A Synthesis of Neamine

Akihiko HARAYAMA, Tsutomu TSUCHIYA, and Sumio UMEZAWA*
Institute of Bioorganic Chemistry, 1614 Ida, Nakahara-ku, Kawasaki 211
(Received May 31, 1979)

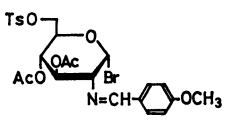
Neamine and its position isomer were synthesized from 3,4-di-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-6-*O*-tosyl-α-D-glucopyranosyl bromide and racemic 5,6(4,5)-*O*-cyclohexylidene-2-deoxy-1,3-bis-*N*-(ethoxycarbonyl)streptamine. Influence of the *N*-protecting groups of 2-deoxystreptamine derivatives for glycosylation was discussed. The unusual $\Delta[M]_{TACu}$ value of the position isomer of neamine was ascribed to the interaction between the 6′-amino group and other groups.

Neamine[1] isolated from *Streptomyces cultures* is a constituent of several aminoglycoside antibiotics including neomycins, kanamycin B and butirosins. Its first synthesis starting from paromamine[2] was reported by Umezawa *et al.*[3] Kohno *et al.*[4] also reported a synthesis of neamine from a protected 2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl bromide and a blocked deoxystreptamine derivative, using the 2,4-dinitrophenyl group as a non-participating group at C-2 of the glycosyl bromide. Interest in neamine synthesis lies in the efficient and stereocontrolled synthesis of α-glycoside having an amino group at C-2 of the glycosyl portion. This paper reports another total synthesis of neamine with the use of a glycosyl bromide carrying a *p*-methoxybenzylidene group (Schiff base) at C-2 as a non-participating group.

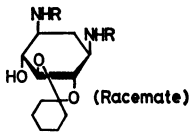
We used 3,4-di-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-6-*O*-tosyl-α-D-glucopyranosyl bromide[5] (**1**) and racemic 5,6(4,5)-*O*-cyclohexylidene-2-deoxy-1,3-bis-*N*-(ethoxycarbonyl)streptamine[6] (**5**). Condensation of **1** and **5** in the presence of mercury(II) cyanide and Drierite in benzene–dioxane followed by treatment of the condensation product with dilute sulfuric acid and reaction with ethyl chloroformate gave a mixture of two α-glycosides, *viz.*, 4-*O*-[3,4-di-*O*-acetyl-2-deoxy-2-(ethoxycarbonylamino)-6-*O*-tosyl-α-D-glucopyranosyl]-2-deoxy-1,3-bis-*N*-(ethoxycarbonyl)streptamine (**6**) and its 6-*O*-isomer (**6′**) in 47% yield based on **5**.

Treatment of the above mixture with sodium azide gave a mixture of 6′-azido derivative (**7**, **7′**). Hydrolysis with sodium hydroxide gave a mixture of **8** and **8′**, which, by catalytic hydrogenation followed by chromatographic separation, afforded neamine (**9**) and its 6-*O*-isomer (**9′**). Separation of **8** and **8′** was troblesome. However, they were separated in low yields for the structural determination of **9** and **9′**. α-Anomeric configurations of **8** and **8′** were confirmed by means of the PMR spectrum ($J_{1,2}$=3.5 Hz each). The physical properties of **9** were identical with those of natural neamine. The synthetic neamine (**9**) also gave an antibacterial spectrum identical with that of natural neamine, the isomer (**9′**) showing very weak antibacterial activity. The azido derivatives (**8** and **8′**) showed no antibacterial activities.

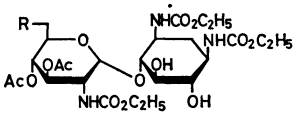
In order to confirm the structure of **9** and **9′**, rotations of **9** and **9′** in TACu[7] were measured. Neamine and synthetic **9** gave expected $\Delta[M]_{TACu}$ values (≈−200°), indicating copper-complexing between 1-NH_2 and 6-OH and between 2′-NH_2 and 3′-OH, respectively. The contributions of both rotations are approximately equal in magnitude and opposite in sign, thus cancelling the values. However, **9′** gave $\Delta[M]_{TACu}$ −990° and not the expected value (−1800°). An explanation is that the copper-complexing between 3-NH_2 and 4-OH is disturbed by the presence of the 6′-NH_2 group. For the sake of confirmation the $\Delta[M]_{TACu}$ values of **8** and **8′** were measured. Since **8** and **8′** have no 6′-NH_2 group, they should give the expected $\Delta[M]_{TACu}$ values which were found to be −190° and −1820°, respectively, as expected, showing that **8** and **8′** are 4-*O*- and 6-*O*-glycosyl isomers of 2-deoxystreptamine, respectively.



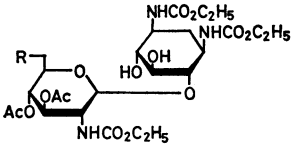
1



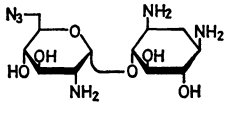
2 R = $CO_2CH_2C_6H_5$
4 R = $CO_2CH_2CH{=}CH_2$
5 R = $CO_2C_2H_5$



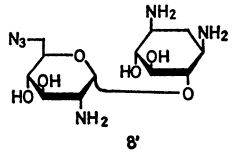
6 R=OTs
7 R=N_3



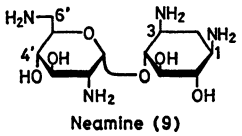
6′ R=OTs
7′ R=N_3



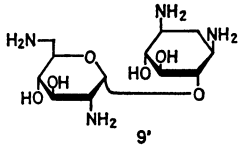
8



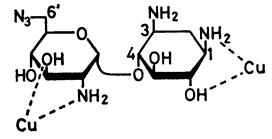
8′

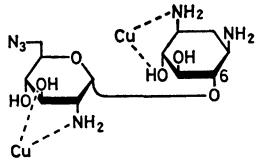


Neamine (9)



9′





Cu-complex of **8** and **8′**

Discussion

Use of the *p*-methoxybenzylidene group as a non-participating group at the C-2 amino group of glycosyl halide is based on the fact that a Schiff base derivative gives α-glycosides in the synthesis of paromamine[6] in an excellent yield.

We have examined the influence of several *N*-blocking groups of the 2-deoxystreptamine (aglycon) on the glycosidation. Condensation of 1,3-bis-*N*-(benzyloxycarbonyl)-4,5(5,6)-*O*-cyclohexylidene-2-deoxystreptamine[8] (**2**) with **1** under similar conditions gave the condensation product in a smaller yield (≈30%) which was also led to neamine.[9] 1,3-Bis-*N*-(allyloxycarbonyl)-4,5(5,6)-*O*-cyclohexylidene-2-deoxystreptamine (**4**) was prepared from 2-deoxystreptamine by the reaction with allyl chloroformate and subsequent cyclohexylidenation. Condensation of **4** with **1**, however, did not proceed satisfactorily (condensation yield, *ca.* 30%). The *N*-ethoxycarbonyl group was thus found to give the best condensation yield among the protecting groups tested.

Experimental

Thin-layer chromatography (TLC) was carried out on Wakogel B-5 with sulfuric acid spray for detection. For column chromatography, silica gel (Wakogel C-200) was used. Paper chromatography (PPC) was performed on Toyo-Roshi No. 50 with 1-butanol–pyridine–water–acetic acid (6: 4: 3: 1) and spots were visualized by spraying 0.5% ninhydrin in pyridine. PMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer.

*1,3-Bis-*N*-(allyloxycarbonyl)-2-deoxystreptamine (**3**).* Allyl chloroformate (3 ml) was added dropwise under stirring to an ice-cold suspension of 2-deoxystreptamine dihydrochloride (2.0 g) and anhydrous sodium carbonate (5.2 g) in aqueous acetone (1: 1, 40 ml). The mixture was stirred for 3 h at room temperature. The reaction mixture was concentrated and the residue extracted with hot dioxane. The dioxane solution was then concentrated, the residue being washed with ether to give a solid (2.4 g). Recrystallization from water gave needles, 1.8 g (65%), mp 223—224 °C; IR (KBr): 1700 (*ν* NH*CO*O), 1650 (sh, *ν* C=C), 1550 (amide II), 990 (*δ* CH=) cm^{-1}. PMR (pyridine-d_5): *δ* 1.95 (1H q, J=12 Hz, H-2_{ax}), 2.83 (1H double t, J=4.5, 4.5 and 12 Hz, H-2_{eq}), 4.6—4.8 (4H m, CH_2CH=CH_2), 5.0—5.5 (4H m, CH_2CH=CH_2), 5.98 (2H double quintet,[10] CH_2–CH=CH_2), 8.12 (2H d, J≈7 Hz, NH; disappeared on deuteration).

Found: C, 51.01; H, 6.66; N, 8.35%. Calcd for $C_{14}H_{22}N_2O_7$: C, 50.90; H, 6.71; N, 8.48%.

*Racemic 1,3-Bis-*N*-(allyloxycarbonyl)-4,5(5,6)-*O*-cyclohexylidene-2-deoxystreptamine (**4**).* A mixture of **3** (2.0 g), 1,1-dimethoxycyclohexane (1.3 ml) and *p*-toluenesulfonic acid (200 mg) was refluxed for a while, half the volume of the solvent then being distilled. The distillate was treated with Molecular Sieves 5A in order to remove methanol co-distilled, and then returned to the reaction vessel. The reaction mixture was treated likewise twice. After addition of triethylamine (4 ml), the mixture was concentrated to give a syrup. The syrup was dissolved in chloroform, washed with water, dried over sodium sulfate, and concentrated to give a solid. Recrystallization of the solid from acetone–hexane gave granular crystals, 1.66 g (67%), mp 106—108 °C: PMR (pyridine-d_5): *δ* 1.1—2.2 (11H, cyclohexylidene and H-2_{ax}), 2.80 (1H double t, H-2_{eq}); 4.6—4.8 (4H), 5.0—5.45 (4H) and 5.7—6.2 (2H) (allyl); 8.25 (1H d) and 8.48 (1H d) (NH; disappeared on addition of D_2O).

Found: C, 58.28; H, 7.42; N, 6.55%. Calcd for $C_{20}H_{30}N_2O_7$: C, 58.52; H, 7.37; N, 6.82%.

*4-O- and 6-O-[3,4-Di-O-acetyl-2-deoxy-2-(ethoxycarbonylamino)-6-O-tosyl-α-*D*-glucopyranosyl]-2-deoxy-1,3-bis-*N*-(ethoxycarbonyl)-streptamine (**6** and **6′**).* A mixture of **1** (991 mg, 1.66 mmol), **5** (400 mg, 1.04 mmol), mercury(II) cyanide (757 mg), and calcium sulfate (Drierite, 600 mg) in dry benzene–dioxane (2: 1, 4 ml) was stirred at room temperature overnight. Mercury(II) cyanide (300 mg) was added and the mixture was stirred for 20 h. The mixture was centrifuged, the insoluble matter being washed with dioxane. The organic solution combined was concentrated to give a syrup which was washed with water to give a pale-yellow solid (1.35 g). The solid, on TLC with benzene–acetone (5: 1), showed major spots at R_f 0.42 and 0.49, and weak spots at R_f 0.22 (**5**) and 0.77 (**1**).

0.5 M sulfuric acid (≈2 ml) was added (pH≈2) to a solution of the solid in acetone (27 ml), and the resulting suspension was stirred at room temperature overnight. The cyclohexylidene and the *p*-methoxybenzylidene groups of the condensation products were removed by this procedure. The reaction mixture was concentrated and the residue was washed with ether to give a solid (TLC, R_f 0). To a suspension of the solid in aqueous acetone (1: 1) were added ethyl chloroformate (0.22 ml) and anhydrous sodium carbonate (240 mg) and the mixture was stirred at room temperature overnight. The resulting mixture was concentrated and the residue was dissolved in chloroform. The solution was washed with water, dried over sodium sulfate, and concentrated to give a syrup. Addition of benzene to the syrup gave a precipitate which was removed by centrifugation. Concentration of the benzene solution gave a syrup (927 mg). The syrup on TLC with benzene–acetone (1: 1) gave spots of R_f 0.51 and 0.45 (**6**, **6′**) and 0.34 (slight) and 0.26 (slight). The syrup was chromatographed with benzene–acetone (2: 1) to give the syrup of a mixture of **6** and **6′**, 376 mg (47% based on **5**). IR (KBr): 1750 (*ν* CH_3CO), 1710 (*ν* NH*CO*O–), 1530 (amide II), 1240 (*ν* CH_3CO), 1180 (ν_s SO_2) cm^{-1}; PMR ($CDCl_3$): triplet-like signals centered at *δ* 1.23 (9H, J≈8 Hz, $COCH_2CH_3$); *δ* 1.84 (1.5 H), 1.90 (1.5 H) and 1.98 (3H) (each s, Ac), 2.47 (3H s, $CH_3C_6H_4SO_2$).

Found: C, 49.29; H, 5.87; N, 5.24; S, 3.83%. Calcd for $C_{32}H_{47}N_3O_{17}S$: C, 49.42; H, 6.09; N, 5.40; S, 4.12%.

*4-O- and 6-O-[3,4-Di-O-acetyl-6-azido-2,6-dideoxy-2-(ethoxycarbonylamino)-α-*D*-glucopyranosyl]-2-deoxy-1,3-bis-*N*-(ethoxycarbonyl)streptamine (**7**, **7′**).* Sodium azide (290 mg) was added to a solution of a mixture of **6** and **6′** (350 mg) in DMF (7 ml), and the mixture was stirred at 80 °C for 3 h. The solution on TLC with benzene–acetone (1: 1) gave a single spot at R_f 0.47. Concentration gave a syrup which was dissolved in chloroform. The solution was washed with a saturated sodium chloride solution, then with water, dried over sodium sulfate, and concentrated to give a syrup. The syrup was dissolved again in benzene–ethyl acetate (3: 1, 40 ml) and the solution was washed with water, dried (sodium sulfate), and concentrated to give a syrup, 234 mg (80%). IR (KBr): 2100 (N_3), 1740, 1700, 1530, 1240 cm^{-1}; PMR ($CDCl_3$): *δ* 1.27 (9H t, J=7 Hz, $CO_2CH_2CH_3$), 2.05 (6H m, half-height width is 4.5 Hz, Ac).

Found: C, 46.54; H, 6.12; N, 12.69%. Calcd for $C_{25}H_{40}N_6O_{14}$: C, 46.29; H, 6.22; N, 12.96%.

*4-O- and 6-O-(2-Amino-6-azido-2,6-dideoxy-α-*D*-glucopyranosyl)-2-deoxystreptamine (**8**, **8′**).* A suspension of a mixture of **7** and **7′** (91.6 mg) in 1 M sodium hydroxide solution (5 ml)

was refluxed. The resulting clear solution after 15 min was refluxed for further 2 h. Dowex 50 WX 2 (H^+ form) resin was added until the solution became neutral and the mixture was poured into a column containing the same resin (NH_4^+ form, ≈1 ml). The column after being washed with water (100 ml) was treated with 0.5 M aqueous ammonia. The fractions containing ninhydrin-positive products were collected and concentrated to give a pale-brown syrup (42 mg). The syrup on PPC gave two spots at R_f 0.20 and 0.18 and a faint spot at 0.32. The syrup was charged on a column of CM-Sephadex C-25 (NH_4^+ form, 6 ml) and the column was washed with water. A ninhydrin-positive syrup (9.1 mg) was obtained from the washings. Its absorption spectrum (IR, 1640 cm^{-1}) indicated the product to be a 1,3-ureylene derivative.[11] The column was then treated with aqueous ammonia with gradual increase in concentration (0→0.15 M) to give a colorless syrup of **8** and **8′**, 28.1 mg (52%).

The syrup (59.3 mg) was charged again on a column of CM-Sephadex C-25 (NH_4^+ form, 23 ml) and eluted with aqueous ammonia (0→0.1 M). The elution was checked by PPC. Compound **8** (R_f 0.18) was eluted between 170—180 ml, **8′** (R_f 0.20) between 190—200 ml and the mixture of **8**, **8′** between 180—190 ml. Concentration of the respective fractions gave syrups of **8** (15.7 mg), **8′** (16.0 mg) and a mixture of both (23.4 mg).

8: $[\alpha]_D^{25}$ +71° (*c* 1, water), $\Delta[M]_{TACu\text{-}NH_3}^{436}$† −190°. IR (KBr): 2100 (N_3), 1590 (broad, δ NH_2) cm^{-1}. PMR (D_2O +ND_3): δ 1.18 (1H q, J=12 Hz, H-2_{ax}), 1.97 (1H double t, J=4.5, 4.5 and 12 Hz, H-2_{eq}), 2.5—3.1 (3H m, H-1, 3,2′), 5.23 (1H d, J=3.5 Hz, H-1′). Irradiation at δ 2.8 turned the doublet of H-1′ into a singlet.

Found: C, 39.36; H, 6.50; N, 21.37%. Calcd for $C_{12}H_{24}N_6O_6 \cdot 1/2H_2CO_3$: C, 39.58; H, 6.64; N, 22.15%.

8′: $[\alpha]_D^{25}$ +85° (*c* 1, water), $\Delta[M]_{TACu\text{-}NH_3}^{436}$ −1820°. IR (KBr): 2100, 1590 cm^{-1}. PMR (D_2O+ND_3): δ 1.24 (1H q, J=12 Hz, H-2_{ax}), 1.98 (1H double t, J=4.5, 4.5 and 12 Hz, H-2_{eq}), 2.5—3.1 (3H m, H-1, 3,2′), 5.13 (1H d, J=3.5 Hz, H-1′). Irradiation at δ 2.8 turned the doublet of H-1′ into a singlet.

Found: C, 39.44; H, 6.53; N, 21.56%. Calcd for $C_{12}H_{24}N_6O_6 \cdot 1/2H_2CO_3$: C, 39.58; H, 6.64; N, 22.15%.

Neamine(9)(4-O-(2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl)-2-deoxystreptamine) and Its Isomer(9′)(6-O-(2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl)-2-deoxystreptamine). A few drops of acetic acid were added to an aqueous solution (7 ml) of a mixture of **8** and **8′** (67.2 mg) obtained after the first Sephadex column treatment. The solution (pH≈3) was then treated with hydrogen at atmospheric pressure for 30 min in the presence of palladium black. The solution on TLC (DC-Fertigplatten Kieselgel 60, E. Merck, Darmstadt) with 1-butanol–ethanol–chloroform–17% aqueous ammonia=4:7:2:7 showed two major spots at R_f 0.17 (**9′**) and 0.28 (**9**), and minor spots at R_f 0.66, 0.72 and 0.82. The spots corresponding to **8** and **8′** (R_f 0.51 and 0.48, respectively) completely disappeared. Concentration of the solution gave a syrup, which, by chromatography on a CM-Sephadex C-25 (NH_4^+ form) column with aqueous ammonia (0→0.25 M), gave **9** (25 mg, 36%) and **9′** (23 mg, 34%) which were eluted in this order.

9: $[\alpha]_D^{25}$ +69° (*c* 0.8, water), $\Delta[M]_{TACu\text{-}NH_3}^{436}$ −180° (natural neamine, −210°). PMR (D_2O+ND_3): δ 1.22 (1H q, J=12 Hz, H-2_{ax}), 2.01 (1H double t, J≈4.5, ≈4.5 and 12 Hz, H-2_{eq}), 2.5—3.1 (5H, H-1, 3, 2′, 6′, 6′?), 5.33 (1H d, J=3.5 Hz, H-1′). All signals were superimposable on those of natural neamine carbonate.

Found: C, 40.68; H, 7.21; N, 14.24%. Calcd for $C_{12}H_{26}N_4O_6 \cdot H_2CO_3$: C, 40.62; H, 7.34; N, 14.58%.

9′: $[\alpha]_D^{25}$ +71° (*c* 0.6, water), $\Delta[M]_{TACu\text{-}NH_3}^{436}$ −990°. PMR (D_2O+ND_3): δ 1.27 (1H q, J=12 Hz, H-2_{ax}), 1.98 (1H q, J≈4.5, ≈4.5, and 12 Hz, H-2_{eq}), 2.5—3.2 (5H), 5.17 (1H d, J=3.5 Hz).

Found: C, 40.64; H, 7.54; N, 14.89%. Calcd for $C_{12}H_{26}N_4O_6 \cdot H_2CO_3$: C, 40.62; H, 7.34; N, 14.58%.

Antibacterial spectra of the synthetic neamine (**9**), natural neamine and the isomer (**9′**) expressed by minimal inhibitory concentration (mcg/ml): *Staphylococcus aureus* FDA 209P: 6.25, 12.5, 25; *Bacillus subtilis* NRRL B-558: <1.56, <1.56. 6.25; *Klebsiella pneumoniae* PCl 602: 25, 25, 100; *Escherichia coli* NIHJ: 25, 25, 200; *Escherichia coli* K-12: 25, 25, 200; *Salmonella typhi* T-63: 6.25, 6.25, 50; *Pseudomonas aeruginosa* A3: >200, >200, >200.

The authors wish to thank Mr. Saburo Nakada, Department of Chemistry, Keio University, for the microanalysis, and Dr. Masa Hamada, Institute of Microbial Chemistry, for the bioassay.

References

1) R. L. Peck, C.E. Hoffhine, Jr., Gale, and K. Folkers, *J. Am. Chem. Soc.*, **71**, 2590 (1949); R. L. Peck, C. E. Hoffhine, Jr., P. H. Gale, and K. Folkers., *ibid.*, **75**, 1018 (1953).

2) T. H. Haskel, J. C. French, and Q. R. Bartz, *J. Am. Chem. Soc.*, **81**, 3480 (1959).

3) S. Umezawa, K. Tatsuta, T. Tsuchiya, and E. Kitazawa, *J. Antibiot.*, **20**, 53 (1967); K. Tatsuta, E. Kitazawa, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, **40**, 2371 (1967).

4) H. Kohno, H. Fukami, and M. Nakajima, *Agric. Biol. Chem.*, **39**, 1091 (1975).

5) S. Umezawa and Y. Nishimura, *J. Antibiot.*, **30**, 189 (1977).

6) S. Umezawa, T. Miyazawa, and T. Tsuchiya, *J. Antibiot.*, **25**, 530 (1972).

7) S. Umezawa, T. Tsuchiya, and K. Tatsuta, *Bull. Chem. Soc. Jpn.*, **39**, 1235 (1966).

8) T. Kurisu, M. Yamashita, Y. Nishimura, T. Miyake, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, **49**, 285 (1976).

9) S. Umezawa, *Pure Appl. Chem.*, **50**, 1453 (1978).

10) NMR spectra catalog compiled by N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, Varian Associates, 1962, Compound No. 34.

11) T. Yamaguchi, T. Tsuchiya, and S. Umezawa, *J. Antibiot.*, **30**, 71 (1977).

12) T. Usui, T. Tsuchiya, and S. Umezawa, *J. Antibiot.*, **31**, 991 (1978).

† A small amount of aqueous ammonia was added[12] to the TACu solution of the sample until the solution gave a constant $[M]_{TACu}^{436}$ value.