was washed with DMF, and the combined filtrate and wash was concentrated to a small volume and treated with acetone. A resulting white precipitate gave 0.08 g (15%) of **7** after it was washed thoroughly with acetone and dried under vacuum. The resin was suspended in 10 mL of water, neutralized with 10% hydrochloric acid and washed with water and DMF. The combined washes and filtrate were treated with Amberlite IR-45 (OH^-) for 30 min. The mixture was filtered and the filtrate was concentrated to a small volume and diluted with acetone. A product that precipitated was recrystallized from aqueous methanol–acetone to give 0.345 g (76%) of **8**: mp >250 °C dec; $[\alpha]_{546}^{22}$ +76.10° (*c* 1.10, DMF); IR 1770 (carbamate, five-membered) and 1720 cm^{-1} (carbamate, six-membered).

Anal. Calcd for $C_{15}H_{20}N_4O_9\cdot 1.5H_2O$: C, 52.15; H, 5.38; N, 13.11. Found: C, 52.25; H, 5.53; N, 13.40.

3-*N*-Acetyl-1,6:2′,3′:4′,6′-*N*,*O*-carbonylneamine (9). A stirred suspension of compound **8** (0.10 g, 0.25 mmol) in 5 mL of dry methanol was treated with 0.5 mL of dry methanol and 0.5 mL of acetic anhydride and the resulting mixture was stirred at 25 °C for 20 h. It was concentrated to dryness and the residue was crystallized from aqueous ethanol to give 0.082 g (74%) of **9**: mp >240 °C dec; $[\alpha]_{546}^{22}$ +70.0° (*c* 0.5, DMF); IR 1765 (carbamate, five-membered), 1720 (carbamate, five-membered), 1650 (NHCO I), and 1525 cm^{-1} (NHCO II).

Anal. Calcd for $C_{17}H_{22}N_4O_{10}\cdot 4H_2O$: C, 39.69; H, 4.83; N, 10.89. Found: 39.61; H, 5.81; N, 10.92.

1,3-*N*,*N*-Carbonylneamine (10). A solution of tetra-*N*-phenoxycarbonylneamine (**5**; 0.20 g, 0.025 mmol) in 10 mL of dry methanol was treated with sodium hydroxide (0.16 g, excess) and the resulting solution was heated under reflux for 26 h, cooled, neutralized with acetic acid, and concentrated to dryness. A DMF extract of the residue was filtered, concentrated to a small volume, and diluted with acetone, whereupon a white solid precipitated. Two recrystallizations from DMF–ether gave 0.072 g (86%) of **10**, mp 278 °C dec; IR 1720 and 1525 (NHCONH I and II), 1585 cm^{-1} (NH_2); NMR (Me_2SO-d_6) showed the NHCONH protons as a broad peak at δ 6.50–6.74.

Anal. Calcd for $C_{13}H_{24}N_4O_7$: C, 44.82; H, 6.89; N, 16.09. Found: C, 44.99; H, 6.62; N, 16.11.

Tetra-*N*-benzyloxycarbonylneamine. A mixture of neamine hydrochloride (0.5 g, 1.06 mmol) and sodium bicarbonate (1.7 g) in 70% aqueous methanol (20 mL) was cooled with stirring in an ice bath and benzyl chloroformate (2.0 g, 11.72 mmol) was added dropwise with the aid of 5 mL of methanol. The reaction mixture was stirred for 2 h at 0 °C and then evaporated to dryness under reduced pressure. The residual product was extracted three times with 25 mL of dry dioxane. The dioxane layer was concentrated to a small volume and treated with ether. The resulting white solid was washed several times with dry ether to give a pure product: 0.76 g (84%); mp 259 °C dec [lit.[6] mp 259 °C dec]; $[\alpha]_{546}^{22}$ +51.0° (*c* 1.0, DMF); IR (KBr) 3580–3220 (OH and NH), 1710, 1700 and 1695 (NHCO I), and 1530 cm^{-1} (NHCO II). This method was simpler and faster than that reported in the literature.[6]

3,2′-Di-*N*-benzyloxycarbonyl-1,6:4′,6′-*O*,*N*-carbonylneamine (11). A solution of tetra-*N*-benzyloxycarbonylneamine (1.0 g, 1.10 mmol) in 15 mL of dry DMF was cooled to 0 °C in an ice bath. The reaction vessel was evacuated and filled with dry nitrogen. Sodium hydride (0.15 g, 50% in oil, 3.125 mmol) was added and the reaction mixture was stirred 30 min at this temperature and 2 h at room temperature (25 °C). After 1 h of stirring the reaction mixture solidified and more dry DMF was added. The reaction mixture was neutralized with glacial acetic acid, concentrated under reduced pressure, and treated with ice cold water. The resulting white solid was filtered and washed thoroughly with water and ether to yield **11**: 0.66 g (86%); mp 230 °C dec; TLC showed a single spot (acetone–DMF); $[\alpha]_{546}^{22}$ +32.57° (*c* 0.7, DMF); IR (KBr) 3600–3200 (OH and NH), 1770 (carbamate, five-membered), 1725 (carbamate, six-membered), 1710 (NHCO I), and 1540 cm^{-1} (NHCO II); NMR (Me_2SO-d_6) δ 5.1 (6s, anomeric proton), 7.3 (s, aromatic protons) and 8.2 (6s, carbamate protons). A small sample was recrystallized from DMF and ether.

Anal. Calcd for $C_{30}H_{34}N_4O_{12}$: C, 55.05; H, 5.37; N, 8.60. Found: C, 55.20; H, 5.67; N, 8.40.

3,2′-Di-*N*-benzyloxycarbonyl-1,6:4′,6′-*N*,*O*-carbonyl-3′-*O*-(2-tetrahydropyranyl)neamine (12). A stirred solution of **11** (0.5 g, 0.78 mmol) in 5 mL of DMF was treated with 5 mL of 2,3-dihydropyran and 20 mg of *p*-toluenesulfonic acid. The mixture was stirred at 25 °C for 1 h, treated with 0.1 mL of triethylamine, and concentrated to dryness. A chloroform extract of the residue was washed with water, dried over $MgSO_4$, and concentrated. Addition of ether to the residue gave a white solid which showed on TLC (4:1 benzene–acetone on silica gel) one major spot and a fast-moving minor spot. The solid was purified on a short silica gel column. After elution of the impurity with benzene the product was eluted with 4:1 benzene–acetone. Recrystallization from chloroform–ether gave 0.455 g (80%) of **12**: mp 158–166 °C dec; $[\alpha]_{546}^{22}$ +22.84° (*c* 1.12, $CHCl_3$); NMR δ 5.2 (anomeric proton of sugar), 6.1 (anomeric proton of tetrahydropyranyl group).

Anal. Calcd for $C_{35}H_{42}N_4O_{13}$: C, 57.85; H, 5.78; N, 7.17. Found: C, 5.77; H, 5.85; N, 7.40.

3,2′-Di-*N*-benzyloxycarbonyl-1,6:4′,6′-*N*,*O*-carbonyl-3-*O*-*p*-toluenesulfonylneamine (13). A solution of **11** (0.550 g, 0.85 mmol) and recrystallized *p*-toluenesulfonyl chloride (1.0 g, 5.2 mmol) in 25 mL of dry pyridine was stirred at 25 °C for 24 h. The brown solution was concentrated under reduced pressure and the residual gum was triturated several times with ether. An excess of cold water was added to precipitate a slightly colored product. The TLC in acetone showed one major product with a minor fast moving spot. The crude product was purified on a silica gel column with benzene–acetone (1:1) and recrystallization from acetone–petroleum ether (30–60 °C) to give the monotosylate **13**: 0.480 g (70%); mp 170–172 °C; $[\alpha]_{546}^{22}$ (*c* 0.85, DMF); IR 1770 (carbamate, six-membered) 1710 (NHCO I), 1600 (aromatic C=C), 1520 (NHCO II), and 1175 cm^{-1} (SO_2); NMR (Me_2SO-d_6) δ 2.3 (3, s, CH_3), 4.9 (2, d aromatic protons), 7.3 (2, d, aromatic protons), and 7.6 (m, NH).

Anal. Calcd for $C_{37}H_{40}O_{14}N_4S$: C, 55.78; H, 5.02; N, 7.03; S, 6.02. Found: C, 55.81; H, 5.18; N, 6.78; S, 4.07.

3,2′-Di-*N*-benzyloxycarbonyl-1,6:4′,6′-*N*,*O*-carbonyl-3′-*O*-*p*-nitrobenzoylneamine (14). A stirred solution of **11** (0.43 g, 0.67 mmol) in 10 mL of dry pyridine was treated with *p*-nitrobenzoyl

chloride (0.30 g, 1.6 mmol). After 18 h another 0.30 g of *p*-nitrobenzoyl chloride was added and the solution was stirred at 25 °C for 20 h. It was then concentrated and the residue was washed with ether. Trituration of the gummy product with water gave a white solid which was shown by TLC (1:1 benzene–acetone on silica gel) to contain one major product and several minor ones. Column chromatography on silica gel with benzene and increasing proportions of acetone afforded compound **14**. Recrystallization from *p*-dioxane–hexane gave 0.32 g (60%) of white solid: mp 211–212 °C dec; $[\alpha]_{546}^{22}$ +36.0° (*c* 1.0, DMF); IR 1720 and 1340 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 8.10–8.40 (4, m, protons of *p*-nitrobenzoyl group).

Anal. Calcd for $C_{37}H_{37}N_5O_{15}\cdot H_2O$: C, 54.88; H, 4.82; N, 8.65. Found: C, 54.78; H, 4.69; N, 8.45.

3,2′-Di-*N*-benzyloxycarbonyl-1,6:4′,6′-*N*,*O*-carbonyl-3′-*O*-trimethylacetylneamine (15). A solution of compound **11** (0.214 g, 0.33 mmol) in 5 mL of dry pyridine was treated with 0.4 mL (excess) of trimethylacetyl chloride. The mixture was stirred at 24 °C for 38 h and concentrated under reduced pressure, and the residue was treated with water to give a solid. TLC (3:2 acetone–benzene) on silica gel showed one major product and several minor ones. The major product was isolated by preparative TLC with the same solvent system and then it was crystallized from *p*-dioxane and petroleum ether (30–60 °C). This procedure gave 0.080 g (33%) of **15** as white crystals: mp 258 °C dec; $[\alpha]_{546}^{22}$ +40.8° (*c* 0.35, DMF); IR 1735 cm^{-1} (COO).

Anal. Calcd for $C_{35}H_{42}N_4O_{13}$: C, 57.85; H, 5.78; N, 7.71. Found: C, 57.64; H, 5.70; N, 7.49.

2″,3″,5″-Tri-*O*-benzoyl-3,2′-di-*N*-benzyloxy-1,6:4′,6′-*N*,*O*-carbonyl-3′-*O*-(2-tetrahydropyranyl)ribostamycin (16). A mixture of **12** (0.36 g, 0.49 mmol), vacuum dried mercuric cyanide (.032 g), Drierite (2.5 g, dried over an open flame for 2 h), and chloroform (60 mL, alcohol free) was stirred at 25 °C for 20 h. A solution of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl chloride (prepared[13] from 600 mg of the corresponding acetate) in 6 mL of dry chloroform was added and the mixture was stirred under reflex for 48 h. Another portion of the halosugar in chloroform (half of the original amount) was added and reflux was continued for 24 h. Another portion of the halosugar in chloroform (half of the original amount) was added and reflux was continued for 24 h. TLC (1:1 benzene–acetone) then showed no starting material remaining. The mixture was filtered through a pad of diatomaceous earth and this pad was washed with dry chloroform. The combined filtrate and wash was concentrated to dryness and the semisolid residue was chromatographed on a column of silica gel with benzene containing an increasing proportion of ethyl acetate (0–100%) as solvent. Concentration of the main fraction (eluted by 4:1 chloroform–methanol) gave white solid which yielded after recrystallization from ethyl acetate–petroleum ether (30–60 °C) 0.36 g (61%) of **16**: mp 135–145 °C dec; $[\alpha]_{546}^{22}$ +86.77° (*c* 0.93, $CHCl_3$); IR 1730 cm^{-1} ($C_6H_5CO_3$); NMR ($CDCl_3$) δ 5.5 (m, anomeric proton of tribenzoylribosyl group).

Anal. Calcd for $C_{61}H_{62}N_4O_{20}$: C, 62.56; H, 5.35; N, 4.78. Found: C, 62.27; H, 5.34; N, 4.88.

2″,3″,5″-Tri-*O*-benzoyl-3,2′-di-*N*-benzyloxycarbonyl-1,6:-4′,6′-*N*,*O*-carbonyl-3′-*O*-*p*-toluenesulfonylribostamycin (17). A stirred mixture of **13** (0.75 g, 0.94 mmol), vacuum dried mercuric cyanide (0.75 g), Drierite (5.0 g, dried 2 h over an open flame), 50 mL of chloroform, and 25 mL of dioxane was treated with 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl chloride (prepared[17] from 1.2 g of the corresponding acetate) in 10 mL of dry chloroform. The mixture was stirred at reflux for 80 h and filtered through a pad of diatomaceous earth. This pad was washed thoroughly with chloroform–dioxane (1:1) and the combined filtrate and wash was concentrated. A chloroform extract of the residue was chromatographed on a column of silica gel with benzene containing increasing amounts of ethyl acetate (0–100%) as solvent. The major product, eluted by 1:1 benzene–acetone, was crystallized from chloroform–hexane to give 0.74 g (63%) of **17** as white solid: mp 205–207 °C dec; IR 1730 cm^{-1} ($C_6H_5CO_2$); NMR ($CDCl_3$) δ 5.6 (m, anomeric proton of tribenzoylribosyl group).

Anal. Calcd for $C_{63}H_{60}N_4O_{21}S$: C, 60.96; H, 4.84; N, 4.52; S, 2.58. Found: C, 61.04; H, 5.01; N, 4.54; S, 2.60.

Ribostamycin (2). From Compound 16. A solution of 1.0 g of **16** in 10 mL of dioxane and 10 mL of water was warmed at 60 °C and treated with 20 mL of aqueous 1 N barium hydroxide solution. The mixture was stirred at this temperature for 16 h with additional barium hydroxide solution added occassionally to maintain strong alkalinity. The mixture was neutralized by carbon dioxide gas and then filtered through a pad of diatomaceous earth. This pad was washed with aqueous DMF and the combined filtrate and wash was concentrated under reduced pressure. Addition of water gave a brownish solid that was collected, washed with water and acetone, and dried in air. The resulting brownish solid (0.70 g) had IR absorption at 1700 and 1520 cm^{-1} (NHCO I and II), but no bands for cyclic carbamate or benzoate ester groups. It was dissolved in 20 mL of dioxane and 20 mL of water, treated with 4 mL of 1 N hydrochloric acid and 0.30 g of 10% palladium-on-charcoal, and hydrogenated at 50 psi and 25 °C for 24 h. The catalyst was removed by filtration and the filtrate was neutralized with Amberlite IR-45 resin (OH^-) and concentrated to a small volume. Addition of methanol and acetone to the residue gave a slightly colored product which was purified by a sequence of operations involving column chromatography on Amberlite IR-C50 (NH_4^+) with 0–0.5 N ammonium hydroxide solution as solvent, crystallization from aqueous methanol–acetone, chromatography on a column of Amberlite IR-400 (OH^-) with water as solvent, concentration to a small volume, and addition of methanol and acetone. This procedure gave 72 mg (19%) of ribostamycin (**2**) as a white solid whose IR absorption spectrum was identical with an authentic sample obtained from Mieji Laboratories. The two samples had identical R_f values on TLC in the systems chloroform–methanol–28% ammonium hydroxide–water (1:4:2:1), lower layer of the system chloroform–methanol–28% ammonium hydroxide (1:1:1), and upper layer of the preceding system. The synthetic sample had $[\alpha]_{546}^{22}$ +36.0° (*c* 0.52, H_2O), whereas the authentic sample had +37.8° under the same conditions.

From Compound 17. To a solution of **17** (0.70 g) in 25 mL of dry methanol was added small chips of sodium metal (0.010 g). The mixture was stirred at 25 °C for 16 h, neutralized with hydrochloric acid, and concentrated to dryness. A DMF extract of the residue was filtered through a pad of diatomaceous earth, concentrated to a small volume, and diluted with ether. The white solid that formed (0.55 g) was dissolved in 10 mL of water and 10 mL of dioxane, treated with 10 mL of 0.05 M barium hydroxide solution, and stirred at 60 °C for 3 h. Another 10 mL of the barium hydroxide solution was added and stirring at 60 °C was continued for 15 h. The mixture was neutralized with carbon dioxide gas and then filtered through a pad of diatomaceous earth. A DMF wash of this pad was combined with the filtrate and concentrated to a small volume. Addition of acetone gave a white solid (0.47 g) that showed no benzoate or cyclic carbamate carbonyl absorption in the infrared spectrum.

The white solid was dissolved in a mixture of liquid ammonia (120 mL) and ethylamine (20 mL). Sodium metal (1.0 g) was added and the dark blue solution that formed was stirred for 2 h at −30 °C. Water was added to discharge the color and the ammonia was allowed to evaporate. The residue was diluted with 10 mL of water and neutralized with Amberlite IR-C50 resin (NH_4^+), and the entire slurry was transferred to a column. It was washed with water and eluted with 2 N ammonium hydroxide until the eluate was no longer ninhydrin positive. The concentrate from this eluate was dissolved in a minimum volume of water, filtered through a pad of diatomaceous earth, and rechromatographed on the same resin with gradient elution by 0–0.3 N ammonium hydroxide. The fractions that gave spots on TLC (1:4:2:1 chlorofrom–methanol–28% ammonium hydroxide–water on silica gel) with R_f values identical with that of authentic ribostamycin were concentrated to a small volume and diluted with acetone. The white solid that precipitated (59 mg, 23%) had an infrared absorption spectrum identical with that of authentic ribostamycin and with the sample prepared from compound **16**. It had a specific rotation of $[\alpha]_{546}^{22}$ +36.7° (*c* 0.45, H_2O) which compares with a value of $[\alpha]_{546}^{22}$ +37.8 (*c* 0.51, H_2O) for the authentic sample.

1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]tetra-*N*-benzyloxycarbonylneamine (19). A solution of **18**[17] (1.0 g, 2.29 mmol) and 3.0 g of sodium carbonate in 50 mL of 70% aqueous methanol, cooled at 0 °C and stirred, was treated dropwise with a solution of benzyl chloroformate (5.0 g, 29.3 mmol) in 5 mL of methanol. The mixture was stirred at 0 °C for 2 h and at 25 °C for 18 h, concentrated under reduced pressure, and extracted three times with warm dioxane. This extract was concentrated under reduced pressure and the residue was treated with ether. A white solid that formed was washed thoroughly with ether to give 2.1 g (95%) of **19**: mp 234–236 °C dec; $[\alpha]_{546}^{22}$ +35.5° (*c* 1.0, DMF); IR 1710, 1895, and 1650 (NHCO I), 1530 cm^{-1} (NHCO II); NMR (Me_2SO-d_6) δ 5.05 (2, s, CH_2), 7.4 (5, br, aromatic).

Anal. Calcd for $C_{48}H_{57}N_5O_{16}$: C, 60.06; H, 5.99; N, 7.30. Found: C, 59.95; H, 6.03; N, 7.41.

1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]-3,2′-di-*N*-benzyloxycarbonyl-4′,6′:2″,4″-*N*,*O*-carbonylneamine (20). An ice-cooled solution of **19** (1.0 g, 1.06 mmol) in 15 mL of dry DMF was treated with 50% sodium hydride in oil suspension (0.15 g, 3.1 mmol) and the mixture was stirred under nitrogen at 25 °C for 2 h. Additional dry DMF was added when the mixture became gelatinous. The mixture was worked up as described in the preparation of compound **11** to give a crude solid that was purified by reprecipitation from DMF with ether. This procedure gave 0.72 g (90%) of **20** as a white solid: mp >232

°C dec; $[\alpha]_{546}^{22}$ +27.88° (c 1.04, DMF); IR 1730 and 1720 (carbamate, six-membered), 1700 (NHCO I) and 1540 cm^{-1} (NHCO II).

Anal. Calcd for $C_{34}H_{41}N_5O_{14}$: C, 54.91; H, 5.52; N, 9.42. Found: C, 54.92; H, 5.89; N, 9.20.

1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]-3,2′-di-*N*-benzyloxycarbonyl-4′,6′:2″,4″-*N*,*O*-carbonyl-6,3′-di(2-tetrahydropyranyl)neamine (21). To a solution of **20** (2.0 g, 2.69 mmol) in 20 mL of dry DMF was added 10 mL (excess) of 2,3-dihydropyran and 100 mg of *p*-toluenesulfonic acid. The mixture was stirred at 24 °C for 1.5 h, neutralized with triethylamine, and concentrated under reduced pressure. Treatment of the residue with water gave a solid that was purified further by precipitation from chloroform solution with ether. The resulting white solid was chromatographed on a silica gel column. A minor impurity was eluted with benzene and then the desired product was eluted with methanol. This procedure gave 1.53 g (61%) of **21** as white solid: mp 148–152 °C dec; $[\alpha]_{546}^{22}$ −5.2° (c 1.15, $CHCl_3$).

Anal. Calcd for $C_{44}H_{57}N_5O_{16}$: C, 57.95; H, 6.30; N, 7.68. Found: C, 58.12; H, 6.51; N, 7.77.

2″,3″,5″-Tri-*O*-acetyl-3,2′-di-*N*-benzyloxycarbonyl-4′,6′:2‴,4‴-*N*,*O*-carbonyl-6,3′-(2-tetrahydropyranyl)butirosin B (23). A mixture of **21** (0.30 g, 0.33 mol), mercuric cyanide (0.6 g), Drierite (2.0 g), and dry methylene chloride (20 mL) was stirred at 25 °C for 2 h and treated with 2,3,5-tri-*O*-acetyl-1-ribosyl chloride (1.0 mmol) in 3 mL of dry methylene chloride. The resulting mixture was stirred at reflux for 48 h, diluted with methylene chloride, and filtered through a pad of diatomaceous earth. Concentration of the filtrate to a small volume and dilution with ether gave a white solid that showed one major and several minor spots on TLC (9:1 chloroform–methanol on silica gel). Purification on a silica gel column with the same solvent system gave a white solid that was recrystallized from chlororform–ether. This procedure gave 0.154 mg (40%) of **23**: mp 108–113 °C dec; $[\alpha]_{546}^{22}$ +12.0° (c 1.0, $CHCl_3$); IR 1730 and 1720 (carbamate, six-membered), 1700 (NHCO I) and 1530 cm^{-1} (NHCO II).

Anal. Calcd for $C_{55}H_{71}N_5O_{23}$: C, 56.45; H, 6.11; N, 5.98. Found: C, 56.14; H, 5.98; N, 5.72.

Butirosin B (22). A solution of 150 mg of **23** in 10 mL of dioxane was heated at 60 °C with 10 mL of 0.1 N barium hydroxide. After 1-h intervals two additional 10-mL portions of 0.1 N barium hydroxide were added. Following a total reaction time of 7 h, the mixture was neutralized with carbon dioxide gas and filtered through a pad of diatomaceous earth. The pad was washed with DMF and the combined filtrate and wash was concentrated to a small volume and diluted with acetone. The white solid that formed showed no absorption characteristic of acetate or cyclic carbamate groups in its infrared spectrum.

A solution of the white solid in 10 mL of dioxane and 10 mL of water was treated with 0.15 g of 10% palladium-on-charcoal and 2.5 mL of acetic acid. The mixture was shaken with hydrogen at 50 psi and 25 °C for 24 h and filtered, and the filtrate was concentrated under reduced pressure. Trituration of the residue with acetone gave a white solid that showed one spot identical in R_f with authentic butirosin B[17] on TLC (1:4:2:1 chloroform–methanol–27% ammonium hydroxide–water on silica gel). This solid was purified by successive column chromatographic separations on Amberlite IR-C-50 (NH_4^+) with 0.1–0.5 N ammonium hydroxide, Amberlite GC-50 (NH_4^+) with 0.1–0.5 N ammonium hydroxide, and Amberlite IR-400 (OH^-) with water. Concentration of the final eluate gave a white solid that was crystallized from aqueous methanol–acetone. This procedure gave 14 mg (20%) of butirosin B **(22)** with a specific rotation of $[\alpha]_{546}^{22}$ +21.8° (c 0.54, H_2O) which compares with $[\alpha]_{546}^{22}$ +22.8° (c 0.46, H_2O) for the authentic sample. The two samples were identical in their infrared absorption spectra and R_f values on TLC (1:1:1 chloroform–methanol—27% ammonium hydroxide on silica gel).

Acknowledgments. This investigation was supported by Grant No. AI 12733 and AI 13998, awarded by the National Institute of Allergy and Infectious Diseases, DHEW. We thank Bristol Laboratories for generous supplies of 1-*N*-[(*S*)-4-amino-2-hydroxybutyrl]neamine, butirosin B, and ribostamycin.

Registry No.—**1,** 22854-78-0; **2,** 25546-65-0; **3** HCl, 41547-94-8; **5,** 66787-76-6; **6,** 66787-77-7; **7,** 66787-78-8; **8,** 66787-79-9; **9,** 66787-80-2; **10,** 51902-04-6; **10** 2′,6′-di-*N*-phenoxycarbonyl derivative, 66787-81-3; **11,** 66787-82-4; **12,** 66787-83-5; **13,** 66787-84-6; **14,** 66787-85-7; **15,** 66787-86-8; **16,** 66787-87-9; **17,** 66787-88-0; **18,** 50474-68-5; **19,** 52621-63-3; **20,** 66787-89-1; **21,** 66787-90-4; **22,** 34291-03-7; **23,** 66787-91-5; phenyl chloroformate, 1885-14-9; *p*-nitrophenoxycarbonyl chloride, 7693-46-1; benzyl chloroformate, 501-53-1; 2,3-dihydropyran, 110-87-2; *p*-toluenesulfonyl chloride, 98-59-9; *p*-nitrobenzoyl chloride, 122-04-3; trimethylacetyl chloride, 3282-30-2; 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl chloride, 29706-90-9; 2,3,5-tri-*O*-acetyl-1-ribosyl chloride, 53402-29-2.

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(21) Kindly provided by Bristol Laboratories.