AMINOGLYCOSIDE ANTIBIOTICS. 5. REGIOSPECIFIC SYNTHESIS OF 1-*N*-[(*S*)-4-AMINO-2-HYDROXYBUTYRYL]-2′′-DEOXYKANAMYCIN B FROM NEAMINE

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(Received for publication April 16, 1982)

The synthesis of pseudotrisaccharides from pseudodisaccharides such as neamine and paromamine has been an important area of aminoglycoside antibiotic research in recent years. It has resulted in the syntheses of known antibiotics including kanamycin B,[1] tobramycin,[2] ribostamycin[3] and butirosin B,[4] and it has provided a means to prepare analogues in which a new sugar unit is introduced. Examples of such analogues include 6-*O*-(β-D-ribofuranosyl)paromamine,[5] 2′′-deoxygentamicin C_2[6] and 2′′-deoxykanamycin B.[7]

As an extension of our studies on the regiospecific synthesis of novel pseudotrisaccharides from neamine, we decided to prepare an amikacin analogue (**6**) in which the kanosamine moiety was replaced by a different sugar. The corresponding 2-deoxykanosamine was chosen on the basis of the availability of starting material.[8] However, during the course of our investigation, the isolation from four different Gram-negative species of a new aminoglycoside 2′′-adenylyltransferase whose substrate range includes amikacin was reported.[9] Although the implication of this discovery to the clinical effectiveness of amikacin is not clear at this time, it added to our desire to synthesize and test compound **6**.

The synthesis of **6** from neamine requires two regiospecific introductions of functional groups: the 4-amino-2-hydroxybutyryl group at N-1 and the glycosidation with 2′′-deoxykanosamine at O-6. Precedent for the first of these operations was found in the synthesis of butirosin B from ribostamycin,[10] and precedent for the second operation was found in the synthesis of kanamycin B and 2′′-deoxykanamycin B from neamine.[1,7] The main problems remaining were to do these operations in the correct sequence and to ensure that all functional groups were protected except for the ones involved in the operations.

A starting compound that contained protecting groups suitable for the proposed synthesis was found to be **1**, which we had used previously in a ribostamyicn synthesis.[4] Further protection of the 3′- and 5-hydroxyl groups of **1** by treatment with sodium hydride and benzyl bromide in *N*,*N*-dimethylformamide was accompanied by benzylation of the two *N*-benzyloxycarbonyl groups to give **2**; however, this unanticipated occurrence caused no subsequent problems in the synthesis of **6**. Now that all of the amino and hydroxyl groups of neamine were protected, it was possible to selectively hydrolyze the 5-membered cyclic carbamate ring of **2** and expose only the 1-amino and 6-hydroxyl groups. Substitution of the side chain onto N-1 of the resulting compound **2** was accomplished by using the *N*-hydroxysuccinimide ester of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid according to the known method.[11] The product **4** now had two free hydroxyl groups, but the one in the side chain was protected readily by cyclic carbamate formation.[4] Condensation of the resulting compound **5** with bromo derivative **11** of 2-deoxykanosamine under KÖENIGS-KNORR conditions then gave the glycoside **7**. Deprotection by hydrolysis with 0.2 M barium hydroxide in dioxane-water, followed by catalytic hydrogenolysis afforded the desired product, 1-*N*-[(*S*)-4-amino-3-hydroxybutyryl]-2′′-deoxykanamycin B (**6**) as a hemi-carbonic acid salt.

Bromo derivative **11** was prepared from 4,6-*O*-benzylidene-2,3-dideoxy-3-trifluoroacetylamino-α-D-arabinohexopyranoside (**8**),[8] a by-product obtained in the HORTON L-daunosamine synthesis,[12] by a route involving hydrolysis to the 4,6-diol **9**, reprotection to give the di-(*p*-nitrobenzoate) **10**, and treatment with hydrogen bromide in acetic acid.

The structure of **6** was verified by elemental analysis, IR spectrum, and a 250 MHz ^{1}H NMR spectrum that showed an exact count for 45 hydrogens. The 1′′ anomeric proton unfortunately appeared as a broad singlet, probably because of the carbonate salt, which made proof of the α-glycosidic linkage difficult. However, the width of the signal (at δ 5.05) was the same

Scheme 1.

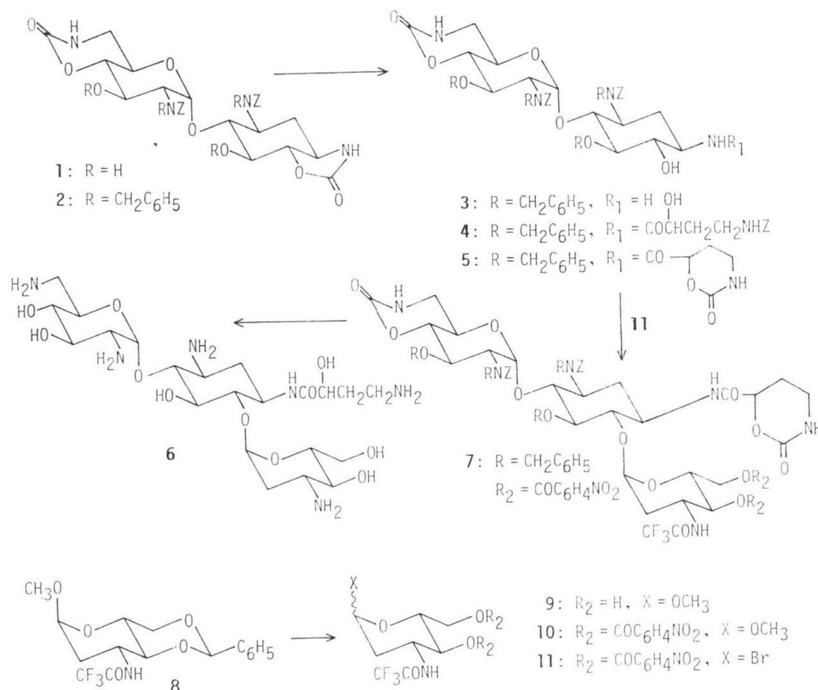

Table 1. Antibacterial activities of **6** and amikacin.*

Organism**		MIC (μg/ml)		Organism**		MIC (μg/ml)	
		6	Amikacin			**6**	Amikacin
Staphylococcus aureus	A-9537	>63	0.5	*Enterobacter cloacae*	A-21006	>63	2
	A-22210	>63	4	*Klebsiella pneumoniae*	A-20468	>63	0.5
	A-20240	>63	4	*Proteus mirabilis*	A-9900	>63	4
	A-22058	>63	16	*Proteus rettgeri*	A-9637	>63	0.25
	A-22231	>63	1		A-21207	>63	4
Escherichia coli	A-22356	>63	1		A-22754	>63	32
	A-22480	>63	0.5	*Providencia stuartii*	A-21210	>63	1
	A-9632	>63	1		A-20894	>63	8
	A-20665	>63	4	*Pseudomonas aeruginosa*	A-21508	>63	0.5
	A-20683	>63	8		A-9843a	>63	0.5
	A-20895	>63	2		A-20610	>63	1
	A-22045	>63	0.5		A-20897	>63	1
	A-21218	>63	4		A-20653	>63	2
	A-20732	>63	1		A-20741	>63	2
	A-20520	>63	4		A-21294	>63	8
Enterobacter cloacae	A-9565	>63	8		A-22233	>63	4
	A-20364	>63	2		A-21509	>63	16

* Antibacterial activities in Mueller-Hinton medium by the serial dilution method. The assays were performed at Bristol Laboratories, Syracuse, N.Y., U.S.A. For further details of the assay method see Reference 13.

** Numbers refer to the coding system for bacterial strains used by Bristol Laboratories.

as that of the 1′ anomeric proton at δ 5.25. If the anomeric linkage had been β, a broader peak would have resulted because of coupling between the 1″ proton and the axial 2″ proton.

Compound **6** was tested against five strains of *Staphylococcus aureus* and 29 strains of seven different Gram-negative bacteria, including some strains relatively resistant to amikacin. The results, given in Table 1, show that **6** was inactive at a dose of 63 μg/ml against all of these strains. In contrast, the amikacin standard[18] was active at concentrations of 0.25 to 32 μg/ml. Although some loss in potency relative to amikacin against the sensitive strains was expected, the complete lack of activity for **6** was surprising in view of previous experience with 2″-deoxyaminoglycosides. Thus, 2″-deoxygentamicin C_2 was found to be less potent than the parent compound, but more active against certain 2″-adenylylating strains of bacteria.[6] A similar result was reported for 2″,3′,4′-trideoxykanamycin B.[7]

Although compound **6** clearly is not an interesting antibacterial agent, we suggest that the route developed for its synthesis from neamine might be valuable for the general synthesis of amikacin analogues in which the kanosamine unit is replaced by another sugar.

Experimental

Melting points were determined on a Laboratory Instruments Mel-Temp apparatus and are uncorrected. IR spectra were taken on a Beckman IR-23 spectrometer with samples prepared as potassium bromide pellets. Absorptions are reported in reciprocal centimeters. Proton nuclear magnetic resonance spectra (PMR) were taken on either a Varian EM-360L 60 MHz spectrometer or a Bruker WM 250 MHz spectrometer and absorptions are reported as parts per million downfield from TMS. Optical rotations were measured on a Perkin Elmer 241MC automatic polarimeter under the indicated conditions. Elemental analyses were performed by Chemalytics, Inc., Galbraith Laboratories, Inc. or the University of Arizona Analytical Center.

3,2′-Di-*N*-benzyloxycarbonyl-3,2′-di-*N*-benzyl-5, 3′-di-*O*-benzyl-1,6:6′, 4′-*N*,*O*-carbonylneamine (**2**)

A solution of **1**[4] (0.31 g, 0.5 mmole) in 15 ml of dry *N*,*N*-dimethylformamide was treated with sodium hydride (0.19 g, 4.0 mmole, 50% mineral oil dispersion). The mixture was stirred at 25°C for 45 minutes and treated with benzyl bromide (1.03 g, 6 mmole) added dropwise with stirring. After 24 hours, the excess benzyl bromide was decomposed by 25 ml of methanol. The mixture was concentrated under reduced pressure and the residue was extracted with chloroform. The extract was washed with water, dried over sodium sulfate and concentrated. Purification of the residual solid by chromatography on silica gel with benzene - ethyl acetate (1:1) as solvent, followed by crystallization, gave 0.34 g (70%) of **2** as white solid, mp 122~123°C, $[\alpha]^{26}_{546}$ +41.8° (*c* 1.0, $CHCl_3$). IR 1775 (5-membered cyclic carbamate), 1720 (6-membered cyclic carbamate), 1610 cm^{-1} (phenyl). PMR ($CDCl_3$) δ 7.30 (30H, aromatic), 51.0 (8H, *O*-benzyl), 4.60 (4H, *N*-benzyl).

Anal. Calcd. for $C_{58}H_{58}N_4O_{12}$: C 69.46, H 5.79, N 5.59.
Found: C 69.74, H 5.63, N 5.49.

3,2′-Di-*N*-benzyloxycarbonyl-3,2′-di-*N*-benzyl-5,3′-di-*O*-benzyl-6′,4′-*N*,*O*-carbonylneamine (**3**)

A solution of **2** (1.01 g, 1.0 mmole) in 50 ml of dioxane was stirred at 50°C and treated with 20 ml of 0.05 M barium hydroxide solution, added slowly. After 4 hours the reaction appeared to be complete according to thin-layer chromatography (benzene - ethyl acetate, 1:1 on silica gel). The mixture was cooled, neutralized with CO_2, and concentrated under reduced pressure. A chloroform extract of the residue was filtered, concentrated to a small volume, and chromatographed on silica gel with benzene - ethyl acetate (1:1) as solvent. The main fraction was concentrated and the residue was crystallized from chloroform to give 0.88 g (88%) of **3** as white powder, mp 133~135°C (dec.), $[\alpha]^{26}_{546}$ +72.0° (*c* 1.0, $CHCl_3$). IR 3600~3150 (OH and NH), 1700 cm^{-1} (6-membered cyclic carbamate). PMR ($CDCl_3$) δ 7.25 (30H, aromatic), 5.1 (8H, *O*-benzyl), 4.5 (4H, *N*-benzyl).

Anal. Calcd. for $C_{57}H_{60}N_4O_{11}$: C 70.08, H 6.15, N 5.74.
Found: C 70.25, H 5.96, N 5.54.

3, 2′-Di-*N*-benzyloxycarbonyl-1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-3, 2′-di-*N*-benzyl-5, 3′-di-*O*-benzyl-6′, 4′-*N*, *O*-carbonylneamine (**4**)

A mixture of **3** (1.85 g, 1.9 mmole), *N*-[(*S*)-4-

benzyloxycarbonylamino-2-hydroxybutyryloxy]-succinimide (0.80 g, 24 mmole), tetrahydrofuran (14 ml) and triethylamine (2 drops) was stirred 16 hours at room temperature and concentrated under reduced pressure. A chloroform extract of the residue was washed with water, dried over sodium sulfate, and concentrated to 5 ml. The addition of 20 ml of petroleum ether gave white solid which was crystallized from chloroform-hexane to furnish 1.61 g (70%) of **4** as white powder, mp 79~81°C. IR 3600~3200 (OH, NH), 1715 (6-membered carbamate), 1680 cm^{-1} (amide). PMR ($CDCl_3$) δ 7.20 (35H, aromatic), 5.10 (10H, *O*-benzyl), 4.60 (4H, *N*-benzyl).

Anal. Calcd. for $C_{69}H_{73}N_5O_{15}$:
C 68.37, H 6.02, N 5.78.
Found: C 68.57, H 5.95, N 5.63.

1,*N*-[(*S*)-4,2-*N*,*O*-carbonyl-4-amino-2-hydroxybutyryl]-3, 2′-di-*N*-benzyloxycarbonyl-3, 2′-di-*N*-benzyl-5, 3′-di-*O*-benzyl-6′, 4′-*N*, *O*-carbonylneamine (**5**)

An ice-cooled solution of **4** (5.0 g, 4.13 mmole) in 50 ml of *N*,*N*-dimethylformamide under an atmosphere of nitrogen was treated with sodium hydride (0.30 g, 12.3 mmole, 50% mineral oil dispersion). The mixture was stirred 0.5 hours at 0°C and 2 hours at room temperature, neutralized to pH 7 with acetic acid and concentrated to 5 ml. On addition of cold water (25 ml) a white solid precipitated. It was collected, washed well with water, dried and crystallized from chloroform - petroleum ether (1: 2) to give 3.2 g (71%) of **5** as white powder, mp 110°C. IR 3440, 3120 (OH, NH), 1715 (6-membered carbamate), 1680 cm^{-1} (amide). PMR ($CDCl_3$) δ 7.13 (30H, aromatic), 5.10 (8H, *O*-benzyl), 4.60 (4H, *N*-benzyl).

Anal. Calcd. for $C_{62}H_{65}N_5O_{14}$:
C 67.45, H 5.89, N 6.34.
Found: C 67.19, H 5.85, N 6.36.

Methyl 2,3-Dideoxy-3-trifluoroacetylamino-*α*-*arabino*hexopyranoside (**9**)

A mixture of **8**[8] (5.0 g, 13.8 mmole), water (112 ml) and acetic acid (44 ml) was stirred at 80°C for 3 hours, filtered, and concentrated under reduced pressure. Traces of acetic acid were removed by coevaporation with toluene (10 ml) and drying under vacuum. Crystallization of the resulting solid from ethyl acetate gave 3.4 g (90%) of **9** as white solid, mp 173~174°C, $[\alpha]^{25}_{546}$ +131.7° (*c* 1.0, $CHCl_3$). IR 3300, 3100(OH, NH), 1700 cm^{-1} (CF_3CO_2). PMR (D_2O) δ 3.3 (s, 3H, 1-OCH_3).

Anal. Calcd. for $C_9H_{14}F_3NO_5$:
C 39.55, H 5.12, N 5.12.
Found: C 39.52, H 5.27, N 5.07.

Methyl 2,3-Dideoxy-4,6-di-*O*-(*p*-nitrobenzoyl)-3-trifluoroacetylamino-*α*-D-*arabino*hexopyranoside (**10**)

A solution of **9** (2.0 g, 7.3 mmole) in pyridine was cooled to 0°C and treated with *p*-nitrobenzoyl chloride (2.8 g, 15 mmole). The mixture was stirred at room temperature for 16 hours and concentrated under reduced pressure. The residue was dissolved in methylene chloride, washed with sodium bicarbonate solution and with water, dried over sodium sulfate and concentrated. Recrystallization of the residual solid from methylene chloride and hexane gave 3.3 g (80%) of **10** as pale yellow solid, mp 210~212°C, $[\alpha]^{25}_{546}$ +121.6° (*c* 0.9, CH_3OH). IR 3300 (NH), 1740 (benzoyl), 1700 cm^{-1} (CF_3CO_2). PMR ($CDCl_3$) δ 8.2 (8H, aromatic), 3.4 (3H, 1-OCH_3).

Anal. Calcd. for $C_{23}H_{20}F_3N_3O_{11}$:
C 48.34, H 3.53, N 7.36.
Found: C 48.26, H 3.69, N 7.29.

1-*N*-[(*S*)-4, 2-*N*, *O*-Carbonyl-4-amino-2-hydroxybutyryl]-3, 2′-di-*N*-benzyloxycarbonyl-3, 2′-di-*N*-benzyl-5,3′-di-*O*-benzyl-6′,4′-*N*,*O*-carbonyl-2″-deoxy-4″, 6″-di-*O*-(*p*-nitrobenzoyl)-3″-trifluoroacetylkanamycin B (**7**)

A solution of **10** (1.25 g, 2.2 mmole) in 12 ml of dry methylene chloride was cooled in an ice bath and treated with a saturated solution of hydrogen bromide in acetic acid (12 ml). The mixture was allowed to warm to room temperature over 1.5 hours and then it was concentrated under reduced pressure. The residue was dissolved in methylene chloride, washed with water and 5% sodium bicarbonate, dried over sodium sulfate and concentrated as petroleum ether was added. This procedure gave 1.0 g of bromosugar **11**, as white powder with mp 138~140°C (dec). The instability of this product prevented its purification for analysis. It was used directly for the preparation of **7**.

A mixture of **5** (1.0 g, 0.9 mmole), mercuric cyanide (1.01 g), Drierite (2.0 g) and methylene chloride (30 ml) was stirred at 25°C under nitrogen for 4 hours, then treated with a solution of bromosugar **11** (0.5 g, 0.8 mmole) in 20 ml of methylene chloride. The mixture was stirred at reflux temperature for 24 hours and treated with another 0.5 g portion of **11**. After 3 days the

mixture was cooled, diluted with methylene chloride, and filtered. Concentration of the filtrate to 5 ml and addition of 40 ml of petroleum ether gave a yellowish solid that showed three spots on TLC (chloroform - methanol, 9: 1). Chromatography on silica gel with benzene - ethyl acetate (1: 1) as solvent gave in the main fraction a yellowish solid, which was recrystallized from chloroform and petroleum ether to furnish 0.45 g (30%) of **7** as nearly white powder, mp 120~123°C. IR 3400, 3300(NH), 1745, 1725 and 1710 (carbonyl), 1520 and 1350 cm^{-1} (NO_2). PMR ($CDCl_3$) δ 8.3 (8H, aromatic), 7.3 (30H, aromatic), 5.10 (8H, *O*-benzyl), 4.60 (4H, *N*-benzyl).

Anal. Calcd. for $C_{80}H_{80}F_3N_8O_{26}$: C 61.38, H 4.93, N 6.82.
Found: C 61.21, H 4.60, N 6.49.

1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]-2′′-deoxykanamycin B (**6**)

A solution of **7** (0.20 g, 0.12 mmole) in 10 ml of dioxane was heated at 50°C and treated with 10 ml of 0.2 M barium hydroxide solution added in portions. At 1 hour intervals two additional 10 ml portions of barium hydroxide solution were added. After a total time of 5 hours, the mixture was neutralized with CO_2, filtered through diatomaceous earth, and concentrated under reduced pressure to 3 ml. Addition of 20 ml of acetone to the concentrate gave a light yellow solid. This solid was treated with 20 ml of dioxane, 5 ml of water, 1.5 ml of acetic acid and 0.20 g of 10% palladium-on-carbon and the mixture was shaken with hydrogen at 3.5 kg/cm^2 on a Parr apparatus for 24 hours. The mixture was filtered through diatomaceous earth and the filtrate was concentrated under reduced pressure. Traces of remaining solvents were removed by addition of 5 ml of toluene and reconcentration. The nearly-white solid residue was purified by successive column chromatographic separations on Amberlite IRC-50 (NH_4^+) and Amberlite CG-50 (NH_4^+) with 0.1 N~0.5 N ammonium hydroxide as the solvent for both columns. Recrystallization of the product from water - acetone gave 0.024 g (35%) of **6** as nearly-white solid, mp 201°C (dec), $[\alpha]^{25}_{436}$ +94° (*c* 0.5, H_2O). IR 3340 (broad, OH, NH), 2960 (H_2CO_3), 1650 cm^{-1} (amide). PMR (250 MHz, D_2O) δ 5.25 (broad s, 1′-H anomeric), 5.05 (broad s, 1′′-H anomeric), 2.7~3.9 (19H including CHN, CHO, 2-H_{ax}, 2′′-H_{ax}), 1.72~2.3 (7H including 2′′-H_{eq}, CH_2N, OH).

Anal. Calcd. for $C_{22}H_{44}N_6O_{11}\cdot\frac{1}{2}H_2CO_3$: C 46.07, H 7.51, N 14.02.
Found: C 46.11, H 7.20, N 14.05.

Acknowledgement

We wish to thank Bristol Laboratories for generous samples of certain intermediates and for the antibacterial assays. These assays were conducted by THERESA PURSIANO.

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