An Improved Synthesis of 3′,4′-Dideoxykanamycin B

Toshio YONETA,* Seiji SHIBAHARA, Tomio MATSUNO, Susumu TOHMA, Shuzo FUKATSU, Shigeo SEKI, and Hamao UMEZAWA†

Central Research Laboratories, Meiji Seika Kaisha, Ltd., Morooka-cho, Kohoku-ku, Yokohama 222

†*Institute of Microbial Chemistry, Shinagawa-ku, Tokyo 141*

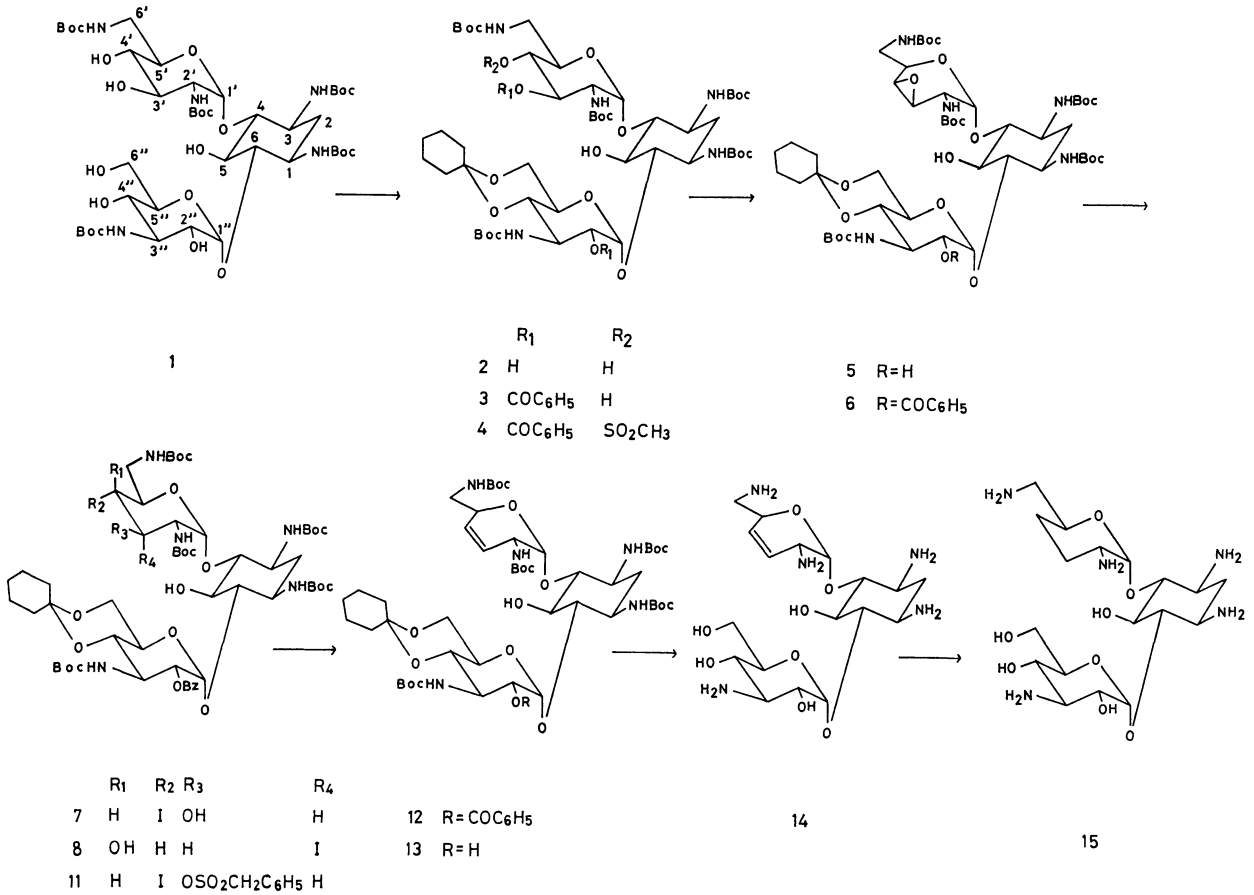
(Received July 15, 1978)

A new synthetic route has been exploited for the large scale production of 3′,4′-dideoxykanamycin B starting with kanamycin B. The key stage in the synthesis involves the formation in excellent yield of the 3′,4′-anhydro-4′-*epi* derivative (**5**) followed by convertion to the 3′-*ene* derivative through the iodohydrin. Compound **5** was prepared by the treatment of 3′,2″-di-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-4′-*O*-methylsulfonyl-penta-*N*-*t*-butoxycarbonyl-kanamycin B with sodium methoxide.

The resistance mechanism of most strains resistant to kanamycin has been elucidated by Umezawa *et al.* and attributed to the transphosphorylation from adenosine triphosphate to the 3′-hydroxyl group of kanamycin by intracellular enzymes produced by the resistant organisms.[1-3] 3′-Deoxykanamycin[4] and 3′,4′-dideoxykanamycin B (**15**)[5,6] prepared on the basis of this mechanism have been shown able to inhibit growth of these resistant strains. The latter has already been clinically used for infections of resistant bacteria.

A previous method for the preparation of 3′,4′-dideoxykanamycin B (**15**) involves the treatment of the 3′,4′-di-*O*-sulfonyl derivative with sodium iodide and zinc according to the Tipson-Cohen method.[7] In the present paper, other methods which do not require zinc for the 3′-*ene* formation have been investigated and it has been found that Watanabe's method,[8,9] involving the cleavage of an epoxide ring to iodohydrin followed by *O*-sulfonylation is one method for the large scale preparation of the 3′-*ene* compound.

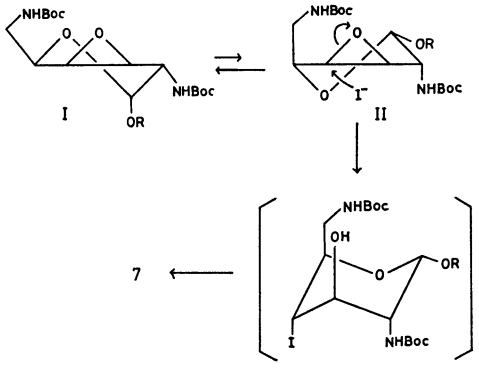
The five amino groups of kanamycin B were protected by the *t*-butoxycarbonyl (Boc) group using *O*-*t*-butyl *S*-4,6-dimethyl-2-pyrimidinyl thiocarbonate (Boc-*S* reagent)[10] to yield quantitatively the *N*-penta-Boc derivative (**1**). The protecting group can be readily removed under mild acidic conditions without ureide formation[6] at *N*-1 and *N*-3 of the 2-deoxystreptamine, which occured during removal of the *N*-ethoxycarbonyl group. The 4″- and 6″-hydroxyl groups of **1** were selectively protected by the formation of the cyclohexylidene



Scheme 1.

derivative in the usual manner.[6] Since the 4-hydroxyl group of hexopyranosides is generally least reactive toward benzoylation with benzoyl chloride in pyridine,[11] the 3'- and 2"-hydroxyl groups of the 4",6"-*O*-cyclohexylidene derivative (**2**) were preferentially benzoylated with benzoyl chloride (2.7 mol to **2**) in pyridine at 5 °C to give the expected 3',2"-dibenzoate product (**3**) in 73% yield. The reaction of the dibenzoate **3** with methylsulfonyl chloride gave the 4'-methylsulfonate (**4**) whose NMR spectrum showed the presence of two benzoyl and one methylsulfonyl group. The treatment of **4** with methanolic sodium methoxide gave the desired 3',4'-anhydro-4'-*epi* derivative (**5**) in a quantitative yield, which was subsequently converted into the 2"-benzoate (**6**) by re-*O*-benzoylation. Successive treatment of **6** with sodium iodide, sodium acetate and acetic acid in acetone[8,9] afforded the iodohydrin (**7**) bearing both iodo and hydroxyl groups in equatorial positions. Formation of the anhydro-ring opening isomer (**8**) was not observed in this reaction.

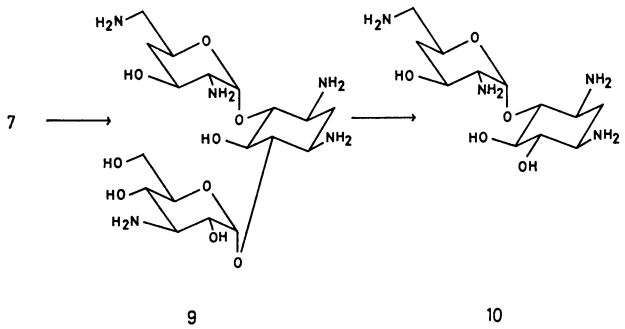
The structure of **7** was established by conversion into 4'-deoxykanamycin B (**9**). Catalytic hydrogenation of the crude iodohydrin (**7**) followed by successive treatment with methanolic sodium methoxide and 95% trifluoroacetic acid gave **9** and a small amount of less polar compounds. Hydrolysis of **9** with 6 M hydrochloric acid afforded 4'-deoxyneamine (**10**) which was identical with seldomycin factor 2,[12] showing that the hydroxyl and iodo groups of **7** are located at the 3'- and 4'-positions, respectively, in both equatorial positions.



Scheme 2.

The conformation of **6** could be represented as **I** and **II**, as shown in Scheme 2. The trans-diequatorial iodohydrin (**7**) derives from conformer **II** by the attack of the iodide ion and subsequent ring inversion of the product. It has been reported[13] that the direction of the ring opening of 3,4-anhydro-*galacto*-hexopyranosides is controlled by the bulkiness of the entering reagents. In the present case, the attack of the bulky iodide ion is seriously hindered by the anomeric axial group in conformer **I** and hence conformer **II**, with the less-hindered 4'-position is more susceptible to attack although the latter is expected to be less stable than the former.

The iodohydrin (**7**) was treated with α-toluenesulfonyl chloride[6] in pyridine and the resulting 3'-α-toluenesulfonate (**11**) was heated at 90 °C for 20—30 min to give the 3'-*ene*-derivative (**12**) in 76% yield. When the corresponding 3'-methylsulfonate or 3'-*p*-toluenesulfonate was used, only a poor yield of **12** was obtained.



Scheme 3.

Removal of the 2"-*O*-benzoyl group in **12** with methanolic sodium methoxide afforded compound **13**. *N*-*t*-Butoxycarbonyl and 4",6"-*O*-cyclohexylidene groups of **13** were removed by treatment with 95% trifluoroacetic acid to afford the 3'-*ene*-kanamycin B (**14**). Catalytic hydrogenation of **14** afforded 3',4'-dideoxykanamycin B (**15**), the NMR, TLC and biological activity of which were identical with that of an authentic sample.[5] The total yield of **15** from kanamycin B was more than 40% on a large scale.[14]

Experimental

All melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter and NMR spectra with a Varuan XL-100 spectrometer at 100 MHz. Thin-layer chromatography (TLC) was performed on Merck silica gel plate No. 5714.

Penta-N-t-*butoxycarbonylkanamycin B (1).* To a solution of kanamycin B (4.98 g) in aqueous triethylamine (34%, 35 ml), was added Boc-S reagent (18.7 g) in 1,4-dioxane (36 ml) and the mixture stirred for 24 h at room temperature. The syrupy mixture was poured into water (200 ml) and the resulting precipitate filtered, washed successively with 0.1 M-hydrochloric acid and water, and dried to give a white powder of **1** (9.88 g, 97%). The crude product was dissolved in hot ethanol and the solution cooled to give analytically pure **1** as a white powder; mp 253—260 °C (dec), $[\alpha]_D^{25}+70°$ (*c* 1.0, DMF).

Found: C, 52.31; H, 7.62; N, 7.24%. Calcd for $C_{43}H_{77}N_5O_{20}$: C, 52.47; H, 7.90; N, 7.12%.

*4",6"-*O*-Cyclohexylidene-penta-*N*-t-butoxycarbonylkanamycin B (2).* To a solution of **1** (1.48 g) in *N*,*N*-dimethylformamide (7.4 ml), was added *p*-toluenesulfonic acid hydrate (75 mg) and 1,1-dimethoxycyclohexane (2.1 ml). The mixture was allowed to stand at room temperature overnight. The resulting solution contained three compounds [TLC, chloroform–methanol (15: 1), R_f 0.22 (**2**), R_f 0.35 (trace), R_f 0.07 (starting material)]. After neutralization with triethylamine (0.6 ml), the solution was concentrated to a syrup and water added. The resulting precipitate was filtered, washed with water and dried. The crude product was dissolved in hot methanol and the solution cooled to give pure **2** (1.48 g, 93%); mp 245 °C (dec), $[\alpha]_D^{25}+74°$ (*c* 1.0, DMF).

Found: C, 54.83; H, 8.08; N, 6.24%. Calcd for $C_{49}H_{85}N_5O_{20}$: C, 55.29; H, 8.06; N, 6.58%.

*3',2"-Di-*O*-benzoyl-4",6"-*O*-cyclohexylidene-penta-*N*-t-butoxycarbonylkanamycin B (3).* To a solution of **2** (1.63 g) in

dry pyridine (16 ml), was added benzoyl chloride (0.27 ml) and the mixture kept overnight at 5 °C. Benzoyl chloride (0.2 ml) was added dropwise at 5 °C until the starting material disappeared. After the addition of water (0.15 ml), the solution was concentrated to a syrup and water added. The resulting precipitate was filtered, washed with water and dried (2.0 g). Column chromatography over silica gel (50 g) with chloroform–methanol (130: 1) afforded a white powder of **3** (1.42 g, 73%) together with the compound (160 mg) showed a high R_f value. Mp 164—167 °C, $[\alpha]_D^{25}+86°$ (*c* 1.0, $CHCl_3$); NMR (CD_3OD): δ 7.34—8.16 (10H, m, C_6H_5).

Found: C, 59.54; H, 7.20; N, 5.04%. Calcd for $C_{63}H_{93}N_5O_{22}$: C, 59.45; H, 7.38; N, 5.50%.

3',2''-Di-O-benzoyl-4'',6''-O-cyclohexylidene-4'-O-methylsulfonyl-penta-N-t-*butoxycarbonylkanamycin B (4).* A solution of **3** (966 mg) and methylsulfonyl chloride (0.29 ml) in pyridine (11.3 ml) was kept at 40 °C for 1 h. After the addition of water (0.1 ml), the solution was concentrated to a syrup and water added. The resulting precipitate was filtered, washed with water and dried (1.0 g). Column chromatography over silica gel (30 g) with chloroform–methanol (150: 1) afforded a white powder of **4** (647 mg, 67%); mp 218—221 °C (dec), $[\alpha]_D^{25}+92°$ (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$): δ 2.90 (3H, s, SO_2CH_3), 7.38—8.20 (10H, m, C_6H_5).

Found: C, 56.79, H; 6.93; N, 4.71; S, 2.76%. Calcd for $C_{64}H_{95}N_5O_{24}S$: C, 56.92; H, 7.09; N, 5.18; S, 2.37%.

3',4'-Anhydro-4'',6''-O-cyclohexylidene-4'-epi-*penta*-N-t-*butoxycarbonylkanamycin B (5).* To a solution of **4** (1.28 g) in methanol (10.5 ml), was added sodium methoxide (524 mg), and the solution stirred at room temperature for 3 h. After neutralization with concd hydrochloric acid at 0 °C, the resulting suspension was concentrated and precipitated by the addition of water. The resulting precipitate was filtered, washed with water and dried (1.03 g, quantitative). The crude product was dissolved in hot ethanol and the solution cooled to give analytically pure **5** as a white powder; mp 232—234 °C (dec), $[\alpha]_D^{25}+44°$ (*c* 0.5, DMF).

Found: C, 55.93; H, 8.06; N, 6.43%. Calcd for $C_{49}H_{83}N_5O_{19}$: C, 56.24; H, 8.01; N, 6.69%.

3',4'-Anhydro-2''-O-benzoyl-4'',6''-O-cyclohexylidene-4'-epi-*penta*-N-t-*butoxycarbonylkanamycin B (6) and the Iodohydrin Formation.* To a solution of **5** (1.28 g) in dry pyridine (20 ml), was added benzoyl chloride (0.5 ml), and the solution kept at 5 °C for 30 min. After the addition of water (0.2 ml), the solution was concentrated to a syrup and water added. The resulting precipitate was filtered, washed with water and dried to give a white powder of **6** (1.39 g, one spot on TLC). A mixture of **6** (1.39 g), sodium iodide (907 mg), sodium acetate (52 mg), acetic acid (0.9 ml) and acetone (39 ml) was refluxed for 6.5 h. The solvent was removed by evaporation under reduced pressure and the residue triturated with water. The resulting precipitate was filtered, washed with water and dried (1.51 g). Column chromatography on silica gel (55 g) with chloroform–methanol (150: 1) gave a white powder (816 mg, 52%) of the crude iodohydrin containing **7**.

Found: I, 10.39%. Calcd for $C_{56}H_{88}IN_5O_{22}$: I, 9.95%.

4'-Deoxykanamycin B (9). The crude iodohydrin (550 mg) in a mixture of methanol (10 ml), 1,4-dioxane (6 ml) and water (5 ml) was hydrogenated for 6 h under atmospheric pressure with Raney nickel (2.3 g). After removal of the catalyst, sodium methoxide (60 mg) was added and the solution kept at room temperature for 2 h. After neutralization with 1 M hydrochloric acid, the solution was concentrated to dryness and the residue triturated with water. The resulting precipitate was filtered, washed with water and dried. The white powder (451 mg) was dissolved in 95% trifluoroacetic acid (4.5 ml) and allowed to stand at room temperature for 30 min. The resulting solution contained three compounds [TLC, 1-butanol–ethanol–chloroform–17% ammonium hydroxide (4: 5: 2: 5), R_f 0.23 (major), R_f 0.21 (minor), R_f 0.17 (minor)] was concentrated to dryness and the residue dissolved in water (13 ml). The aqueous solution was neutralized with 4 M sodium hydroxide and charged onto a column of Amberlite CG-50 (NH_4^+ form, 6 ml). After washing with water, the column was developed with 0.3 M ammonium hydroxide. A mixture of minor components was eluted first (32 mg). From the next fraction 119 mg of the compound **9** was obtained as a colorless solid; mp 227 °C (dec), $[\alpha]_D^{25}+128°$ (*c* 1.3, H_2O); NMR (D_2O): δ 5.83 (1H, d, J=4 Hz, H-1'), 5.55 (1H, d, J=4 Hz, H-1''), 3.45 (1H, q, J=4 and 10.5 Hz, H-2'), 2.38—2.71 and 1.68—2.10 (each 2H, m, H-2 and H-4').

Found: C, 42.64; H, 7.82; N, 13.41%. Calcd for $C_{18}H_{37}N_5O_9\cdot H_2CO_3$: C, 43.08; H, 7.49; N, 13.23%.

4'-Deoxyneamine (10). A solution of **9** (217 mg) in 6 M hydrochloric acid (4 ml) was kept at 100 °C for 45 min. The solution was concentrated to dryness, water (7 ml) added to the residue followed by neutralization with 1M sodium hydroxide. The solution was charged onto a column of Amberlite CG-50 (NH_4^+ form, 6 ml). The column was washed with water and developed with 0.3 M ammonium hydroxide. The eluate containing **10** was concentrated to give 128 mg of a colorless powder; mp 206 °C (dec), $[\alpha]_D^{25}+80°$ (*c* 0.5, H_2O) [lit,[12] mp 208—209 °C (dec), $[\alpha]_D^{25}+90°$ (*c* 1.0, H_2O)]; NMR (D_2O): δ 5.80 (1H, d, J=4 Hz, H-1'), 3.20 (1H, q, J=4 and 10 Hz, H-2'), 1.85 (1H, q, J=11.2 and 11.6 Hz, H-4' axial).

Found: C, 44.20; H, 8.05; N, 16.26%. Calcd for $C_{12}H_{26}N_4O_5\cdot\frac{1}{2}H_2CO_3$: C, 44.49; H, 8.08; N, 16.60%.

2''-O-Benzoyl-4'',6''-O-cyclohexylidene-3',4'-dideoxy-3'-ene-*penta*-N-t-*butoxycarbonylkanamycin B (12).* To a solution of the crude iodohydrin **7** (954 mg) in pyridine (18 ml), was added α-toluenesulfonyl chloride (643 mg), and the solution kept at 5 °C for 30 min. After the addition of methanol (0.36 ml), the resulting solution was heated at 90 °C for 30 min. The solution was concentrated and mixed with water and the resulting precipitate filtered, washed with water and dried (987 mg). Column chromatography on silica gel (30 g) with chloroform–methanol (150: 1) afforded the pure compound **12** (645 mg, 76%) as a white powder; mp 219 °C (dec), $[\alpha]_D^{25}+32°$ (*c* 0.2, CH_3OH); NMR (C_5D_5N): δ 5.96 (2H, s, olefinic protons).

Found: C, 59.44; H, 7.77; N, 5.90%. Calcd for $C_{56}H_{87}N_5O_{19}$: C, 59.30; H, 7.73; N, 6.17%.

4'',6''-O-Cyclohexylidene-3',4'-dideoxy-3'-ene-*penta*-N-t-*butoxycarbonylkanamycin B (13).* To a solution of **12** (397 mg) in methanol (20 ml), was added sodium methoxide (90 mg), and the solution kept at room temperature for 30 min. After neutralization with 1 M hydrochloric acid, the solvent was removed by evaporation and the residue triturated with water. The resulting precipitate was filtered, washed with water and dried (371 mg). Reprecipitation from ethanol–water afforded a white powder (278 mg, 79%) of **13**; mp 215—217 °C, $[\alpha]_D^{25}+30°$ (*c* 1.0, CH_3OH).

Found: C, 57.20; H, 8.07; N, 6.38%. Calcd for $C_{49}H_{83}N_5O_{18}$: C, 57.11; H, 8.14; N, 6.80%.

3',4'-Dideoxy-3'-enekanamycin B (14). A solution of **13** (398 mg) in 95% trifluoroacetic acid (4 ml) was kept at room temperature for 30 min. The solvent was removed by evaporation and the residue dissolved in water (10 ml). The aqueous solution was neutralized with 1M sodium hydroxide and charged onto a column of Amberlite CG-50 (NH_4^+ form, 9 ml). The column was washed with water and developed

with 0.3 M ammonium hydroxide. The eluate containing **14** was evaporated to give a colorless solid (130 mg, 81%); mp 161—181 °C (dec), $[\alpha]_D^{25}+48.8°$ (*c* 0.9, H_2O); NMR (D_2O): δ 5.55 (1H, d, J=4 Hz, H-1″), 5.88 (1H, d, J=4 Hz, H-1′), 6.10 (2H, s, olefinic protons).

Found: C, 44.18; H, 7.71; N, 14.52%. Calcd for $C_{18}H_{35}N_5O_8 \cdot \frac{1}{2}H_2CO_3 \cdot H_2O$: C, 44.57; H, 7.63; N, 14.06%.

*3′,4′-Dideoxykanamycin B (**15**).* Compound **14** (114 mg) in water (5 ml), was hydrogenated with platinum oxide (8 mg) under atmospheric pressure overnight. After removal of the catalyst, the solution was charged onto a column of Amberlite CG-50 (NH_4^+ form, 3 ml). After washing with water, the column was eluted with 0.3 M ammonium hydroxide to give a colorless solid (103 mg, 90%) of **15**, $[\alpha]_D^{25}+130°$ (*c* 1.0, H_2O).

This compound was confirmed to be identical with an authentic sample of 3′,4′-dideoxykanamycin B in all respects including biological activity.

The authors wish to thank Dr. Sumio Umezawa of the Institute of Bio-organic Chemistry, for his helpful discussion. We also would like to thank Mr. Katsuyoshi Iwamatsu for the decoupling experiments.

References

1) H. Umezawa, *Adv. Carbohydr. Chem. Biochem.*, **30**, 183 (1974).

2) H. Umezawa, "Drug Action and Drug Resistance in Bacteria. II. Aminoglycoside Antibiotics," Univ. Tokyo Press, Tokyo (1975), p. 211.

3) H. Umezawa, M. Okanishi, S. Kondo, K. Hamana, R. Utahara, K. Maeda, and S. Mitsuhashi, *Science*, **157**, 1559 (1967).

4) S. Umezawa, Y. Nishimura, H. Hineno, K. Watanabe, S. Koike, T. Tsuchiya, and H. Umezawa, *Bull. Chem. Soc. Jpn.*, **45**, 2847 (1972).

5) S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Jpn.*, **45**, 3624 (1972).

6) T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Carbohydr. Res.*, **49**, 141 (1976).

7) R. S. Tipson and A. Cohen, *Carbohydr. Res.*, **1**, 338 (1965).

8) R. U. Lemieux, E. Fraga, and K. A. Watanabe, *Can. J. Chem.*, **46**, 61 (1968).

9) K. A. Watanabe, R. S. Goody, and J. J. Fox, *Tetrahedron*, **26**, 3883 (1970).

10) T. Nagasawa, K. Kuroiwa, K. Narita, and Y. Isowa, *Bull. Chem. Soc. Jpn.*, **46**, 1269 (1973).

11) J. M. Williams and A. C. Richardson, *Tetrahedron*, **23**, 1369, 1641 (1970); M. W. Horner, L. Hough, and A. C. Richardson, *J. Chem. Soc., C*, **1970**, 1336.

12) R. S. Egan, A. C. Sinclair, R. L. Vault, J. B. McAlpine, S. L. Meuller, P. C. Goodley, R. T. Mauritz, L. A. Mitscher. K. Shirahata, S. Saito, and T. Iida, *J. Antibiot.*, **30**, 31 (1977),

13) K. Capek, J. Nemec, and J. Jary, *Collect. Czech. Chem. Commun.*, **33**, 1758 (1968).

14) In the case of large scale preparation, all intermediary products were used for subsequent steps without purification except compound **14** which was readily purified by resin column chromatography.