Chemical Transformation of Seldomycin 5 into 3′-Episeldomycin 5 and Its Antibacterial Activity

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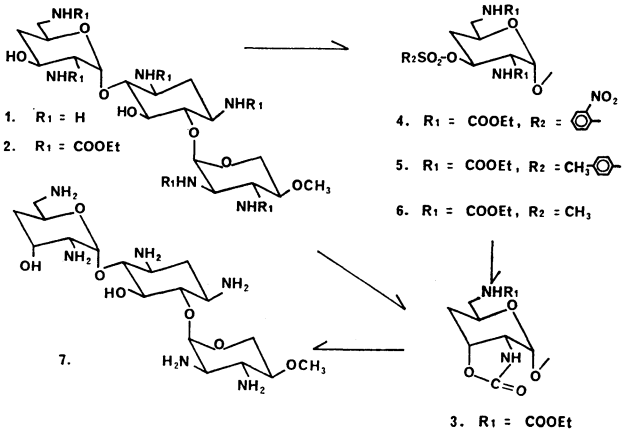
3′-Episeldomycin 5 (**7**) was synthesized from seldomycin 5. Reaction of hexa-*N*-ethoxycarbonylseldomycin 5 (**2**) with *o*-nitrobenzenesulfonyl chloride gave penta-*N*-ethoxycarbonylseldomycin 5-2′,3′-epicyclic carbamate (**3**) in one step. Hexa-*N*-ethoxycarbonyl-3′-*O*-tosylseldomycin 5 or hexa-*N*-ethoxycarbonyl-3′-*O*-mesylseldomycin 5, prepared from **2**, was also converted into **3** by being heated in weakly basic solvents such as *N*,*N*-dimethylformamide or pyridine. Compound **3** was hydrolyzed to give **7**. Structure of **7** was confirmed by PMR, CMR, MS, and Δ[M] values. Compound **7** was found to be active against resistant bacteria which carry 3′-*O*-phosphorylating enzymes. Δ[M] values for vicinal amino groups were shortly discussed.

Seldomycins are new aminoglycoside antibiotic complex produced by *Streptomyces hofuensis* and five factors are known among which factor 5 is the most active component.[1] Structure of seldomycin 5 has been shown to be 4-*O*-[2,6-diamino-2,4,6-trideoxy-α-D-*xylo*-hexopyranosyl]-6-*O*-[2,3-diamino-2,3-dideoxy-4-*O*-methyl-α-D-xylopyranosyl]-2-deoxystreptamine (**1**).[2] Seldomycin 5 has been shown to exhibit strong antibacterial activity against both gram-positive and gram-negative bacteria, but to be inactivated, similarly to other aminoglycoside antibiotics having 3′-hydroxyl group,[3] by resistant strains carrying 3′-*O*-phosphorylating enzymes.[1] Consideration of spatial specifity manifested by most enzymes led us to transform **1** into 3′-episeldomycin 5 (**7**) which has an axial hydroxyl group at 3′ position and expect it to be active against resistant strains carrying those enzymes. Hanessian *et al.* had transformed paromamine into 3′-epiparomamine by oxidation of the 3′-hydroxyl group to a carbonyl group and reducing it by sodium borohydride, but the antibacterial activity of the product had not been reported.[4]

Results and Discussion

Compound **1** was converted into hexa-*N*-ethoxycarbonyl(ecb)-seldomycin 5 (**2**) as an *N*-protected derivative. Reaction of **2** with *o*-nitrobenzenesulfonyl chloride in pyridine at 60—65 °C for 45 h surprisingly gave penta-*N*-*ecb*-seldomycin 5-2′,3′-epicyclic carbamate (**3**) in 57.9% yield. When this reaction was run at 13.5 °C for 20 h, hexa-*N*-*ecb*-seldomycin 5-3′-*O*-(*o*-nitrobenzenesulfonate) (**4**) was isolated in 70.3% yield and **4** was then converted into **3** by being heated in pyridine at 65 °C for 43 h in 80.4% yield. These results indicate that direct formation of **3** from **2** at higher temperature occurs by way of the formation of **4** *in situ*. The critical temperature above which the direct formation of **3** mainly occurs within 45 h was determined to be about 55 °C. Although the anchimeric ability of a benzyloxycarbonylamino group to a vicinal carbon atom which has an eliminating group such as halogen, mesyloxy, and epoxide is well known,[5] direct conversion of **2** into **3** may be, to our best knowledge, the first instance of epimerization of a free hydroxyl group in one step. On the other hand, reaction of **2** with *p*-toluenesulfonyl chloride or methanesulfonyl chloride in pyridine at 55—65 °C for 23 h gave hexa-*N*-*ecb*-3′-*O*-tosylseldomycin 5 (**5**) or hexa-*N*-*ecb*-3′-*O*-mesylseldomycin 5 (**6**) in 96.0 or 90.6% yield, respectively. Conversion of **5** or **6** into **3** was achieved by heating them in weakly basic solvents such as pyridine, *N*,*N*-dimethylformamide, at 80—110 °C for 18—19.5 h in 75.8 or 38.6% yield, respectively.

The structure of **3** was elucidated by the following evidences. (i) elemental analysis, (ii) disappearance of the signals due to the sulfonate residues in PMR and IR, (see experimental section), (iii) appearance of a new peak at 1760 cm^{-1} in IR due to a carbonyl group of a five-membered cyclic carbamate, (iv) In the CMR spectrum of **3** (Table 1), a new signal appeared at 158.8 ppm which is attributable to the carbonyl carbon of the newly formed cyclic carbamate. There were 6.5 and 9.8 ppm upfield shifts in the C-2′ and C-4′ signals, respectively, relative to the corresponding signals in the spectrum of **2**, while 7.2 ppm downfield shift in the C-3′ signal was observed resulting from the transformation of **2** into **3**. (v) hydrolysis to **7**. The reactions described above are summarized in Scheme 1 and possible mechanism for the formation of the epicyclic carbamate ring is shown in Scheme 2. This mechanism requires water for the formation of **3**, which is supposed to be contained in the solvents used. In fact both the pyridine and DMF that we used were revealed (Karl Fisher method) to contain about 650 ppm water, which is stoichiometrically enough for the



Scheme 1.

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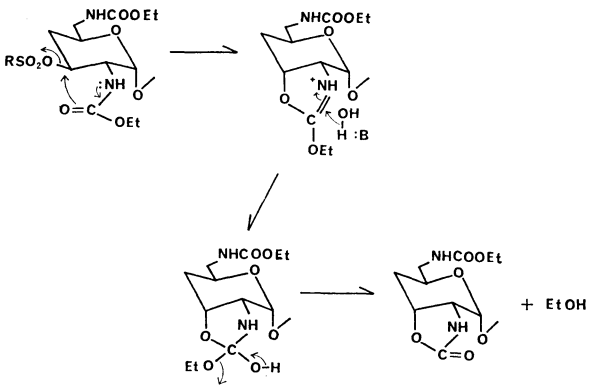
Table 1. CMR chemical shifts of compounds **1**, **2**, **3**, and **7**

Compound	**2**[a]	**3**[a]	Δ	**1**[b,c]	**7**[b]	Δ
C-1′	99.3	96.5	−2.8	101.9	102.6	0.7
C-2′	57.6	51.1	−6.5	57.6	51.8	−5.8
C-3′	64.4	71.6	7.2	69.0	67.9	−1.1
C-4′	37.0	27.2	−9.8	37.0	35.3	−1.7
C-5′	66.6	65.2	−1.4	70.8	66.0	−4.8
C-6′	44.4	44.0	−0.4	45.6	45.6	0.0
C-1	50.7	51.1	0.4	51.1	51.0	−0.1
C-2	35.0	34.7	−0.3	36.5	36.5	0.0
C-3	49.9	49.5	−0.4	50.1	49.9	−0.2
C-4	82.3	81.7	−0.6	87.6	88.7	1.1
C-5	74.6	74.6	0.0	75.1	75.1	0.0
C-6	79.4	78.7	−0.7	86.8	86.9	0.1
C-1″	96.7	96.5	−0.2	99.9	100.0	0.1
C-2″	54.4	54.3	−0.1	56.2	56.2	0.0
C-3″	53.1	53.2	0.1	54.8	54.7	−0.1
C-4″	76.1	76.2	0.1	80.1	80.2	0.1
C-5″	59.5	59.6	0.1	60.8	60.8	0.0
OCH_3	57.6	57.7	0.1	58.7	58.7	0.0
$COOCH_2\underline{CH_3}$[d]	14.6	14.6	0.0	—	—	—
$COO\underline{CH_2}CH_3$[d]	59.5	59.6	0.1	—	—	—
$\underline{CO}OCH_2CH_3$[e]	155.4	155.2	—	—	—	—
	156.0	155.3	—	—	—	—
	—	156.0	—	—	—	—
new CO	—	158.8	—	—	—	—

a) Measured in DMSO-d_6 solution with DMSO-d_6 as internal reference and shown by ppm from TMS, using $\delta_{TMS}=39.5-\delta_{DMSO}$. b) Measured in D_2O solution with dioxane as internal reference and shown by ppm from TMS, using $\delta_{TMS}=67.4-\delta_{dioxane}$. c) CMR data of this compound had been reported in Ref. 2. Each chemical shifts obtained here are consistent with those reported in Ref. 2 within 0.5 ppm deviation. d) All the methyls or methylenes of the ecb residues showed the single chemical shift. e) The carbonyls of the ecb residues in **2** or **3** showed the two or three chemical shifts, respectively.



Scheme 2. Possible mechanism for the formation of the epicyclic carbamate ring.

reactions.

Alkaline hydrolysis of **3** caused simaltaneous deethoxycarbonylation and fission of the epicyclic carbamate ring to give **7**. The structure of **7** was supported by the following evidences. (i) High resolution mass spectrum gave the (M+1) peak at 451.2912 (*m/e*), which means **7** is an isomer of **1**. Its fragmentation pattern is quite similar to that of **1**.[2] (ii) In the CMR spectrum of **7** (Table 1), the four signals due to the C-2′—C-5′ carbons appeared in the range of 1.1—5.8 ppm upfield compared with the corresponding signals in the spectrum of **1**. These upfield shifts resulting from epimerization of the 3′-hydroxyl group are consistent with those reported by Roberts and Dorman[6] or Perlin *et al.*[7] (iii) In the PMR spectrum of **7** (Table 2), the signals due to the H-2′, H-3′, and H-4′ax showed 0.3—0.4 ppm downfield shifts, respectively, from the corresponding shifts in the spectrum of **1**. These downfield shifts are well compared with the empirical rule proposed by Lemiuex and Stevens.[8] (iv) Optical rotatory behavior (Δ[M] value) in copper-ammonium solutions[9,10] also supported the structure of **7**. Compound **1** or **7** has two sites where the formation of copper complex are possible, *i.e.*, a pair of adjacent 2′-amino and 3′-hydroxyl groups (site A) and a pair of adjacent 2″- and 3″-amino groups (site B). Supposing that the complex formation occurs equally at site A and site B, contributions of each site to the apparently observed Δ[M] values could be calculated as shown in Table 3. These results indicate that the projected angle[9] at site A in **7** is +60° (counterclockwise), while it is −60° (clockwise) in **1**. It seems to be noteworthy that the sign of $\Delta[M]_{cupra\ B}$ for the vicinal amino groups at site B (the projected angle: clockwise) is minus. That is in accord with the sign conventionally observed for vicinal amino and hydroxyl groups or vicinal hydroxyl groups in a chair-formed pyranose or cyclitol. On the other hand the $\Delta[M]_{CuAm}$ value for the vicinal amino groups is nearly zero.

Table 2. Selected PMR chemical shifts (τ value) of compounds **1** and **7**[a]

	H-1′	H-2′	H-3′	H-4′$_{ax}$	H-4′$_{eq}$	H-5′
Compound **1**	4.70	7.4	6.3	8.72	8.1	6.0
Compound **7**	4.86	7.1	6.0	8.3	8.1	5.9

a) Measured in D_2O solution with DSS as internal standard (100 MHz). Each chemical shifts were confirmed by spin decoupled method.

Table 3. Δ[M] value of compounds **1** and **7**

	CuAm		Cupra B	
	1	**7**	**1**	**7**
$\Delta[M]_{observed}$	−950	+780	−1100	+620
Contribution of the site A[a]	−870	+870	−860	+860
Contribution of the site B[b]	−90	−90	−240	−240

a) $(\Delta[M]_{\text{observed with 7}}-\Delta[M]_{\text{observed with 1}})/2$.
b) $(\Delta]M]_{\text{observed with 7}}+\Delta[M]_{\text{observed with 1}})/2$.

Table 4. The minimum inhibitory concentrations of compounds **7** and **1** (mcg/ml)

Strain	Inactivating enzyme[a]	**7**	**1**
Staphylococcus aureus 209-P		1.56	0.78
Staphylococcus aureus Smith		1.56	0.78
Escherichia coli NIHJC-2		12.5	6.25
Escherichia coli GN-2411-5		25	12.5
Escherichia coli R-5	APH(3′)-I	25	>100
Escherichia coli R-12	ANT(2″)	25	6.25
Escherichia coli R-16	APH(3′)-I	6.25	>100
Escherichia coli R-17	AAC(6′)-I	25	25
Escherichia coli R-18	APH(3′)-II	12.5	12.5
Escherichia coli R-19	AAC(3)-I	>100	>100
Escherichia coli R-20	APH(3′)-I	12.5	>100
Proteus vulgaris JJ		50	6.25
Pseudomonas aeruginosa BmH#1		12.5	12.5
Pseudomonas aeruginosa TI-13a	APH(3′)-I	50	>100
Klebsiella pneumoniae KY4274		12.5	>100
Klebsiella pneumoniae Y-58	ANT(2″)	12.5	50
Providencia 164	AAC(2′)-I	>100	>100
Serratia marcescens 1065	AAC(6′)-IV	>100	>100

a) APH: aminoglycoside antibiotic phosphotransferase, ANT: aminoglycoside antibiotic nucleotidyltransferase, AAC: aminoglycoside antibiotic acetyltransferase. The figure in parenthesis indicates the position where the antibiotic is enzymatically modified.

No Δ[M] values for vicinal amino groups has been hitherto reported.

Table 4 shows *in vitro* antibacterial activities of **7** and **1**. It is obvious that epimerization of the 3′-hydroxyl group of **1** improves, as expected, its antibacterial activity against these resistant strains carrying 3′-*O*-phosphorylating enzyme [APH(3′)-I],[11] but does not improve its activity against other kinds of resistant strains and sensitive strains. LD_{50} value of **7** was estimated to be 183 mg/kg (mice, intravenously), while that of **1** was 667 mg/kg, namely, **7** is unexpectedly more toxic than **1** in acute toxicity.

After we had completed this work, Mallams *et al.* reported the synthesis of 3′-epigentamicin X_2, starting from 3,4,6-tri-*O*-acetyl-D-allal, with similar objective in mind.[12]

Experimental

General. Mps were determined with Yanaco micro melting point apparatus and uncorrected. IR spectra (with KBr pellet) were taken with Shimazu IR-27G or Perkin-Elmer 125 model. Varian T-60 or JEOL PS-100 was employed for NMR measurements with TMS as internal reference or in D_2O with DSS as internal reference. Samples of **1** and **7** were decarbonated by the usual manner[13] just before the NMR measurements. Optical rotations were measured with Hitachi-Perkin-Elmer 141 polarimeter at ambient temperature. Mass spectra were obtained with JEOL JMS-01SG-2 model at 75 eV using direct insertion probe. Thin layer chromatography (tlc) was carried out with Merck TLC plate and spots were visualized with iodine vapor and or UV light and or ninhydrin. Water content of solvent was measured with Tsutsui Rikagaku Kikai's Karl-Fisher automatic titrator. Evaporation of solvents was done *in vacuo* at bath temps of below 45 °C. The solutions of CuAm and Cupra B were prepared according to the literatures.[10],[11] Antibacterial activity was determined with the agar dilution method at pH 7.2 specified by the Japan Society of Chemotherapy.

Hexa-N-ecb-*seldomycin 5 (**2**).* To an ice-cooled solution of **1** (13.5 g) in water (195 ml) and acetone (195 ml) containing sodium carbonate (anhydrous, 45.6 g), was added with vigorous stirring ethyl chroloformate (43.2 g) in ten minutes. After all the ethyl chloroformate was added, the reaction mixture was stirred for 20 h at 19 °C. The white ppt formed was filtered, washed with water (800 ml) and ether (500 ml), successively. The ppt was suspended again in water (600 ml) and stirred for 1 h at room temp. The undissolved material was filtered, washed with water (1000 ml) and dried *in vacuo* over phosphorus pentaoxide overnight to give a white powder of **2**, 18.9 g. Mp>300 °C, $[\alpha]_D^{17}$ +76.1° (*c* 0.315, DMF), R_f value on TLC (chloroform–methanol 12 : 1); 0.55.

Found: C, 48.50; H, 7.20; N, 9.36%. Calcd for $C_{36}H_{62}N_6O_{19}$: C, 48.96; H, 7.09; N, 9.52%.

Hexa-N-ecb-*3′-O-tosylseldomycin 5 (**5**).* To a solution of **2** (3.00 g) in pyridine (150 ml), *p*-toluenesulfonyl chloride (9.90 g) was added and the solution was allowed to stand at 55—65 °C for 23 h. A single spot appeared on TLC (R_f= 0.67, chloroform–methanol 12 : 1). After addition of water (10 ml), the solution was evaporated and the residue was treated with water (150 ml), the water-insoluble material was filtered, washed with water (200 ml) and then with ether (150 ml), dried *in vacuo* overnight to give a white powder, 3.42 g. Analytical sample was recrystallized from aq ethanol. Mp 193—197 °C, $[\alpha]_D^{19}$ +72.3° (*c* 0.361, DMF);

Found: C, 49.51; H, 6.65; N, 8.08; S, 2.98%. Calcd for $C_{43}H_{68}N_6O_{21}S$: C, 49.79; H, 6.62; N, 8.10; S, 3.09%. IR: 1175 cm^{-1}, PMR (in DMSO-d_6): τ 2.50 (4H, q), 7.60 (3H,s).

Hexa-N-ecb-*3′-O-mesylseldomycin 5 (**6**).* To a solution of **2** (5.20 g) in pyridine (500 ml), methanesulfonyl chloride (11.8 g) was added and the solution was allowed to stand at 24 °C for 3.5 h. On TLC with chloroform–methanol 9 : 1 a spot (R_f=0.80) appeared. After addition of water (10 ml), the solution was concentrated to *ca.* 20 ml. Water (150 ml) was added to the concentrate and the ppt formed was filtered, washed with water (80 ml) and dried *in vacuo* overnight

to give a white powder, 5.14 g. Analytical sample was recrystallized from aq ethanol. Mp 242—243 °C, $[\alpha]_D^{24}$ +118° (*c* 0.347, DMF).

Found: C, 46.30; H, 6.81; N, 8.68; S, 3.62%. Calcd for $C_{37}H_{64}N_6O_{21}S$: C, 46.23; H, 6.73; N, 8.75; S, 3.34%. IR: 1170 cm^{-1}. PMR (in DMSO-d_6): τ 6.87 (3H, s).

Hexa-N-ecb-*3'*-O-*(o-nitrophenylsulfonyl)seldomycin 5* (**4**). To a solution of **2** (1.00 g) in pyridine (50 ml), *o*-nitrobenzenesulfonyl chloride (3.02 g) was added and the solution was kept at 13 °C for 20 h. Similar processing as described above gave a solid of crude **4**, which showed a major spot (R_f=0.36, chloroform–methanol 18 : 1) and two faster moving spots on TLC. Recrystallization of the crude **4** from aq ethanol gave a pure product which showed a single spot on tlc. mp 169—171 °C, $[\alpha]_D^{14}$ +34.6 ° (*c* 0.327, DMF).

Found: C, 47.08; H, 6.07; N, 8.91, S, 3.49%. Calcd for $C_{42}H_{65}N_7O_{23}S$: C, 47.22; H, 6.15; N, 9.18; S, 3.00%. PMR (in DMSO-d_6): τ 2.0 (4H, m).

Penta-N-ecb-*seldomycin 5-2',3'-Epicyclic Carbamate* (**3**).
i) *form* **2**: To a solution of **2** (2.78 g) in pyridine (150 ml) *o*-nitrobenzenesulfonyl chloride (7.50 g) was added and the solution was kept at 60—65 °C for 45 h. On TLC with chloroform–methanol 12 : 1 a major spot of **3** (R_f=0.24) and a small spot of **4** (R_f=0.54) appeared. After a small amount of water (1.5 ml) was added, the solution was evaporated to dryness. The residue was chromatographed on a column of silica gel (Merck, 170 g) with chloroform–methanol 30 : 1—25 : 1. The portion containing **3** was evaporated to give a white powder which showed a single spot on TLC, 1.53 g. Mp 247—248 °C, $[\alpha]_D^{16}$ +47.8 ° (*c* 0.320, DMF).

Found: C, 48.37; H, 6.73; N, 9.91%. Calcd for $C_{34}H_{56}N_6O_{18}$: C, 48.79; H, 6.76; N, 10.04%. IR: 1760 cm^{-1}.

ii) *from* **5**: The compound **5** (2.90 g) was dissolved in DMF (58 ml) and the solution was heated at 110 °C for 19.5 h. A small amount of ppt was filtered off and the filtrate was evaporated to dryness. The residue was chromatographed on a column of silica gel (180 g) with chloroform–methanol 30 : 1—20 : 1. From earlier fractions 0.445 g of unreacted **5** was recovered. The portion containing **3** was evaporated to give 1.49 g of a white solid. Mp 249—250 °C. IR spectrum was identical with that of **3** obtained in i).

iii) *from* **6**: The solution of **6** (1.00 g) in a mixture of DMF (18 ml) and water (2ml) was kept at 110 °C for 18 h. Recovered **6** (0.47 g) and a white powder of **3** (0.18 g) was respectively isolated after similar processing and chromatography as described in ii). mp 248—249 °C IR spectrum was identical with that of **3** obtained in i).

iv) *from* **4**: The solution of **4** (0.72 g) in pyridine (40 ml) was kept at 65—70 °C for 43 h. The solvent was evaporated to dryness and the residue was chromatographed as described in i). Pure **3** was obtained as a white powder, 0.45 g. Mp 248—251 °C. IR spectrum was identical with that of **3** obtained in i).

Experiment Concerning Temperature Dependence of the Reaction of **2** *with* o-*Nitrobenzenesulfonyl Chloride*. Eleven solutions of **2** (18—20 mg each) and *o*-nitrobenzenesulfonyl chloride (48—53 mg each) in pyridine (1.00 ml each) were kept at 11 different temps between 67 and 9.5 °C, respectively, using Toyo Kagaku Sangyo's Temperature Gradient Incubator TN-3. After 44 h the solutions were inspected by TLC (chloroform–methanol 12 : 1). The samples reacted at 9.5, 14.5, 20, and 26 °C showed a single spot of **4** on TLC and even a trace spot of **3** was not detected. At 31.5, 37.5, and 41.5 °C a major spot of **4** and a trace spot of **3** were visible. At 49.5 and 53 °C the spots of **4** and **3** appeared in apparently 1 : 1 ratio. At 57 and 67 °C a major spot of **3** and a tiny spot of **4** appeared.

3'-Episeldomycin 5 Free Base (**7**). The compound **3** (1.15 g) was suspended in a mixture of 2N-aq sodium hydroxide (25.5 ml) and methanol (25.5 ml) and heated at 110 °C in a sealed glass tube. After 15 minutes **3** dissolved completely and heating was continued for 100 minutes. A white ppt formed was filtered off, washed with aq methanol. The filtrate and washings combined were neutralized with concd hydrochloric acid and evaporated to dryness. The residue was dissolved in water (10 ml) and adjusted to pH 4.80 by hydrochloric acid. A small amount of ppt formed was filtered off and the filtrate was charged on a column of Amberlite CG-50 (ammonium cycle, 50 ml). After washing the column with water (370 ml), it was eluted stepwisely by 0.1- and 0.2 M aqueous ammonia. From a portion eluted by 0.1 M-ammonia a white powder (0.13 g) was obtained which showed no antibacterial activity. $[\alpha]_D^{18}$ +126° (*c* 0.300, water). This compound was supposed to be 1*N*,3*N*-carbonyl-3'-episeldomycin 5.[14] Fractions eluted by 0.2 M -ammonia gave a white powder of pure **7** (0.14 g) after evaporation. Mp 189—194 °C, $[\alpha]_D^{20}$ +95.1° (*c* 0.377, water). PMR (in D_2O): τ 4.86 (d, J=4 Hz, H-1'), 5.00 (d, J=3 Hz, H-1").

Found: C, 45.52; H, 7.75; N, 16.82%. Calcd for $C_{18}H_{38}N_6O_7$ 1/2 H_2CO_3: C, 46.13; H, 8.18; N, 17.45%.

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References

1) T. Nara, M. Yamamoto, S. Takasawa, S. Sato, T. Sato, I. Kawamoto, R. Okachi, I. Takahashi, and A Morikawa, *J. Antibiot.*, **30**, 17 (1977).
2) J. B. McAlpine, A. C. Sinclair, R. S. Egan, R. L. DeVault, R. S. Stanaszek, M. Cirovic, S. L. Mueller, P. C. Goodley, R. J. Mauritz, N. E. Wideburg, L. A. Mitcsher, K. Shirahata, H. Matsushima, S. Sato, and T. Iida, *J. Antibiot.*, **30**, 39 (1977).
3) H. Umezawa, *Adv. Carbohydr. Chem. Biochem.*, **30**, 183 (1974).
4) S. Hanessian, R. F. Butterworth, and T. Nakagawa, *Carbohydr. Res.*, **26**, 261 (1973).
5) i) L. Goodman, *Adv. Carbohydr. Chem. Biochem.*, **22**, 139 (1967); ii) H. E. Khadem, *ibid.*, **25**, 375 (1970).
6) D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355 (1970).
7) A. S. Perlin, B. Casu, and H. J. Koch, *Can. J. Chem.*, **48**, 2596 (1970).
8) R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, **44**, 249 (1966).
9) R. E. Reeves, *J. Am. Chem. Soc.* **71**, 2116 (1949).
10) S. Umezawa, T. Tsuchiya, and K. Tatsuta, *Bull. Chem. Soc. Jpn.*, **39**, 1235 (1966).
11) S. Mitsuhashi, L. Rosival, and V. Krcmery, Ed., "Drug Inactivating Enzymes and Antibiotic Resistance," Springer-Verlag, Berlin (1975), p. 115.
12) M. Kugelman, A. K. Mallams, and H. F. Vernay, *J. Chem. Soc. Perkin Trans.*, *1*, **1976**, 1113.
13) J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Am. Chem. Soc.* **95**, 7464 (1973).
14) Private communication from Dr. J. McAlpine, Abbott Laboratories.