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Selective O-Benzoylation in Aminoglycoside Antibiotics[1]

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It was shown that the 3′ and 4′ hydroxy groups of N-protected butirosin or kanamycin derivatives resist benzoylation in an aqueous medium, furnishing an effective method for chemical modifications of these antibiotics.

Keywords——benzoylation; selective reaction; cyclohexylidenation; aminoglycoside antibiotics; butirosin; kanamycin

Butitosin, an aminoglycoside antibiotic complex mainly consisting of butirosin A (**1a**), exhibits broad inhibitory activity against gram-positive and gram-negative bacteria.[3] In a preceding paper,[4] we reported that 3′,4′[5]-dideoxybutirosin A (**1b**) was chemically synthesized from butirosin and showed significant activities against both sensitive and resistant strains *in vitro* and *in vivo*, indicating that removal of the 3′- and 4′-hydroxy groups confers a capability of overcoming butirosin resistant microorganisms of the 3′-O-phosphorylation type[6] on the butirosin molecule. As a sequel to these preceding papers,[4,7,8] this paper deals with some improvement in the process of synthesizing 3′,4′-dideoxybutirosin (**1b**) and related compounds.

In chemical modification of butirosin at the 3′ and/or 4′ positions, initial protection of the hydroxy groups with a cyclic ketal provides an effective method; for example, a tetra-N-benzyloxycarbonyl derivative of butirosin (**2a**) was treated with 1,1-dimethoxycyclohexane to give the 3′,4′: 3″,5″-di-O-cyclohexylidene derivative (**2b**). Successively, blocking of the remaining hydroxy functions in **2b** by acetylation and removal of the 3′,4′-cyclohexylidene group with acid gave a protected derivative **2c** having only a 3′,4′-glycol system which was submitted to chemical modification at the 3′ and 4′ positions.

However, the initial cyclohexylidenation of **2a** resulted in an unavoidable formation of some by-products including polycyclohexylidene compounds which required a wasteful chromatographic separation and lowered the yield of the desired dicyclohexylidene compound (**2b**), as described before.[4] Supposedly, formation of these by-products arose from participation of an active 2‴-hydroxy group of the side chain in this reaction. Consequently, we have investigated a selective protection of the active hydroxy group by acylation prior to cyclohexylidenation in order to reduce such complication; and, after several unfruitful attempts

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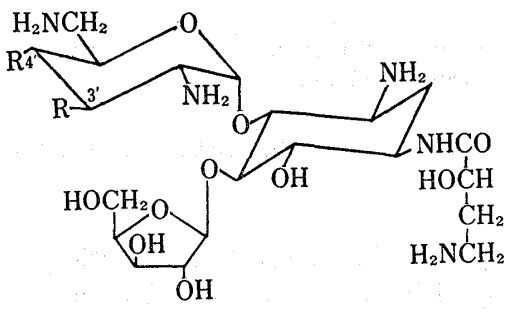
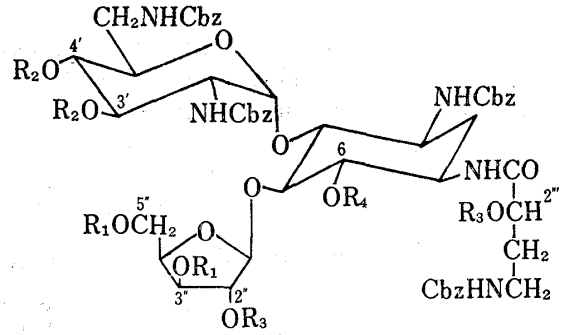
2) Location: *Hiromachi 1-2-58, Shinagawa-ku, Tokyo, 140, Japan.*
3) See the foot-notes cited in the referencece 4.
4) H. Saeki, Y. Shimada, Y. Ohashi, M. Tajima, S. Sugawara, and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1145 (1974).
5) The numbering of the positions was made based on the proposal of Hichens and Rinehart, Jr. (See *J. Am. Chem. Soc.*, **85**, 1547 (1963)) as illustrated in the chart.
6) S. Sugawara, S. Inabe, M. Madate, and H. Saeki, *Sankyo Kenkyusho Nempo*, **25**, 56 (1973).
7) H. Saeki, Y. Shimada, N. Takeda, I. Igarashi, S. Sugawara, and E. Ohki, *Sankyo Kenkyusho Nempo*, **25**, 62 (1973).
8) H. Saeki, Y. Shimada, E. Ohki, and S. Sugawara, *J. Antibiotics*, **28**, 530 (1975).

along this line,[9] it was found that benzoylation in aqueous medium furnished an effective method for this purpose as will be shown below.

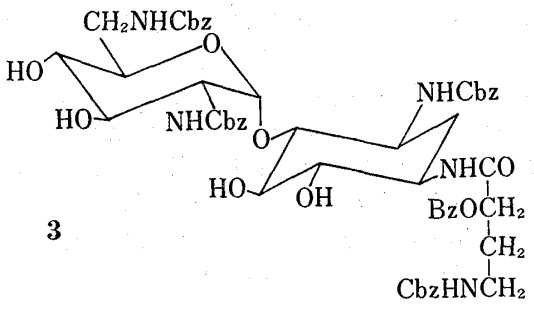
Treatment of tetra-N-benzyloxycarbonylbutirosin A[4] (**2a**) with 1,1-dimethoxycyclohexane in dimethylformamide (DMF) under mild conditions gave a 3″,5″-O-cyclohexylidene derivative (**2d**) quantitatively. Benzoylation of **2d** with excess benzoyl chloride in an aqueous acetone solution was carried out in the presence of potassium carbonate, affording a 2″,2‴-dibenzoate (**2e**) in good yield. The structure of **2e** was certified on the basis that acid hydrolysis of **2e** in methanol gave the known 1-N(2-benzoyloxy-4-benzyloxycarbonylaminobutyryl)-tri-N-benzyloxycarbonylneamine[7] (**3**) along with methyl 2-O-benzoyl-α,β-D-xylofuranosides[12] (**4**). Different from the direct cyclohexylidenation of the butirosin derivative (**2a**)



1a: R=OH
1b: R=H



2a: $R_1=R_2=R_3=R_4=H$
2b: $R_1=R_2=$cyclohexylidene, $R_3=R_4=H$
2c: $R_1=$cyclohexylidene $R_2=H$, $R_3=R_4=Ac$
2d: $R_1=$cyclohexylidene, $R_2=R_3=R_4=H$
2e: $R_1=$cyclohexylidene, $R_2=R_4=H$, $R_3=Bz$
2f: $R_1=R_2=$cyclohexylidene, $R_3=Bz$, $R_4=H$



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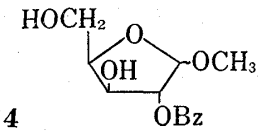

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Chart 1. Cbz=$COOCH_2C_6H_5$, Ac=$COCH_3$, Bz=COC_6H_5

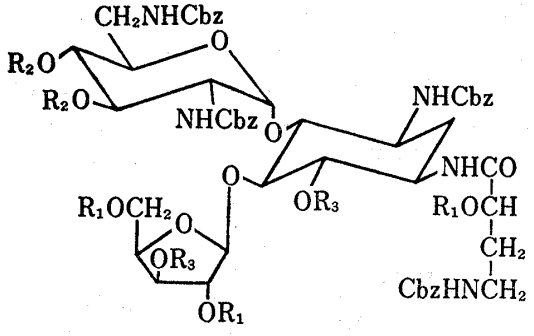
such as described before,[4] treatment of the dibenzoate (**2e**) thus obtained with 1,1-dimethoxycyclohexane gave a 3′,4′: 3″,5″-di-O-cyclohexylidene dibenzoate **2f** exclusively without contamination of by-products even under severe conditions. Saponification of **2f** with base afforded the afore-mentioned 3′,4′: 3″,5″-di-O-cyclohexylidene compound (**2b**) in good yield. Thus, it was found that introducing the partial benzoylation procedure in an earlier step of synthesis

9) Some examples on selective acylation have already been announced in the field of monosaccharides.[10,11] However, application of these methods to our cases did not give satisfactory results.
10) J.M. Sugihara, "Advances in Carbohydrate Chemistry," Vol. 8, ed. by C.S. Hudson and M.L. Wolfrom, Academic Press, New York, 1953, p. 1; R.W. Jeanloz and D.A. Jeanloz, *J. Am. Chem. Soc.*, **79**, 2575 (1957); J.M. Williams and A.C. Richardson, *Tetrahedron*, **23**, 1369 (1967) and references cited therein; G.J.F. Chittenden, *Carbohydr. Res.*, **16**, 495 (1971); N.L. Holder and B. Fraser-Reid, *Synthesis*, **1972**, 83; D.R. Hicks and B. Fraser-Reid, *ibid.*, **1974**, 203.
11) F.A. Carey and K.O. Hodgson, *Carbohydr. Res.*, **12**, 463 (1970).
12) W.D.S. Bowering and T.E. Timell, *J. Am. Chem. Soc.*, **82**, 2827 (1960); R.E. Schaub and M.J. Weiss, *ibid.*, **80**, 4683 (1958).

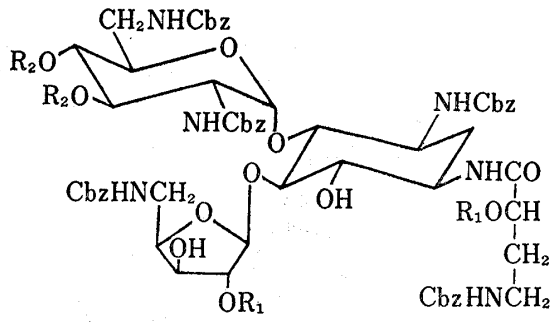
of 3′,4′-dideoxybutirosin (**1b**) reduces or eliminates the wasteful chromatographic separation of the intermediate **2b** and facilitates its preparation in large quantities even if the sequential number of reaction steps increase.

Next, application of the benzoylation procedure to other butirosin derivatives was carried out. Benzoylation of tetra-N-benzyloxycarbonylbutirosin A (**2a**) in aqueous acetone in the presence of potassium carbonate gave a quantitative yield of a 2″,2‴,5″-tribenzoate (**5a**). The structure of **5a** was verified by a sequence of reactions as shown below . Cyclohexylidenation of **5a**, successive acetylation of the remaining 3″,6-hydroxy groups and removal of the cyclohexylidene group gave a tribenzoate diacetate (**5b**). Mesylation of **5b** followed by removal of acyl functions with bases afforded a 3′,4′-O-mesyl derivative (**5c**) which was identified with the sample obtained by hydrolysis of the acetyl derivative of **5c** reported in the previously mentioned paper.[4] The 3′,4′-dimesylate (**5c**) thus obtained is also an important intermediate for synthesis of 3′,4′-dideoxybutirosin A (**1b**).

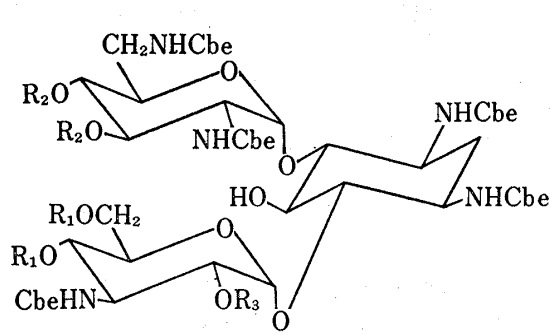
Benzoylation of 5″-amino-penta-N-benzyloxycarbonyl-5″-deoxybutirosin A[13] (**6a**) in aqueous medium afforded a 2″,2‴-dibenzoate (**6b**) almost quantitatively. Cyclohexylidenation of **6b** followed by removal of the benzoyl groups yielded a 3′,4′-cyclohexylidene compound **6c** which was identified with the known sample,[13] thus providing a proof for the structure of **6b**. The 3′,4′-cyclohexylidene compound **6c** also forms an important intermediate for synthesis of 5″-amino-3′,4′,5″-trideoxybutirosin A, an active butirosin analog.[8,13]



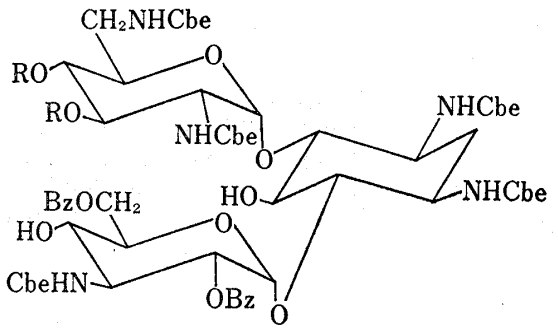
5a : R_1=Bz, R_2=R_3=H
5b : R_1=Bz, R_2=H, R_3=$COCH_3$
5c : R_1=R_3=H, R_2=SO_2CH_3



6a : R_1=R_2=H
6b : R_1=Bz, R_2=H
6c : R_1=H, R_2=cyclohexylidene



7a : R_1=R_2=R_3=H
7b : R_1=cyclohexylidene, R_2=R_3=H
7c : R_1=cyclohexylidene, R_2=H, R_3=Bz
7d : R_1=R_2=cyclohexylidene, R_3=Bz
7e : R_1=isopropylidene, R_2=R_3=H
7f : R_1=isopropylidene, R_2=H, R_3=Bz



8a : R=H
8b : R=cyclohexylidene

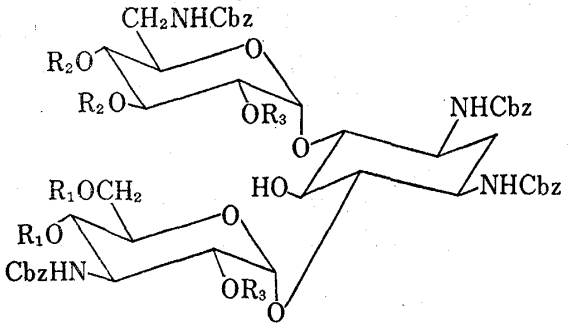
Chart 2. Cbz=$COOCH_2C_6H_5$, Bz=COC_6H_5, Cbe=$COOC_2H_5$

13) P.W.K. Woo, *J. Antibiotics*, **28**, 522 (1975).

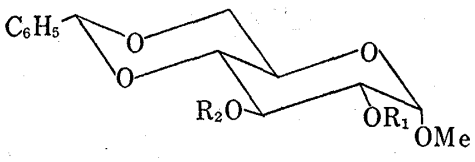
Based on the fact that the 3′ and 4′-hydroxy groups of butirosin derivatives remain intact in benzoylation in aqueous medium, we attempted to apply this procedure in case of kanamycin derivatives. Penta-N-ethoxycarbonylkanamycin B[14] (**7a**) was treated with 1,1-dimethoxycyclohexane under mild conditions, giving a 4″,6″-O-cyclohexylidene compound (**7b**) whose analogous benzoylation afforded a 2″-benzoate (**7c**) in good yield. The benzoate (**7c**) was identified with the sample prepared by the known method[14] including cyclohexylidenation of **7a** at 3′,4′: 4″,6″-positions under an elevated temperature, successive benzoylation of the 2″-hydroxyl group, and partial hydrolyis of the resulting di-O-cyclohexylidene benzoate (**7d**). Similarly, penta-N-ethoxycarbonyl-4″,6″-O-isopropylidenekanamycin B (**7e**) was benzoylated to give a corresponding 2″-benzoate (**7f**) in a good yield.

Different from the preceding examples, direct benzoylation of penta-N-ethoxycarbonylkanamycin B (**7a**) did not proceed selectively but mainly gave a 2″,6″-dibenzoate (**8a**), along with isomeric dibenzoates and tribenzoates which were not characterized. The benzoate **8a** did not give a cyclohexylidene compound under a mild condition, but, with an elevated temperature, **8a** gave a 3′,4′-O-cyclohexylidene compound (**8b**).

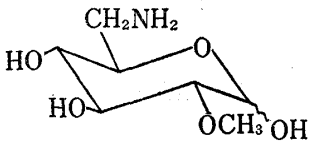
Meanwhile, further reduced selectivity was observed in benzoylation of a kanamycin A derivative having a 1,2,3-triol system in the 6-amino-6-deoxyglucoside part. Treatment of tetra-N-benzyloxycarbonylkanamycin A (**9a**) with 1,1-dimethoxycyclohexane under mild conditions gave a 4″,6″-cyclohexylidene compound (**9b**) in good yield. The structure assignment of **9b** was based on the fact that **9b** was easily degraded on oxidation with sodium periodide. Benzoylation of **9b** was analogously carried out, resulting in formation of a complex benzoate mixture. Without further purification, the reaction product was treated with 1,1-dimethoxycyclohexane under elevated temperature and successive chromatographic purification afforded a dicyclohexylidene dibenzoate (**9c**) in 38% yield calculated from the used **9b**. Referring to the examples given above for the kanamycin B series, the position of one of the



9a : $R_1=R_2=R_3=H$
9b : R_1=cyclohexylidene, $R_2=R_3=H$
9c : $R_1=R_2$=cyclohexylidene, $R_3=Bz$
9d : $R_1=R_2$=cyclohexylidene, $R_3=H$
9e : $R_1=R_2$=cyclohexylidene, $R_3=CH_3$
9f : $R_1=R_2=H$, $R_3=CH_3$



11a : $R_1=Bz$, $R_2=H$
11b : $R_1=H$, R_2=tetrahydropyranyl
11c : $R_1=CH_3$
R_2=tetrahydropyranyl



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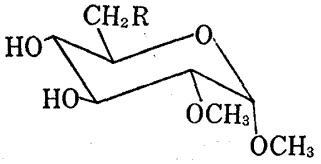

12a : R=OH
12b : $R=N_3$

Chart 3. Cbz=$COOCH_2C_6H_5$, Bz=COC_6H_5

14) S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, **45**, 3624 (1972); Y. Takagi, T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiotics*, **7**, 405 (1973).

benzoyl groups in **9c** would be assignable as 2″ in the kanosamine part. The position of the other benzoyl group was determined as being at 2′ in the 6-amino-6-deoxyglucose part on the basis of the following degradation reaction. Hydrolysis of **9c** with bases afforded 2′,2″,5-triol (**9d**) which was converted into a 2′,2″-di-O-methyl derivative **9e** on treatment with methyl iodide in the presence of silver oxide. The nuclear magnetic resonance spectrum (NMR) of **9e** with peaks at 3.50 and 3.30 ppm indicated the existence of two methoxy groups in the molecule. Removal of the cyclohexylidene groups from **9e** gave a dimethyl derivative (**9f**) whose hydrogenation followed by acid hydrolysis yielded 6-amino-6-deoxy-2-O-methyl-D-glucose (**10**). Identification of the unknown aminosugar was carried out with the sample synthesized by an unequivocal method as follows: Blocking the remaining hydroxy group of 2-O-benzoyl-4,6-benzylidene-α-D-glucopyranoside[11] (**11a**) with a tetrahydropyranyl group, base hydrolysis of the benzoyl group, methylation of the resultant glycoside (**11b**) with methyl iodide and sodium hydride to give a 2-O-methyl compound (**11c**), removal of the blocking group, partial tosylation of the formed glycoside (**12a**), displacement reaction of the 6-tosyloxy group with sodium azide, reduction of the azide (**12b**) into an amine and finally hydrolysis of the glycoside function with acid.

Thus, considering the fact that the 3′ and 4′ hydroxy groups of butirosin or kanamycin derivatives resist benzoylation in an aqueous medium to some degree, it can be seen that an application of the benzoylation procedure in an initial step furnishes an effective method for chemical modifications of these hydroxy functions including deoxygenation.

Experimental

Infrared spectra (IR) were recorded on a JASCO A-2 spectrometer and NMR spectra on a Hitachi Perkin-Elmer R-24 spectrometer. Thin-layer chromatography (TLC) was performed on TLC-plates, Silica gel F_{254} precoated, layer thickness 0.25 mm (E. Merck) and spots were visuallized by UV or by spraying with vanadic acid-sulfuric acid followed by heating or with iodine. Developing solvents used were shown in parenthesis. Columns for ordinary chromatography were prepared with Wakogel C-200 (Wako Pure Chemical Industries, Ltd.). Plates for preparative TLC were provided with Silica gel $60F_{254}$ (E. Merck). Solvents were removed by a rotary flash evaporator at diminished pressure and usually at 15—35°.

Tetra-N-benzyloxycarbonyl-3″,5″-O-cyclohexylidenebutirosin A (**2d**)——A mixture of 10 g of tetra-N-benzyloxycarbonylbutirosin A (**2a**), 15 ml of 1,1-dimethoxycyclohexane, 200 mg of *p*-toluene-sulfonic acid, and 100 ml of DMF was stirred for 1.5 hr at 40—45°. After addition of 1 g of Na_2CO_3 (solid), the mixture was filtered and the solid was washed with $CHCl_3$ several times. Combined filtrate and washings were evaporated *in vacuo* to give a syrup which was dissolved in EtOAc and filtered. Evaporation of the solvent gave 10.68 g (99.5%) of a crude **2d**. The analytical sample was obtained by purification over silica gel (MeOH–$CHCl_3$, 1: 20, v/v). IR ν_{max}^{KBr}: 1700 cm^{-1}. NMR ($CDCl_3$) δ: 7.15 (br. s, 20H), 4.9 (br. s, 8H), 1.4 (br., 10H). *Anal.* Calcd. for $C_{59}H_{73}N_5O_{20}$: C, 60.45; H, 6.28; N, 5.97. Found: C, 60.94; H, 6.59; N, 5.93.

2″,2‴-Di-O-benzoyl-tetra-N-benzyloxycarbonyl-3″-5″-O-cyclohexylidenebutirosin A (**2e**)——To a stirred mixture of 1.0 g of **2d**, 1.5 g of K_2CO_3, 20 ml of acetone, and 12 ml of water was added dropwise 0.7 ml of benzoyl chloride and the mixture was stirred for 30 min at room temperature. After adding 2 ml of MeOH and stirring for 20 min to decompose excess of benzoyl chloride, the mixture was concentrated *in vacuo* to about half volume. Then, the mixture was extracted with EtOAc and the extract was washed with brine, dried and evaporated *in vacuo*. The residue was dissolved again in EtOAc and hexane was added gradually, giving crude **2e** in 95% yield. The analytical sample was obtained by silica gel chromarography (MeOH–$CHCl_3$, 1: 20—30, v/v), amorphous powder. IR ν_{max}^{Nujol}: 1740 cm^{-1}. *Anal.* Calcd. for $C_{73}H_{81}N_5O_{22}$: C, 63.52; H, 5.91; N, 5.07. Found: C, 63.03; H, 6.20; N, 4.80.

Acid Hydrolysis of the Dibenzoate (**2e**)——To a solution of 0.7 g of **2e** in 20 ml of MeOH was added 1 ml of 5.7 N methanolic HCl and the mixture was stirred at 55° for 3.5 hr, then was allowed to stand overnight at room temperature. After dilution with MeOH, the precipitates were collected to give 270 mg of the neamine derivative **3**. Further, concentration of the mother liquor *in vacuo* to half volume gave 102 mg of **3** as a second crop. Reprecipitation of the crude **3** from the DMF solution with water gave the analytical sample, as amorphous powder, mp 247—250°. IR ν_{max}^{Nujol}: 1700 cm^{-1}. NMR ($CDCl_3$) δ: 8.1—7.5 (br., 5H), 7.2 (br. s, 20H), 4.95 (br. s, 8H). *Anal.* Calcd. for $C_{55}H_{61}N_5O_{17}\cdot 1/2H_2O$: C, 61.36; H, 5.69; N, 6.57. Found: C, 61.56; H, 5.82; N, 6.53.

The authentic sample of **3** was prepared by stirring a solution of 120 mg of the known 5,6: 3′,4′-di-O-cyclohexylidene derivative[7] of **3** and 10 mg of *p*-toluenesulfonic acid in 10 ml of MeOH at room temperature

for 2 hr followed by a work-up in the usual manner and was identified with the sample obtained as above by TLC (MeOH–$CHCl_3$, 1: 10, v/v) and by infrared spectrometry.

The mother liquor left by collection of 3 was evaporated *in vacuo* to dryness, giving 98.8 mg of a syrup. The existence of methyl 2-O-benzoyl-α,β-D-xylofuranosides[12] (**4**) in the syrup was confirmed by TLC (MeOH–$CHCl_3$, 1: 10, v/v) and by vapor–phase chromatography of the trimethylsilyl derivatives (retention time 62 min; 2 m column, SE-30 (20%), det. 200°, ov. 180°, N_2: 20 ml/min).

Tetra-N-benzyloxycarbonyl-3′,4′: 3″,5″-di-O-cyclohexylidenebutirosin A (**2b**)——A mixture of 3 g of **2e**, 6 ml of 1,1-dimethoxycyclohexane, 200 mg of *p*-toluenesulfonic acid, and 30 ml of DMF was stirred at 50° for 1.5 hr under a diminished pressure (20 mmHg). Then, after addition of 1 g of Na_2CO_3 (solid), the mixture was stirred for 15 min and filtered. The filtrate was evaporated *in vacuo* and the residue was dissolved in $CHCl_3$ filtered, and evaporated *in vacuo*, giving 3.3 g (98%) of a crude dicyclohexylidene benzoate **2f**. The product **2f** was dissolved in 30 ml of MeOH and 0.5 ml of 2 N methanolic $NaOCH_3$ was added, then the mixture was stirred for 20 min at room temperature. After neutralizing with 2 N HCl, the mixture was evaporated *in vacuo* and the residue was dissolved in EtOAc, filtered, and evaporated *in vacuo*, giving 2.93 g of a crude (**2b**). The product was purified by silica gel chromatography and was identified with the authentic sample[4] by TLC and infrared spectrometry.

2″,2‴,5″-Tri-O-benzoyl-tetra-N-benzyloxycarbonylbutirosin A (**5a**)——To a stirred mixture of 1.0 g of tetra-N-benzyloxycarbonylbutirosin A[4] (**2a**), 1.6 g of K_2CO_3, 20 ml of acetone and 12 ml of water was added dropwise 0.7 ml of benzoyl chloride and the mixture was stirred for 1.5 hr at room temperature. Work-up as described above gave a crude **5a** quantitatively. The analytical sample was obtained by silica gel chromatography (MeOH–$CHCl_3$, 1: 50, v/v), amorphous powder. IR ν_{max}^{Nujol}: *ca.* 1740 cm^{-1}. *Anal.* Calcd. for $C_{74}H_{77}N_5O_{23}$: C, 63.28; H, 5.53; N, 4.99. Found: C, 62.99; H, 5.24; N, 4.40.

3″,6-Di-O-acetyl-2″,2‴,5″-tri-O-benzoyl-tetra-N-benzoyloxycarbonylbutirosin A (**5b**)——A mixture of 21 g of **5a**, 400 mg of *p*-toluenesulfonic acid, 18 ml of 1,1-dimethoxycyclohexane, and 80 ml of DMF was refluxed at 70—80° (bath temp.) under a diminished pressure for 1 hr. The cooled mixture was filtered after addition of 5 ml of 1 N-aq. Na_2CO_3 and evaporated *in vacuo* to dryness. The residue was extracted with EtOAc and the extract was evaporated *in vacuo*. The syrup thus obtained was acetylated in the usual manner with 30 ml of Ac_2O and 100 ml of pyridine. The acetylated product was dissolved in 180 ml of MeOH and 0.5 g of *p*-toluenesulfonic acid was added. The reaction progress was monitored by TLC of the collected aliquots, then the mixture was neutralized by addition of pyridine and evaporated to dryness. The residue was charged on 150 g of silica gel and eluted with MeOH–$CHCl_3$ (1: 50, v/v). Evaporation of the fractions gave 12.6 g of **5b**, amorphous powder. *Anal.* Calcd. for $C_{78}H_{81}N_5O_{25}$: C, 62.94; H, 5.49; N, 4.71. Found: C, 63.51; H, 5.43; N, 4.43.

Tetra-N-benzyloxycarbonyl-3′,4′-di-O-mesylbutirosin A (**5c**)——To an ice-cold solution of 12.5 g of **5b** in 75 ml of pyridine was added dropwise 5 ml of mesyl chloride and the mixture was stirred at room temperature for 3 hr. Work-up in the usual manner gave 12.8 g of a 3′,4′-dimesylate. The crude dimesylate (8.2 g) was dissolved in 80 ml of MeOH and 0.8 ml of 2 N methanolic $NaOCH_3$ was added. After stirring for 1 hr at room temperature, the mixture was diluted with EtOAc, washed with brine and successively with water, and dried. Then, the mixture was evaporated *in vacuo* and the residue was dissolved in a small amount of ether and hexane was gradually added. Thus, 6.67 g of **5c** was obtained. The analytical sample was obtained by preparative TLC (MeOH–$CHCl_3$, 1: 10, v/v), amorphous powder, $[\alpha]_D^{21}$ −18.9° (c=4, MeOH). *Anal.* Calcd. for $C_{55}H_{69}N_5O_{24}S_2 \cdot H_2O$: C, 52.16; H, 5.65; N, 5.53; S, 5.06. Found: C, 52.29; H, 5.69; N, 5.68; S, 5.06.

Saponification of a solution of 1.7 g of 2″,2‴,6′-tri-O-acetyl-3″,5″-O-cyclohexylidene-3′,4′-di-O-mesylbutirosin A, which was reported in the preceding paper,[4] in methanol with $NaOCH_3$ and successive hydrolysis of the cyclohexylidene group with aq. AcOH in the usual manner gave 1.2 g of **5c** which was identified with the sample obtained as above by TLC and by IR and NMR spectrometry.

5″-Amino-2″,2‴-di-O-benzoyl-penta-N-benzyloxycarbonyl-5″-deoxybutirosin A (**6b**)——To a stirred mixture of 220 mg of 5″-amino-penta-N-benzyloxycarbonyl-5″-deoxybutirosin A[13] (**6a**), 320 mg of K_2CO_3, 4 ml of acetone and 1.5 ml of water was added 0.12 ml of benzoyl chloride at room temperature and the mixture was stirred for 30 min. Work-up as described above gave a crude (**6b**) quantitatively. The analytical sample was obtained by preparative TLC (MeOH–$CHCl_3$, 1: 20, v/v), as an amorphous powder. *Anal.* Calcd. for $C_{73}H_{79}N_6O_{23}$: C, 62.88; H, 5.56; N, 5.87. Found: C, 63.02; H, 5.37; N, 5.27.

As described earlier, treatment of **6b** with 1,1-dimethoxycyclohexane and successive hydrolysis of the benzoyl groups with base yielded 5″-amino-penta-N-benzyloxycarbonyl-3′,4′-O-cyclohexylidene-5″-deoxybutirosin A[13] which was identified with the authentic sample by TLC and by spectrometry.

Penta-N-ethoxycarbonyl-4″,6″-O-cyclohexylidenekanamycin B (**7b**)——A solution of 0.5 g of penta-N-ethoxycarbonylkanamycin B[14] (**7a**), 1 ml of 1,1-dimethoxycyclohexane, and 20 mg of *p*-toluenesulfonic acid in 10 ml of DMF was stirred for 2.5 hr at room temperature. Then, the mixture was neutralized by addition of K_2CO_3 (solid) and filtered. The solid was washed with MeOH. The combined filtrate and washings were evaporated *in vacuo* to dryness, giving a crude (**7b**) which was washed with boiling benzene to remove the dicyclohexylidene derivative thereby contaminated. Thus, 512 mg (93.5%) of **7b**, mp 275—280°, were obtained. The analytical sample was obtained by reprecipitation from aqueous dioxane, mp

303—304°, amorphous powder, $[\alpha]_D^{20}$ +91.5° (c=0.8, MeOH). IR ν_{max}^{KBr}: 1700 cm^{-1}. NMR ($CDCl_3$) δ: 1.65 (br., 10H), 1.10 (t, J=7, 15H). *Anal.* Calcd. for $C_{39}H_{65}N_5O_{20}$: C, 50.70; H, 7.04; N, 7.58. Found: C, 49.94; H, 6.97; N, 7.29.

Penta-N-ethoxycarbonyl-2″-O-benzoyl-4″,6″-O-cyclohexylidenekanamycin B (7c)——To an ice-cold solution of 0.3 g of **7b** and 0.35 g of K_2CO_3 in 60 ml of aq. acetone (1: 1, v/v) was added 0.19 ml (5 mole equiv.) of benzoyl chloride with stirring and the mixture was further stirred for 30 min at room temperature. Then, after dilution with water, the resulting precipitates were collected to give 248 mg (74.4%) of **7c**, mp 274—276°, amorphous powder, which was identified with the sample synthesized according to Umezawa, *et al.*[14] by TLC and by spectrometry.

Penta-N-ethoxycarbonyl-2″-O-benzoyl-4″,6″-O-isopropylidenekanamycin B (7f)——Penta-N-ethoxycarbonyl-4″,6″-O-isopropylidenekanamycin B[14] (**7e**, 8 g) and 8 g of K_2CO_3 were dissolved in 1200 ml of warm aqueous acetone (acetone: water=7: 5, v/v) and 5 ml of benzoyl chloride was added at room temperature. Then, the mixture was stirred for 3 hr and was allowed to stand overnight. The resultant precipitates (2.62 g) were collected by filtration and the filtrate was concentrated *in vacuo*, giving a second crop of **7f** (4.71 g). The analytical sample was prepared by washing with benzene and reprecipitation from aq. dioxane, mp 307—310°, and was identical with the authentic sample[14] by TLC and spectrometry. *Anal.* Calcd. for $C_{43}H_{65}O_{21}N_5$: C, 52.27; H, 6.63; N, 7.09. Found: C, 51.87; H, 6.77; N, 7.06.

Penta-N-ethoxycarbonyl-2″,6″-di-O-benzoylkanamycin B (8a)——To a solution of 5 g of **7a** and 7.5 g of K_2CO_3 in 700 ml of aq. acetone (1: 1, v/v) was added dropwise 3 g of benzoyl chloride with vigorous stirring. After further stirring for 1 hr at room temperature, 1 g of benzoyl chloride was added and the mixture was stirred for 1 hr. Then, the mixture was neutralized with 2 N HCl and concentrated *in vacuo* to about half volume. The concentrate was extracted with AcOEt and the extract was evaporated *in vacuo*, giving 5.39 g of a crude **8a**.

A mixture of the crude **8a** thus obtained, 4 g of 1,1-dimethoxycyclohexane, 330 mg of *p*-toluenesulfonic acid, and 130 ml of DMF was stirred for 2.5 hr at 50—52° (bath temp.) under a diminished pressure of 9 mmHg. Then, the mixture was neutralized with triethylamine and evaporated *in vacuo* to dryness. The residue was dissolved in $CHCl_3$ and was charged on 80 g of silica gel. Elution with $CHCl_3$–MeOH (100: 3, v/v) followed by evaporation of the solvent gave 2.2 g of a 3′,4′-cyclohexylidene compound of **8a** (**8b**), which was reprecipitated from aq. dioxane, giving 2.0 g of **8b**, amorphous powder, mp 154°, $[\alpha]_D^{20}$ −88.7° (c=1.16, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (br.), 1710. NMR ($CDCl_3$) δ: 8.1 (br., 4H), 7.5 (br., 6H), 1.5 (br., 10H). *Anal.* Calcd. for $C_{53}H_{73}N_5O_{22}$: C, 56.23; H, 6.45; N, 6.18. Found: C, 56.24; H, 6.68; N, 6.03.

A solution of 2.0 g of **8b** thus obtained, and 80 mg of *p*-toluenesulfonic acid in 100 ml of MeOH was stirred for 1 hr at room temperature. Then, the mixture was neutralized with dil. $NaHCO_3$ and evaporated *in vacuo* to dryness, giving 1.7 g of **8a**. The analytical sample was prepared by further purification over preparative TLC and reprecipitation from aq. dioxane, mp 270—271°. IR ν_{max}^{KBr} cm^{-1}: 3500 (sh.), 3450 (sh.), 3350, 1720, 1690. *Anal.* Calcd. for $C_{47}H_{65}N_5O_{22}$: C, 53.66; H, 6.18; N, 6.66. Found: C, 53.22; H, 6.26; N, 6.36.

Tetra-N-benzyloxycarbonylkanamycin A (9a)——To an ice-cold mixture of 2 g of kanamycin sulfate, 1.7 g of Na_2CO_3, 5 ml of MeOH and 20 ml of water was added 2.7 g of carbobenzoxy chloride and the mixture was stirred for 1 hr with cooling and further for 5 hr at room temperature. Then, after dilution with water, the resulting precipitates were collected and washed with water, giving 1.88 g (63.7%) of **9a**, amorphous powder, mp 250—254°. IR $\nu_{max}^{CHCl_3}$: 1690 cm^{-1}. NMR (d_6-DMSO) δ: 7.2 (br. s, 20H), 5.0 (br. s, 8H). *Anal.* Calcd. for $C_{50}H_{60}N_4O_{19}$: C, 58.82; H, 5.92; N, 5.49. Found: C, 58.12; H, 6.01; N, 5.33.

Tetra-N-benzyloxycarbonyl-4″,6″-O-cyclohexylidenekanamycin A (9b)——A mixture of 5 g of **9a**, 25 ml of DMF, 5 ml of 1,1-dimethoxycyclohexane and 150 mg of *p*-toluenesulfonic acid was stirred for 2 hr at room temperature and work-up as described before gave 4.2 g (78.9%) of **9b**, powder, mp 268—278°. IR ν_{max}^{Nujol}: 1700 cm^{-1}. NMR (d_6-DMSO) δ: 7.25 (br. s, 20H), 4.96 (br. s, 8H), 1.4 (br., 10H). *Anal.* Calcd. for $C_{56}H_{68}N_4O_{19}$: C, 61.08; H, 6.22; N, 5.08; Found: C, 59.25; H, 6.17. N, 4.81.

2′,2″-Di-O-benzoyl-tetra-N-benzyloxycarbonyl-4″,6″-O-cyclohexylidenekanamycin A (9d) and Its 3′,4′-Cyclohexylidene Derivative (9c)——A mixture of 2 g of **9b**, 100 ml of dioxane, 40 ml of water, 50 ml of acetone, 4 g of K_2CO_3 and 4 ml of benzoyl chloride was stirred for 1 hr at room temperature and work-up as described above gave 1.45 g (60.9%) of the crude **9d** as amorphous powder. IR ν_{max}^{Nujol}: 1700 cm^{-1} (br.). NMR (d_6-DMSO) δ: 8.0—7.6 (m, 10H), 7.35 (br. s, 20H), 5.05 (br., 8H), 1.4 (br., 10H).

A mixture of 2 g of the crude **9d** thus obtained, 1.6 ml of 1,1-dimethoxycyclohexane, 10 ml of DMF and 80 mg of *p*-toluenesulfonic acid was stirred for 30 min at 70° (bath temp.) under a diminished pressure of 25 mmHg and the cooled mixture was neutralized by addition of triethylamine and was evaporated *in vacuo*. The residue was extracted with EtOAc several times and the extract was evaporated, giving 2.13 g of a syrup which was chromatographed over silica gel (MeOH–$CHCl_3$, 1: 50, v/v). Thus, 0.83 g (38.8%) of **9c** were obtained as powder, mp 143—150°. IR ν_{max}^{Nujol}: 1720 cm^{-1} (br.). NMR ($CDCl_3$) δ ppm: 7.8—7.3 (m, 10H), 7.15 (br. s, 20H), 4.90 (br. s, 8H), 1.5 (br., 20H). *Anal.* Calcd. for $C_{76}H_{84}N_4O_{21}$: C, 65.71; H, 6.05; N, 4.03. Found: C, 65.35; H, 6.06; N, 4.05.

A solution of 0.6 g of **9c** thus obtained and 9 mg of *p*-toluenesulfonic acid in 23 ml of MeOH was stirred for 45 min at room temperature and then was neutralized with resin (IR-45 (OH^-)) and evaporated *in vacuo*, giving 470 mg (83.9%) of **9d**, mp 245—253°, amorphous powder. IR ν_{max}^{Nujol}: 1700 cm^{-1}. NMR (d_6-DMSO)

δ: 8.0—7.6 (m, 10H), 7.35 (br. s, 20H), 5.05 (br., 8H), 1.4 (br., 10H). *Anal.* Calcd. for $C_{69}H_{76}N_4O_{21}$: C, 63.89; H, 5.86; N, 4.32. Found: C, 63.56; H, 5.70; N, 4.45.

Tetra-N-benzyloxycarbonyl-2′,2″-O-methylkanamycin A (9f)——A solution of 0.9 g of **9c** in 13 ml of MeOH containing 0.3 ml of 2 N methanolic NaOMe was stirred for 3 hr at room temperature and, then, was evaporated *in vacuo* to dryness. The residue was extracted with AcOEt and the extract was evaporated *in vacuo*. Reprecipitation from $CHCl_3$–hexane gave 768 mg of a 2′,2″,5′-triol (**9d**) as powder which was submitted to the next reaction.

A mixture of the crude (**9d**), 25 ml of methyl iodide, and 1.8 g of freshly prepared silver oxide was refluxed for 6 hr and then filtered and evaporated *in vacuo*, giving 765 mg of a dimethyl ether **9e**. NMR ($CDCl_3$) δ: 7.26 (s, 20H), 5.05 (br. s, 4H), 3.50 (s, 3H), 3.30 (s, 3H), 1.6 (br., 20H).

Successively, a solution of 764 mg of **9e** thus obtained in 50 ml of MeOH containing 20 mg of *p*-toluenesulfonic acid was stirred for 3 hr at room temperature. After neutralization with IR-45 (OH^-), the mixture was evaporated *in vacuo* and trituration of the residue with hexane, giving 693 mg of **9f** as amorphous powder, mp 268—269°. *Anal.* Calcd. for $C_{52}H_{64}N_4O_{19}\cdot H_2O$: C, 58.53; H, 6.19; N, 5.25. Found: C, 58.04; H, 6.17; N, 4.96.

Hydrolysis of 2′,2″-O-Methylkanamycin A——Hydrogen was bubbled in a solution of 300 mg of **9f** in a mixture of 15 ml of dioxane and 5 ml of water over 600 mg of 10% Pd-C. Reaction progress was monitored by TLC or ninhydrin test of the collected aliquots. During the hydrogenation, 2 N HCl was added at intervals to maintain the mixture acidic. Then, the mixture was filtered and evaporated *in vacuo* to give 229 mg of 2′,2″-O-methylkanamycin A hydrochloride as a syrup. NMR (D_2O) δ: 5.9 (d, J=3 Hz, 1H), 5.55 (br., 1H) 3.70 (s, 6H). Without further purification, 70 mg of the hydrochloride was dissolved in 2 ml of 6 N HCl and the mixture was refluxed for 6 hr. After decolorizing with activated charcoal, the mixture was neutralized with IR-45 (OH^-) and concentrated *in vacuo*. The existence of **10** in the concentrate was shown by paper partition chromatography (Toyo-roshi No. 51A, BuOH: AcOH: water=4: 2: 1) and by TLC (water–ammonia–MeOH–AcOEt=1: 3: 8: 8) in comparison with the synthesized sample which will be described below.

Methyl 4,6-O-Benzylidene-3-O-tetrahydropyranyl-α-D-glucopyranoside (11b)——A mixture of 2.5 g of methyl 2-O-benzoyl-4,6-benzylidene-α-D-glucopyranoside[11] (**11a**), 10 mg of *p*-toluene sulfonic acid and 10 ml of dihydropyran was stirred for 20 min at room temperature and then was diluted with petroleum ether, giving 2.0 g of a crystalline mass which was recrystallized from EtOH to a 3-tetrahydropyranyl-2-benzoate, mp 154—156°, prisms. IR ν_{max}^{Nujol} cm^{-1}: 1720, 1270, 1095, 1051, 1030. NMR ($CDCl_3$) δ: 8.2—7.1 (m, 10H), 5.5 (s, 1H), 5.0 (br. s, 2H), 3.30 (s, 3H), 1.5 (m, 6H). *Anal.* Calcd. for $C_{26}H_{30}O_8$: C, 66.37; H, 6.43. Found: C, 66.23; H, 6.13.

To a solution of 5 g of the benzoate thus obtained in 150 ml of MeOH was added dropwise 2 ml of 2 N NaOMe in MeOH at room temperature over 15 min period and the mixture was kept for 6 hr at room temperature. Concentration of the solvent afforded 3.3 g of **11b**. The analytical sample was obtained by recrystallization from aq. acetone, mp 145—147.5°, prisms. IR ν_{max}^{Nujol} cm^{-1}: 3440, 1070, 1050, 1030. NMR ($CDCl_3$) δ: 7.4 (s, 5H), 5.5 (s, 1H), 5.1 (m, 1H), 4.8 (m, 1H, anomeric), 3.4 (s, 3H), 1.7 (m, 6H). *Anal.* Calcd. for $C_{19}H_{26}O_7$: C, 62.45; H, 6.70. Found: C, 62.57; H, 6.62.

Methyl 2-O-Methyl-6-O-*p*-toluenesulfonyl-α-D-glucopyranoside——Sodium hydride (1.2 g, 50% mineral oil suspension) was washed with *n*-hexane and suspended in 30 ml of dimethyl sulfoxide and the mixture was heated on a steam bath with stirring. The mixture was cooled room to temperature and a solutilon of 3.0 g of **11b** in 30 ml of dimethyl sulfoxide was added dropwise over 30 min period. After stirring for 30 min, 3.45 g of methyl iodide was added and the mixture was further stirred for 1 hr at room temperature, then diluted with $CHCl_3$. The organic layer was collected, washed with water, dried and evaporated *in vacuo*, giving 3.0 g of the crude 2-O-methyl derivative (**11c**) as a syrup. IR ν_{max}^{Nujol} cm^{-1}: 1080, 1050, 1030. NMR($CDCl_3$) δ: 7.3 (br. s, 5H), 5.45 and 5.40 (two s, 1H), 4.95 (br. s, 1H), 4.7 (d, J=4 Hz, 1H, anomeric), 3.45 and 3.35 (two s, 3H), 3.28 (s, 3H), 1.5 (m, 6H).

A mixture of 2.8 g of the crude (**11c**) obtained as above, 60 mg of *p*-toluenesulfonic acid and 50 ml of MeOH was stirred for 3 hr at room temperature and then, after adding of 1 ml of pyridine, the mixture was evaporated *in vacuo* to give 2.06 g of a syrup which was dissolved in 30 ml of pyridine. To the ice-cold pyridine solution was added portionwise 1.88 g of *p*-toluenesulfonyl chloride and the mixture was kept overnight in a refrigerator. Then, the mixture was diluted with water and work-up in the manner gave 2.34 g of the 2-O-methyl-6-tosylate as a syrup. The analytical sample was obtained by preparative TLC. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450 (br.), 1360, 1070, 1060, 1030. NMR ($CDCl_3$) δ: 7.78 (d, J=9 Hz, 2H), 7.32 (d, J=9 Hz, 2H), 4.76 (d, J=4 Hz, 1H), 4.25 (m, 2H), 3.3 (s, 3H), 2.4 (s, 3H). *Anal.* Calcd. for $C_{15}H_{22}O_6S$: C, 49.71; H, 6.12; S, 8.85. Found: C, 49.36; H, 6.27; S, 9.06.

Methyl 6-Azido-6-deoxy-2-O-methyl-α-D-glucopyranoside (12b)——A stirred mixture of 0.92 g of the 6-tosylate obtained as above, 0.6 g of sodium azide and 10 ml of dimethyl sulfoxide was heated at 100° for 2 hr with N_2 atmosphere, then the mixture was cooled and poured into aq. NaCl and extracted with AcOEt. The extract was washed with aq. NaCl, dried and evaporated *in vacuo*, giving 0.4 g of **12b** as a syrup. IR ν_{max}^{KBr} cm^{-1}: 2100, 1050, (br.), 1040. *Anal.* Calcd. for $C_8H_{15}N_3O_5$: C, 41.20; H, 6.48; N, 18.02. Found: C, 41.23; H, 6.59; N, 17.88.

6-Amino-6-deoxy-2-O-methyl-D-glucose (**10**)——Hydrogen was bubbled into a solution of 0.4 g of **12b** in 5 ml of MeOH over 0.3 g of 10% Pd-C at room temperature for 1.5 hr. After filtration, the mixture was evaporated *in vacuo* and the residue was dissolved in water and charged on 60 ml of CG-50 (NH_4^+). The column was eluted with 0.1—0.5 N ammonia. Each fraction was monitored by ninhydrin test and, after adjusting with 1 N HCl to pH 4, was evaporated *in vacuo*, giving 0.34 g of a hydrochloride as amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3450—2700 (br.), 1130, 1060, 1040.

A stirred mixture of 0.34 g of the crude hydrochloride obtained as above and 6 N HCl was heated at 95° for 3 hr and was diluted with water. After decolorization with activated charcoal and adjusting with dil. NaOH to pH 8, the mixture was charged on 40 ml of CG-50 (NH_4^+) and eluted with 0.1—0.3 N ammonia. Working-up as described above, adjusting with 2 N HCl to pH 4 and lyophylization gave 0.23 g (71%) of a hydrochloride of **10** as amorphous powder which did not show satisfactory analytical data. The hydrochloride thus obtained was treated with carbobenzoxy chloride in the usual manner and the product was purified by preparative TLC (MeOH–$CHCl_3$, 1: 5, v/v), giving a N-benzyloxycarbonyl derivative of **10** as syrup. *Anal.* Calcd. for $C_{15}H_{21}NO_7 \cdot 1/4H_2O$: C, 54.29; H, 6.53; N, 4.22. Found: C, 54.35; H, 6.35; N, 4.20.