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Syntheses of Neamine Derivatives and Their Antibacterial Activities

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1-N-[(*S*)-4-Amino-2-hydroxybutyryl]-neamine (**3**) derived from butirosin, an aminoglycoside antibiotic, has been chemically modified in its amide side chain. The N-benzenesulfonyl derivative (**5**) was hydrazinolysed and the resulting amine (**6**) was reacylated with another amino and/or hydroxy carboxylic acid. Removal of benzenesulfonyl groups were carried out with sodium in liquid ammonia, giving several kinds of analogues. In addition, an hydroxy epimer (**31**) and amino varients (**25** and **30**) in the α-position of the amide side chain of **3** were prepared starting from tetra-N-mono-O-benzenesulfonyl derivative (**23**). Antibacterial activity of these analogues were tested and discussed.

Recent chemical studies have revealed that butirosin[2] (**1**), a fermentation product of *Bacillus circulans* NRRL B-3312 and B-3313, is the first member of aminoglycoside antibiotics having the unique *S*-(—)-4-amino-2-hydroxybutyryl side chain connected to 1[3]-amino group of 2-deoxystreptamine through an amide linkage.[4,5] Thus, it is of interest to examine how the presence of the characteristic side chain in the molecule of butirosin (**1**) fills an important role in its antibacterial activities. As demonstrated in Table I, butirosin has a broad inhibitory activity against gram-positive and -negative bacteria and notably against *Pseudomonas aeruginosa* species.[6] Alkaline hydrolysis[7,8] of butirosin gave a mixture of 5-O-β-D-xylofuranosyl- and 5-O-β-D-ribofuranosyl-neamine[9] (**2**) which retained most of its inhibitory activities at a level similar to kanamycin, but showed a serious loss of potency against *Pseudomonas* species. As previously reported,[8,10] acid hydrolysis of butirosin afforded 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-neamine (**3**), which shows potencies 1/2—1/4 lower than those of butirosin (**1**), but which still retains the anti-*Pseudomonas* activity thus differing from neamine (see Table I). Furthermore, 1-N-(4-amino-2-hydroxybutyryl)-2-deoxystreptamine (**4**) prepared from **1** according to Woo, *et al.*[5] was found to show no antibacterial activity. On the basis

1) Location: *Hiromachi 1-2-58, Shinagawa-ku. Tokyo.*
2) Butirosin is an aminoglycoside complex containing either D-xylose or D-ribose in the pentoside moiety as shown in the Chart.
3) The numbering of the positions in deoxystreptamine was made following the proposal of Hichens and Rinehart, Jr (See *J. Am. Chem. Soc.*, **85**, 1547 (1963)).
4) P.W.K. Woo, H.W. Dion, and Q.R. Bartz, *Tetrahedron Letters*, **1971**, 2617; P.W.K. Woo, *ibid.*, **1971**, 2621.
5) P.W.K. Woo, H.W. Dion, and Q.R. Bartz, *Tetrahedron Letters*, **1971**, 2625.
6) J.D. Howell, L.E. Anderson, G.L. Coffey, G.D. Senos, M.A. Underhill, D.L. Vogler, and J. Ehrlish, *Antimicrob. Ag. Chemother.*, **2**, 73 (1973); C.L. Heifetz, M.W. Fisher, J.A. Chodubski, and M.O'. DeCarlo, *ibid.*, **2**, 89 (1972).
7) Personal communication from Dr. T.H. Haskell. Also see T.H. Haskell, R. Rodebaugh, N. Plessas, D. Watson, and R.D. Westland, *Abstr. Papers, Am. Chem. Soc.*, 1972, No. 164, CARB 22.
8) H. Tsukiura, K. Fujisawa, M. Konishi, K. Saito, K. Numata, H. Ishikawa, T. Miyaki, K. Tomita, and H. Kawaguchi, *J. Antibiotics*, **26**, 351 (1973).
9) Neamine is a trivial name for 4-O-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-2-deoxystreptamine. The conventional numbering of neamine skeleton is shown in formula **3**. One component of the hydrolysis, ribofuranosyl-neamine has been isolated from culture filtrates of *Streptomyces ribosidificus* and is called ribostamycin. See E. Akita, T. Tsuruoka, N. Ezaki, and T. Niida, *J. Antibiotics*, **23**, 173 (1970).
10) S. Sugawara, S. Inaba, M. Madate, and H. Saeki, *Ann. Sankyo Res. Lab.*, **25**, 56 (1973).

Table I. Antibacterial Activities of Neamine Derivatives
M.I.C. on Heart Infusion Agar, 37°, 20 hr.

Test organisms [a]	Butirosin sulfate (1)	Pentofuranosyl neamine hydrochloride (2)	1-N-[(S)-4-Amino-2-hydroxybutyryl]-neamine hydrochloride (3)	1-N-(DL-3-Amino-2-hydroxypropionyl)-neamine hydrochloride (21)	Neamine hydrochloride	Kanamycin sulfate
Bacillus subtilis PCI 219	0.2	0.8	0.8	0.8	1.5	0.2
Staphylococcus aureus 209 P	0.2	0.4	0.4	0.8	0.8	0.2
Staphylococcus aureus 56 (PC,TC,CP)	0.4	1.5	1.5	1.5	3.1	0.8
Staphylococcus epidermidis	0.4	0.8	0.8	0.8	1.5	0.2
Escherichia coli NIHJ	0.8	3.1	3.1	12.5	6.2	1.5
Escherichia coli 665 (SM, KM)	400	>400	400	>400	>200	400
Klebsiella 806	0.8	1.5	3.1	12.5	3.1	1.5
Klebsiella 846 (CP, AB-PC, SM, KM, CER)	0.4	>400	3.1	25	>200	>400
Proteus vulgaris	0.4	0.8	1.5	6.2	3.1	1.5
Salmonella enteritidis Gaertner	1.5	3.1	6.2	12.5	6.2	1.5
Shigella flexneri 2a	1.5	3.1	6.2	25	12.5	3.1
Shigella sonnei	1.5	3.1	6.2	25	12.5	3.1
Pseudomonas aeruginosa Scr.	3.1	400	3.1	6.2	200	25
Pseudomonas aeruginosa 8753 (KM)	3.1	>400	12.5	25	>200	>400
Pseudomonas schuylkilliensis	6.2	>400	6.2	12 5	>200	50

a) Drug resistance is shown in parentheses: PC, penicilin G; TC, tetracycline; CP, chloramphenicol; SM, streptomycin; KM, kanamycin; AB-PC, ampicillin; CER, cephaloridine

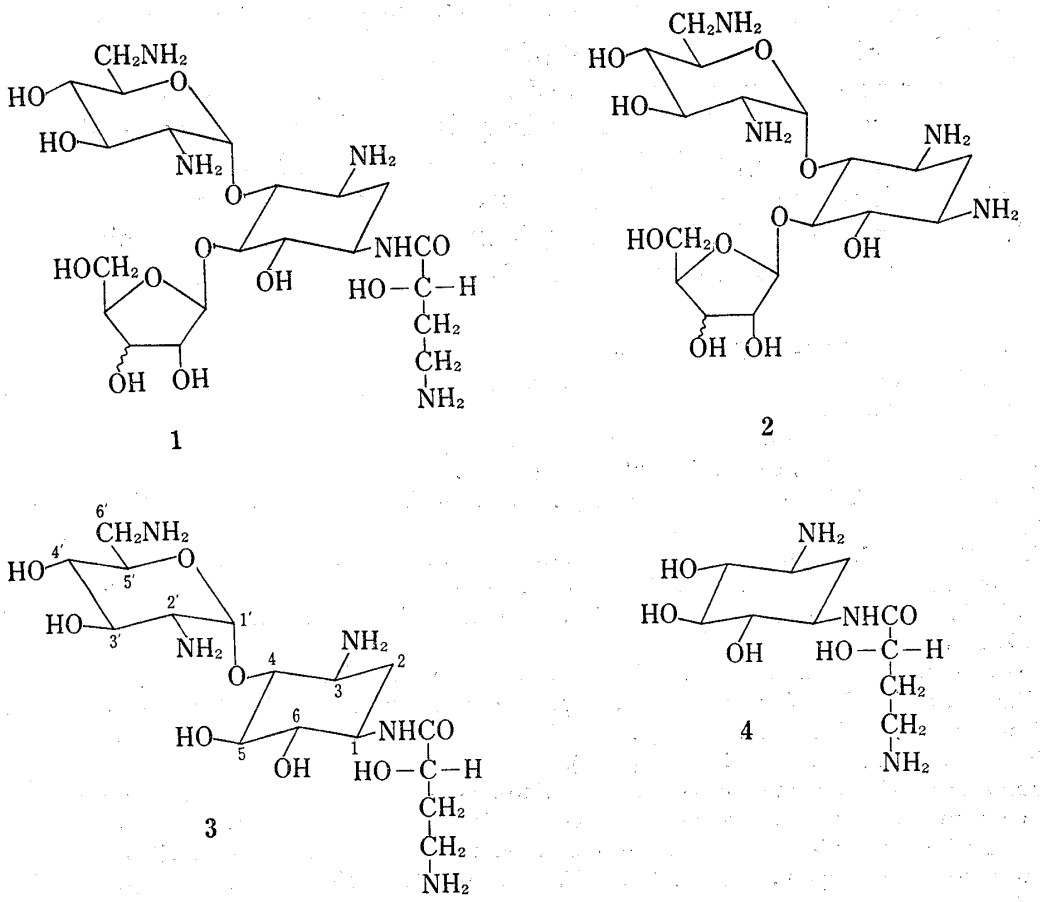
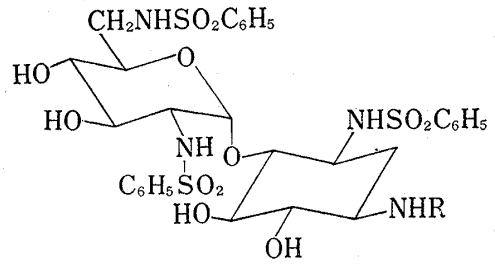
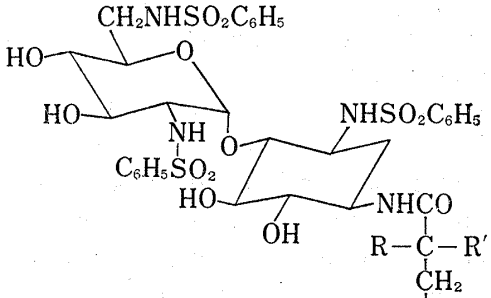


Chart 1

of these facts, it was assumed that the neamine skeleton acylated at the 1-amino group of 2-deoxystreptamine is an essential requirement for the appearance of activity against *Pseudomonas aeruginosa*; and we attempted to exchange the original acyl group of the acyl-neamine (**3**) for another analogous acyl group in view of clarifying structure-activity relationships.[11]

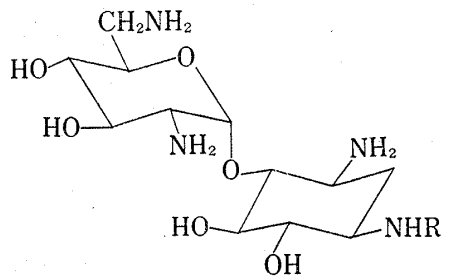
In order to achieve the exchange of the side chain, we chose the benzenesulfonyl group for blocking the four amino functions of the acyl-neamine (**3**) because it was presumed that benzenesulfonamido group is stable enough in alkali to withstand conditions for complete hydrolysis of the amide bond and it can easily be removed by treatment with sodium in liquid ammonia without affecting the molecule of the newly formed acyl-neamine. The reaction of **3** with benzenesulfonyl chloride in aqueous alkaline solution at low temperature yielded a tetra-benzenesulfonyl derivative (**5**) along with minor by-products. The less polar by-product was isolated and determined as a penta-benzenesulfonyl derivative whose structure will be discussed later. The tetra-N-benzenesulfonyl derivative (**5**) was heated in hydrazine hydrate, giving an amine (**6**) without any damage to these benzenesulfonamido groups. The structure of **6** was confirmed by positive ninhydrin test and by its infrared spectrum which did not show the amide I and II bands, but a NH-deformation band at 1600 cm^{-1}. Acetylation of **6** with acetic anhydride in methanol gave an N-acetyl compound (**7**). Other N-acyl derivatives





5 : R=COCH(OH)$CH_2CH_2NHSO_2C_6H_5$ (*S*)
6 : R=H
7 : R=$COCH_3$
8 : R=$COCHOCOCH_3$
9 : R=COCH($OCOCH_3$)CH_3 (DL)
10 : R=$COCH_2NHSO_2C_6H_5$
11 : R=$COCH_2CH_2NHSO_2C_6H_5$
12 : R=$COCH_2CH_2CH_2N_3$
13 : R=COCH(OH)$CH_2NHCOOCH_2C_6H_5$ (DL)
14 : R=COCH(OH)$CH_2CH_2CH_2NHCOOCH_2C_6H_5$ (*S*)

23 : R=$OSO_2C_6H_5$, R′=H
24 : R=H, R′=N_3
26 : R=H, R′=I
27 : R=N_3, R′=H
28 : R=H, R′=$OCOCH_3$
29 : R=H, R′=OH



15 : R=$COCH_3$
16 : R=$COCH_2OH$
17 : R=COCH(OH)CH_3 (DL)
18 : R=$COCH_2NH_2$
19 : R=$COCH_2CH_2NH_2$
20 : R=$COCH_2CH_2CH_2NH_2$
21 : R=COCH(OH)CH_2NH_2 (DL)
22 : R=COCH(OH)$CH_2CH_2CH_2NH_2$ (*S*)
25 : R=COCH(NH_2)$CH_2CH_2NH_2$ (D)
30 : R=COCH(NH_2)$CH_2CH_2NH_2$ (L)
31 : R=COCH(OH)$CH_2CH_2NH_2$ (*R*)

Chart 2

11) During this study, Haskell, *et al.*[7] have studied synthesis of 1-N-analogues of butirosin through an alternate exchange reaction of its amide side chain and have discussed their antibacterial activities.

were obtained by treatment of the amine (**6**) with acid chloride or active ester of appropriately protected carboxylic acid. Thus, acetoxyacetyl (**8**), DL-2-acetoxypropionyl (**9**), benzenesulfonamidoacetyl (**10**), 3-benzenesulfonamidopropionyl (**11**), 4-azidobutyryl (**12**), DL-3-benzyloxycarbonylamino-2-hydroxypropionyl (**13**), and L-5-benzyloxycarbonylamino-2-hydroxyvaleryl derivative (**14**) was prepared as described in the experimental section. Most of these acyl derivatives were purified by means of chromatography over silica gel. Each acyl derivative was treated with sodium in liquid ammonia at −50——60° and the resulting amine was purified through Amberlite CG-50 (H^+ form). Thus, new acyl-neamines (**15**—**22**) were obtained and characterized as their hydrochlorides.

As mentioned above, the penta-benzenesulfonyl derivative was obtained as a minor product on treatment of the acyl-neamine (**3**) with benzenesulfonyl chloride. The same product was prepared as a major product by treatment of **3** with excess of the reagent at room temperature for a long time and was easily purified by recrystallization. The following sequence of reactions verified that the product was designated as **23** as shown in the Chart: Treatment of **23** with sodium azide in dimethyl sulfoxide at 90—100° gave an azide (**24**), which was reduced with sodium inqliuid ammonia to afford a penta-amino compound (**25**). Saponification of **25** with aqueous barium hydroxide gave neamine and 2,4-diaminobutyric acid which were identified with authentic samples respectively by thin-layer chromatography (TLC) and high-voltage paper electrophoresis. Thus, the fifth benzenesulfonyl group in **23** was verified to be at the α-hydroxy of the acyl side chain. The α-carbon of the side chain in butirosin has been determined to have the *S*-configuration; subsequently, the configuration of the α-carbon with an azido group in **24** and with an amino group in **25** can be depicted as *R*, since nucleophilic displacement of the benzenesulfonyloxy group in **23** with an azide ion proceeds *via* S_N2-type reaction and gives an azide (**24**) with an inverted configuration. Treatment of **23** with one molar equivalent of lithium iodide or sodium iodide afforded an *R*-iodo compound (**26**). On treatment with sodium azide as above, **26** was converted into an isomeric *S*-azide (**27**) which was distinguished from the *R*-azide (**24**) obtained before by infrared spectrometry. In addition, treatment of **23** with sodium acetate in dimethyl sulfoxide gave an *R*-acetoxy derivative (**28**). Deacetylation of **28** with a base yielded an *R*-hydroxy derivative (**29**) distinct from the corresponding parent derivative (**5**) in infrared (IR) absorption and in movement on TLC. These compounds (**27** and **29**) were reduced with sodium in liquid ammonia, giving an epimeric penta-amino (**30**) and tetra-amino compound (**31**) respectively which were submitted to biological tests.

Antibacterial Activity

Minimum inhibitory concentration (M.I.C.) assays were conducted in comparison with those of the parent compound, 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-neamine (**3**). 1-N-Acetyl-neamine (**15**), α-hydroxyacyl-neamines (**16** and **17**), and ω-aminoacyl-neamines (**18**, **19**, and **20**) exhibited no marked activity against butirosin-sensitive bacteria, suggesting that the presence of both amino and hydroxy functions in the side chain is needed for the appearance of antimicrobial activity. 2,4-Diaminobutyryl-neamines (**25** and **30**), α-amino varients of **3**, were also inactive. Further, the epimer of **3** having an α-hydroxy group with *R*-configuration showed a significant loss in potency, suggesting that the *S*-configuration of the hydroxy group is required for activity. While the corresponding valeryl analogue (**22**) with one more additional methylene linkage was almost inactive, the racemic 3-amino-2-hydroxypropionyl analogue (**21**) showed less but appreciable activities against butirosin-sensitive bacteria including *Pseudomonas* species as illustrated in Table I. In conclusion, it would be mentioned that the (*S*)-4-amino-2-hydroxybutyryl chain is essential for the maximal antibacterial activity in these 1-N-acyl-neamines. This is parallel to the prediction of Haskell, *et al.*[7] that a limited number of carbons with a highly specific arrangement of the polar groups in the amido side chain are required for the maximal potency in butirosin 1-N-analogues.

Experimental

Melting points are not corrected. IR spectra were recorded on a Hitachi EPI-G3 spectrometer, and optical rotations were measured on a Perkin-Elmer Model 141 automatic polarimeter in 1 dm tubes. TLC was performed on TLC-plates Silica gel F_{254} precoated (E. Merck AG, layer thickness 0.25 mm) and spots were visualized by UV-irradiation and/or spraying with vanadic acid–sulfuric acid reagent.[12] Amino compounds were detected by ninhydrin reagent. For column chromatography on silica gel, Wakogel C-200 (Wako Pure Chemical Ind. Ltd., Osaka, Japan) and commercial $CHCl_3$ stabilized with about 1% of EtOH were used. Solvents were removed by a rotating flash evaporator at diminished pressure and usually at 35—50°.

3,2′,6′-Tri-N-benzenesulfonyl-1-N-[(*S*)-4-benzenesulfonamido-2-hydroxybutyryl]-neamine (5) and 3,2′,6′-Tri-N-benzenesulfonyl-1-N-[(*S*)-4-benzenesulfonamido-2-benzenesulfonyloxybutyryl]-neamine (23)——To a solution of 7.9 g of 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-neamine (3) tetrahydrochloride[10] and 6.3 g of Na_2CO_3 in a mixture of 30 ml of acetone and 70 ml of H_2O was added a solution of 7.1 ml of benzenesulfonyl chloride in 6 ml of acetone over a period of 15 min with stirring and ice-cooling. Then, after addition of 28 ml of acetone, the mixture was stirred at room temperature for 45 min and was acidified with AcOH to pH 5. The resulting precipitates completely separated by further addition of ice-water and standing for 2 hr at room temperature were collected and were dissolved in 120 ml of MeOH–$CHCl_3$ (1: 1, v/v). The mixture was treated with active carbon and was evaporated below 45°. The residue was dissolved in dioxane and charged on 250 g of silica gel. Initially, the column was eluted with $CHCl_3$ for removal of benzenesulfonyl chloride and dioxane. Fractions successively eluted with 5% MeOH/$CHCl_3$ were evaporated to give 0.6 g of **23** which was recrystallized from MeOH as fine needles, mp 194° (decomp.). $[\alpha]_D^{25}$+30.9° (c=2.0, DMF). IR ν_{max}^{Nujol} cm^{-1}: 1660, 1530. *Anal.* Calcd. for $C_{46}H_{53}O_{18}N_5S_5$: C, 49.03; H, 4.71; N, 6.22; S, 14.22. Found: C, 48.86; H, 4.73; N, 5.98; S, 14.27.

Further stepwise elution with 10% and 15% MeOH/$CHCl_3$ and removal of the solvent gave 7.0 g of **5** which was recrystallized from MeOH as fine needles, mp 230° (decomp.). $[\alpha]_D^{20}$+22.9° (c=1.0, DMF). IR ν_{max}^{Nujol} cm^{-1}: 1660, 1530 (amide). *Anal.* Calcd. for $C_{40}H_{49}O_{16}N_5S_4$: C, 48.82; H, 6.75; N, 7.12; S, 13.03. Found: C, 48.51; H, 4.95; N, 6.75; S, 13.12.

These composition of the fractions of chromatography was monitored by TLC: Solvent system, 10% or 20% (v/v) MeOH/$CHCl_3$.

Also, the penta-benzenesulfonyl derivative (**23**) was prepared in a good yield as follows: To a mixture of 5 g of tetrahydrochloride of **3** and 6.4 g of K_2CO_3 in a mixture of 50 ml of H_2O and 25 ml of acetone was added 9.3 g of benzenesulfonyl chloride with stirring at room temperature. The mixture was allowed to stand two days at room temperature and, then, filtered. The filtrate was diluted with a large volume of H_2O and the resulting oily precipitates were washed with H_2O. The precipitates crystallized on trituration with H_2O and gave 7.2 g (82%) of crude **23** which was recrystallized from MeOH, mp 194° (decomp.).

3,2′,6′-Tri-N-benzenesulfonyl-neamine (6)——A solution of 4.5 g of **5** in 20 ml of 80% $NH_2NH_2 \cdot H_2O$ (or 16 ml of 100% $NH_2NH_2 \cdot H_2O$) was refluxed for 7 hr with stirring and then evaporated *in vacuo*, leaving a thick oil which on trituration with EtOH gave 3.3 g of crystals. The crystals were dissolved in MeOH and, after filtration, was evaporated *in vacuo* to dryness. The residue was recrystallized from EtOH to give **6** as granular crystals, mp 143—170°. $[\alpha]_D^{27}$ +27.9° (c=1.03, MeOH). IR ν_{max}^{Nujol}: 1600 cm^{-1} (–NH_2). *Anal.* Calcd. for $C_{30}H_{38}O_{12}N_4S_3$: C, 48.51; H, 5.15; N, 7.54; S, 12.92. Found: C, 49.29; H, 5.39; N, 6.93; S, 12.90.

1-N-Acylation of 3,2′,6′-Tri-N-benzenesulfonyl-neamine (6)——(i) Acetyl Derivative (**7**): To a suspension of 500 mg of **6** in 10 ml of MeOH was added 0.5 ml of Ac_2O with stirring at room temperature. After having been stirred for 25 min, the mixture was dried up *in vacuo*. The residual foam was dissolved in a small amount of hot dioxane, and charged on a column of silica gel (10 g, packed with $CHCl_3$). The column was first eluted with $CHCl_3$ and then with 10% MeOH/$CHCl_3$. The fractions containing **7** were collected and evaporated, giving 500 mg of **7** as granules (on precipitation with MeOH–ether), mp 148—159°. $[\alpha]_D^{25}$+33.1° (c=0.9, DMF). IR ν_{max}^{Nujol} cm^{-1}: 1650, 1540. *Anal.* Calcd. for $C_{32}H_{40}O_{13}N_4S_3$: C, 48.97; H, 5.14; N, 7.14; S, 12.26. Found: C, 48.51; H, 5.37; N, 7.54; S, 11.88.

(ii) 4-Azidobutyryl Derivative (**12**): Synthesis of **12** is described as a general procedure for acylation with acid chlorides. To a solution of 1.5 g of **6** in 50 ml of acetone was added a solution of 1.0 g of $NaHCO_3$ in 15 ml of H_2O. Then, a solution of acid chloride prepared from 0.6 g of 4-azidobutyric acid in 10 ml of acetone was added dropwise with cooling and stirring. After having been stirred for 1 hr at room temperature, the mixture was diluted with H_2O and acidified with AcOH. The resulting solid was collected and after treatment with active charcoal in MeOH when it is needed, the product was recrystallized from EtOH to give 1.7 g of **12** as crystalline mass, mp 234° (decomp. with sintering at 229°). $[\alpha]_D^{20}$+37.3° (c=1.2, DMF). IR ν_{max}^{Nujol} cm^{-1}: 2100 (–N_3), 1640, 1550 (amide). *Anal.* Calcd. for $C_{32}H_{43}O_{13}N_7S_3 \cdot 1/2H_2O$: C, 47.32; H, 5.14; N, 11.36; S, 11.15. Found: C, 46.89; H, 5.11; N, 11.35; S, 11.51.

12) M. Ishidate, M. Matsui, and M. Okada, *Anal. Biochem.*, 11, 176 (1965).

(iii) Acetoxyacetyl Derivative (**8**): By using acetoxyacetyl chloride as acylating agent and by chromatographic purification on silica gel with MeOH–$CHCl_3$ as eluant, **8** was obtained as amorphous powder. $[\alpha]_D^{27}$ +12.5° (c=1.39, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1740 (acetoxy), 1650, 1540 (amide). *Anal.* Calcd. for $C_{34}H_{42}O_{15}N_4S_3$: C, 48.45; H, 5.02; N, 6.65; S, 11.41. Found: C, 48.77; H, 5.34; N, 6.61; S, 10.92.

(iv) DL-2-Acetoxypropionyl Derivative (**9**): Amorphous powder (from $CHCl_3$–ether). $[\alpha]_D^{20}$+34.7° (c=1, DMF). IR ν_{max}^{Nujol} cm^{-1}: 1730 (acetoxy), 1660, 1550 (amide). *Anal.* Calcd. for $C_{35}H_{44}O_5N_4S_3 \cdot H_2O$: C, 48.05; H, 5.30; N, 6.40; S, 10.99. Found: C, 47.65; H, 5.14; N, 6.61; S, 10.93.

(v) Benzenesulfonamidoacetyl Derivative (**10**): Acid chloride prepared from N-benzenesufonylglycine[13] was used. Granular crystals (from MeOH–ether). $[\alpha]_D^{25}$+33.7° (c=1.1, DMF). IR ν_{max}^{Nujol} cm^{-1}: 1660, 1540. *Anal.* Calcd. for $C_{38}H_{45}O_{15}N_5S_4$: C, 48.55; H, 4.83; N, 7.45; S, 13.64. Found: C, 48.28; H, 5.05; N, 7.08; S, 13.32.

(vi) 3-Benzenesulfonamidopropionyl Derivative (**11**): Acid chloride prepared from N-benzenesulfonyl-β-alanine was used. Amorphous powder, mp>220°. $[\alpha]_D^{27}$+28.9° (c=1.5, DMF). IR ν_{max}^{Nujol} cm^{-1}: 1640, 1540. *Anal.* Calcd. for $C_{39}H_{47}O_{15}N_5S_4 \cdot H_2O$: C, 48.29; H, 4.88; N, 7.22; S, 13.22. Found: C, 48.28; H, 4.85; N, 7.01; S, 13.16.

(vii) DL-3-Benzyloxycarbonylamino-2-hydroxypropionyl Derivative (**13**): A solution of 1 g of N-benzyloxycarbonyl derivative, mp 117—119° (from AcOEt), of DL-isoserine,[14] 0.48 g of N-hydroxysuccinimide, and 0.87 g of N,N′-dicyclohexylcarbodiimide in 15 ml of dioxane was allowed to stand in a refrigerator overnight. Precipitates were removed by filtration and the filtrate was dried up to a thick syrup, which was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over anhyd. Na_2SO_4 and evaporated, giving a semi-solid. A solution of this solid in 10 ml of DMF was added to a solution of 1.3 g of **6** in 10 ml of DMF. After having been stirred at room temperature overnight, the reaction mixture was concentrated to a syrup which gave 1.23 g of solid on trituration with H_2O. The crystalline mass obtained by treatment of the solid with EtOH was washed with AcOEt to give 400 mg of **13**, mp 245—246° (with slightly browning at 220°). Its TLC revealed two spots which were very close to each other. The mother liquor and the washings were collected and purified by column chromatography over silica gel using 3% (v/v) MeOH/$CHCl_3$, giving 52 mg of further crops. $[\alpha]_D^{25}$+28.3° (c=1.09, DMF). IR ν_{max}^{Nujol} cm^{-1}: 1700 (benzyloxycarbonyl), 1660, 1530 (amide). *Anal.* Calcd. for $C_{41}H_{49}O_{16}N_5S_3$: C, 51.08; H, 5.12; N, 7.26; S, 9.98. Found: C, 50.87; H, 5.31; N, 7.26; S, 9.36.

(viii) (*S*)-5-Benzyloxycarbonylamino-2-hydroxyvaleryl Derivative (**14**): To a solution of 535 mg of N-benzyloxycarbonyl derivative, mp 106—107°, $[\alpha]_D^{20}$+1.7° (MeOH), of *S*-5-amino-2-hydroxyvaleric acid[15] and 323 mg of N-hydroxysuccinimide in 7 ml of dry dioxane, 413 mg of N,N′-dicyclohexylcarbodiimide was added with vigorous stirring. After addition of the reagent, the mixture was stirred at room temperature overnight. Precipitates were removed by filtration and the filtrate was dried up to a thick syrup which was washed with dry ether. To a solution of this syrup in 4 ml of DMF was added a solution of 1.19 g of **6** in 9 ml of DMF. After having been stirred at room temperature for 1.3 hr, the reaction mixture was concentrated to a syrup which gave 1.12 g of solid on trituration with dil. AcOH. A solution of the solid in hot dioxane was charged on a column of 22 g of silica gel packed with $CHCl_3$ and the column was first washed with $CHCl_3$, then eluted with 5% (v/v) MeOH/$CHCl_3$. The fractions containing pure **14** were collected and dried to an amorphous powder, 544 mg. $[\alpha]_D^{20}$+3.1° (c=1.2, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1700 (benzyloxycarbonyl), 1660, 1530 (amide). *Anal.* Calcd. for $C_{43}H_{53}O_{16}N_5S_3$: C, 52.09; H, 5.38; N, 7.06; S, 9.70. Found: C, 51.92; H, 5.34; N, 7.23; S, 9.84.

1-N-[(*R*)-2-Azido-4-benzenesulfonamidobutyryl]-3,2′,6′-tri-N-benzenesulfonyl-neamine (**24**)——A solution of 0.5 g of **23** and 0.1 g of NaN_3 in 4 ml of dimethyl sulfoxide (DMSO) was stirred and heated at 100° in N_2 atmosphere for 1 hr. The reaction mixture was poured into 100 ml of ice-water and the resultant precipitates were collected after standing at room temperature overnight and dried, giving a crude product, 460 mg, mp 213—218° with bubbling. The precipitates were dissolved in MeOH, treated with charcoal and evaporated to dryness. The residue was triturated with iso-PrOH–ether and gave a crystalline mass (305 mg), mp 211—212° (with bubbling). $[\alpha]_D^{27}$+25.3° (c=1.8, DMF). IR ν_{max}^{Nujol} cm^{-1}: 2100 ($-N_3$). *Anal.* Calcd. for $C_{40}H_{48}O_{15}N_8S_4$: C, 47.61; H, 4.79; N, 11.10; S, 12.71. Found: C, 47.78; H, 4.97; N, 10.96; S, 12.40.

1-N-[(*R*)-4-Benzenesulfonamido-2-iodobutyryl]-3,2′,6′-tri-N-benzenesulfonyl-neamine (**26**)——A solution of 1.0 g (0.9 mmole) of **23** and 134 mg (1 mmole) of LiI in 10 ml of acetone was refluxed with stirring for 5 hr, then concentrated to a small volume and diluted with H_2O. The resulting precipitates were washed with H_2O and dissolved in EtOH. The amorphous powder obtained by removal of the solvent was chromatographed over a column of silica gel (25 g) using $CHCl_3$, 5% and 10% MeOH/$CHCl_3$ as eluant. The fractions containing pure **26** were collected, evaporated, and gave 774 mg of **26**, which was dissolved in EtOH–benzene and the solvent was distilled. This procedure was repeated several times, giving the analytical sample.

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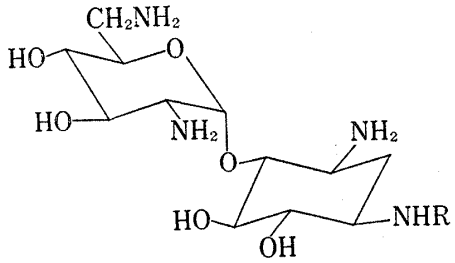
Anal. Calcd. for $C_{40}H_{48}O_{15}N_5S_4I \cdot C_2H_5OH$: C, 44.32; H, 4.61; N, 6.15; S, 11.27; I, 11.15. Found: C, 45.61; H, 4.54; N, 6.00; S, 11.29; I, 11.39.

1-N-[(*S*)-2-Azido-4-benzenesulfonamidobutyryl]-3,2′,6′-tri-N-benzenesulfonyl-neamine (27)—— A solution of 452 mg of **26** and 0.1 g of NaN_3 in 4 ml of DMSO was heated at 90—100° with stirring for 1 hr. The reaction mixture was poured into 80 ml of ice-water containing 0.2 g of sodium thiosulfate, and the resulting suspension was allowed to stand for 2 hr. Precipitated material was collected on a filter and dried (432 mg). The material was dissolved in MeOH and treated with active charcoal and the solution was evaporated to dryness. Trituration of the residue with iso-PrOH–ether gave 243 mg of pure **27**, mp 122—139°. IR ν_{max}^{Nujol}: 2100 cm^{-1} ($-N_3$). $[\alpha]_D^{25}$ +30.2° (c=0.65, DMF). *Anal.* Calcd. for $C_{40}H_{48}O_{15}N_8S_4 \cdot C_3H_7OH$: C, 48.30; H, 5.28; N, 10.48; S, 12.00. Found: C, 48.11; H, 5.26; N, 10.15; S, 12.11.

1-N-[(*R*)-2-Acetoxy-4-benzenesulfonamidobutyryl]-3,2′-6′-tri-N-benzenesulfonyl-neamine (28)——A solution of 1.0 g of **26** and 0.3 g of anhyd. NaOAc in 8 ml of DMSO was heated at 100—110° with stirring in N_2 atmosphere for 2 hr, then poured into 50 ml of ice-water. The resultant precipitates were collected and dried, giving 0.85 g of crude **28** as powder-like crystals. Purification of **28** was carried out by using chromatography on 28 g of silica gel using 5% (v/v) MeOH–$CHCl_3$ as eluant. Re-precipitation of pure **28** thus obtained (352 mg) with MeOH–ether gave powder like crystals, mp 109—131°. $[\alpha]_D^{27}$ +41.8° (c=1.9, DMF). IR ν_{max}^{Nujol}: 1740 cm^{-1} (acetoxy). *Anal.* Calcd. for $C_{42}H_{51}O_{17}N_5S_4$: C, 49.16; H, 5.01; N, 6.83; S, 12.50. Found: C, 48.93; H, 5.21; N, 6.71; S, 12.13.

1-N-[(*R*)-4-Benzenesulfonamido-2-hydroxybutyryl]-3,2′,6′-tri-N-benzenesulfonyl-neamine (29)——The crude **28** obtained from 1.0 g of **23** was dissolved in 8 ml of MeOH containing 1 ml of 1N methanolic NaOMe. The solution was allowed to stand at room temperature for 10 min and then, acidified with AcOH and evaporated to dryness. The residue was chromatographed over a column of silica gel (20 g) using $CHCl_3$, 5% and 10% MeOH–$CHCl_3$ as eluant, giving 365 mg of crude **29** and 354 mg of pure **29**. The pure sample melted at

Table II. Synthesized Acyl-neamines



No.	R	mp (decomp.)	$[\alpha]_D$ in H_2O (c, temp.)	Formula	Analysis (%) Found (Calcd.) C	H	N	Cl
15	$COCH_3$	210—212°	+72.5°(0.67, 27°)	$C_{14}H_{28}O_7N_4 \cdot 3HCl \cdot 2H_2O$	33.68 (32.98	6.91 6.92	10.22 10.99	20.77 20.86)
16	$COCH_2OH$	—	+54.2°(0.45, 20°)	$C_{14}H_{28}O_8N_4 \cdot 3HCl \cdot 3H_2O$	31.21 (30.92	6.47 6.85	10.11 10.30	19.40 19.56)
17	$COCH(OH)CH_3$ (DL)	207—223°	+67.9°(0.98, 27°)	$C_{15}H_{29}O_8N_4 \cdot 3HCl \cdot CH_3OH$	36.14 (35.93	7.12 6.79	10.17 10.48	19.83 19.89)
18	$COCH_2NH_2$	—	+60.1°(1.4, 23°)	$C_{14}H_{27}O_7N_5 \cdot 4HCl \cdot H_2O$	31.56 (31.05	6.37 5.39	12.93 12.93	26.51 26.96)
19	$COCH_2CH_2NH_2$	230—245°	+57.8°(2.0, 27°)	$C_{15}H_{31}O_7N_5 \cdot 4HCl \cdot 2H_2O$	31.50 (31.31	6.69 6.83	11.80 12.17	25.65 24.65)
20	$COCH_2CH_2CH_2NH_2$	130—232°	+58.0°(2.1, 27°)	$C_{16}H_{33}O_7N_5 \cdot 4HCl \cdot H_2O$	33.26 (33.63	6.83 6.88	11.99 12.26	24.67 24.82)
21	$COCH(OH)CH_2NH_2$ (DL)	247—260°	+61.3°(1.3, 25°)	$C_{15}H_{31}O_8N_5 \cdot 4HCl \cdot H_2O$	31.05 (31.42	6.65 6.51	12.07 12.22	24.74 24.43)
22	$COCH(OH)CH_2CH_2CH_2NH_2$ (S)	200—217°	+52.4°(1.0, 20°)	$C_{17}H_{35}O_8N_5 \cdot 4HCl \cdot 2H_2O$	33.26 (32.94	7.02 6.99	11.67 11.31	21.09 22.90)
25	$COCH(NH_2)CH_2CH_2NH_2$ (D)	241—255°	+44.4°(1.3, 25°)	$C_{16}H_{34}O_7N_6 \cdot 5HCl \cdot C_2H_5OH$	33.05 (33.21	7.05 6.97	13.04 12.91	26.76 27.24)
30	$COCH(NH_2)CH_2CH_2NH_2$ (L)	225—245°	+55.4°(1.15, 25°)	$C_{16}H_{34}O_7N_6 \cdot 5HCl \cdot H_2O$	31.01 (30.85	6.49 6.63	13.30 13.49	28.80 28.46)
31	$COCH(OH)CH_2CH_2NH_2$ (R)	200—217°	+52.4°(1.0, 20°)	$C_{17}H_{35}O_8N_5 \cdot 4HCl \cdot 2H_2O$	34.03 (33.95	6.95 6.87	11.23 11.65	23.58 23.58)

237—240° with sintering at 222°. *Anal.* Calcd. for $C_{40}H_{49}O_{16}N_3S_4 \cdot 2H_2O$: C, 47.09; H, 5.24; N, 6.87; S, 12.57. Found: C, 46.98; H, 5.02; N, 6.95; S, 12.63.

These isomers (**5** and **29**) were distinguishable in the region of 1000—800 cm^{-1} of their infrared spectra and also in their *Rf* values of TLC (Solvent system, 10% MeOH/$CHCl_3$, v/v; *Rf* for **5**, 0.32—0.33; *Rf* for **29**, 0.27—0.28).

1-N-Acyl-neamine Hydrochlorides (15—22, 25, 30, 31)——To a solution of about 500 mg of the amino-protected acyl-neamine prepared as above in 5—10 ml of liquid ammonia, 0.2 g of Na pieces were added with stirring at −60°. The solution was further stirred at −45° for 20 min, diluted with MeOH for decomposition of excess Na, and evaporated *in vacuo*. The residue was dissolved in a small amount of H_2O and Amberlite CG-50 (H^+ form) was added with stirring until the supernatant became negative to ninhydrin test. The resin was collected and washed with H_2O and 0.3N NH_4OH successively, then was eluted with 0.5—1.5N NH_4OH. The fractions which were pure on TLC (solvent system: conc. NH_4OH: MeOH=1: 1, v/v) were collected and concentrated *in vacuo* at 45° (bath temp.) until ammonia was completely removed. Adjusting to pH 3—4 with 2N HCl and concentrating gave a syrup which was triturated with EtOH or MeOH or was lyophilized. The physico-chemical properties of the N-acyl neamines are listed in Table II.

Alkaline Degradation of 1-N-(D-2,4-Diaminobutyryl)-neamine (25)——A solution of a few mg of **25** in sat. aq. $Ba(OH)_2$ was heated on a steam bath for 2 hr, then neutralized with solid CO_2 and filtered. The filtrate was examined by TLC (solvent systems, 14% NH_4OH: MeOH=1: 10 or upper layer of $CHCl_3$: MeOH: 14% NH_4OH=2: 1: 1, v/v) and by high-voltage paper electrophoresis (Toyo Roshi No. 51, formic acid: AcOH: H_2O=1: 3: 36, pH 1.74, 3300 V, 26—22.5 mA, 30 min, 10°C; *Rm*(alanine): neamine=2.3; 2,4-diaminobutyric acid=1.6). The results of these examinations revealed the presence of neamine and 2,4-diaminobutyric acid in the filtrate.

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