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SYNTHESIS OF METHYL 3-*O*- AND 2-*O*-CARBAMOYL-α-D-MANNOPYRANOSIDES AND CARBAMOYL-GROUP MIGRATION BETWEEN THEM[1]

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ABSTRACT

Methyl 3-*O*- and 2-*O*-carbamoyl-α-D-mannopyranosides, (2 and 3), were synthesized from methyl α-D-mannopyranoside *via* ammonolysis of a cyclic carbonate or a *p*-nitrophenoxycarbonate, as shown in Charts 1 and 2. Carbamoyl-group migration between the C-2 and C-3 hydroxyl groups, in methyl α-D-mannopyranoside under alkaline conditions, was also studied.

INTRODUCTION

During chemical studies on bleomycin, we isolated[2] methyl 3-*O*-carbamoyl-α-D-mannopyranoside from an acid-catalyzed methanolyzate of bleomycin A_2. Few antibiotics contain *O*-carbamoyl sugar derivatives; examples include novobiocin[3] and venturicidin[4]. In the case of novobiocin, the carbamoyl group migrates, under alkaline conditions, from O-3 to O-2 of the noviose residue and an equilibrium is

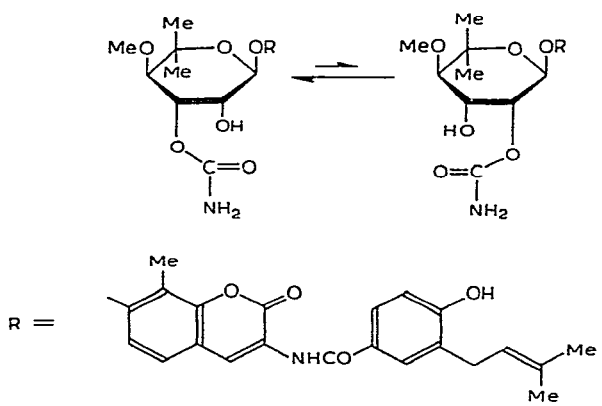

Fig. 1. Carbamoyl-group migration in novobiocin[5].

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eventually established between the two derivatives (Fig. 1). The carbamoyl group plays an important role in the antibacterial activity of novobiocin. Decarbamoyl-novobiocin and iso-novobiocin, which has a carbamoyl group at O-2 of its noviose moiety, have no antibacterial activity[5].

It was considered probable that the carbamoyl function of bleomycin could migrate readily under similar conditions. In order to assess the behavior of the carbamoyl group in bleomycin, we have synthesized methyl 3-*O*- and 2-*O*-carbamoyl-α-D-mannopyranosides (**2** and **3**) as model compounds, and have studied the equilibrium between them under alkaline conditions. This work provided useful information for the alkaline conversion of bleomycin into iso-bleomycin.

RESULTS AND DISCUSSION

Methyl 3-O-carbamoyl-α-D-mannopyranoside (**2**). — This compound was synthesized by ammonolysis of methyl 2,3-*O*-carbonyl-α-D-mannopyranoside (**1**), which had been prepared by the method of Hough *et al*[6]. Treatment of **1** with liquid ammonia gave in quantitative yield a colorless, hygroscopic solid that appeared homogeneous by chromatography and its i.r. spectrum showed the presence of carbamoyl-group bands (at 1610 and 1710 cm^{-1}). The n.m.r. spectrum of the product was almost identical with that of **2** isolated from bleomycin A_2, but the spectrum suggested the presence of a small proportion of a contaminant later found to be the 2-carbamate **3**.

Crude **2** gave a crystalline, chromatographically homogeneous acetate **4** which, after recrystallization from isopropyl alcohol, was identical with a sample derived from natural **2**.

In contrast, t.l.c. of the benzoate **5**, derived from crude **2**, did show the presence of a small proportion of a presumed isomer, which was isolated by column chromatography on silica gel and determined to be the benzoate (**7**) of **3** (Chart 1).

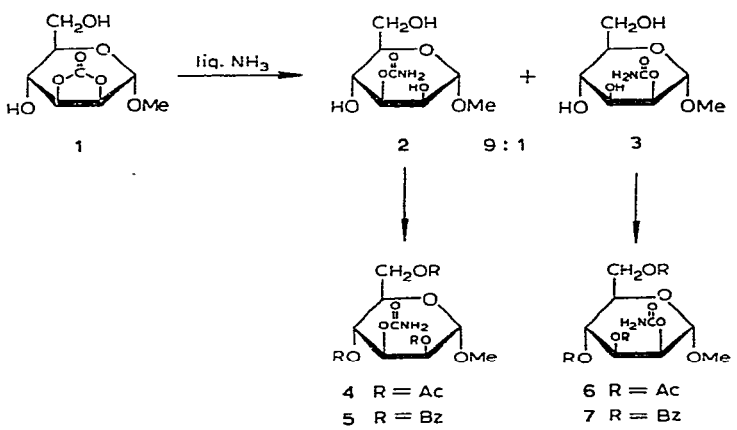

Chart 1.

Methyl 2-O-carbamoyl-α-D-mannopyranoside (**3**). — Compound **3** was synthesized from methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-(*p*-nitrophenoxycarbonyl)-α-D-mannopyranoside **10** by ammonolysis (Chart 2). In order to obtain **10**, it was necessary at the outset to protect the C-3, C-4, and C-6 hydroxyl groups of methyl α-D-mannopyranoside. Garegg *et al.*[7] reported that acetylation of methyl 4,6-*O*-ethylidene-α-D-mannopyranoside gave six times more of the 3-acetate than of the 2-acetate, suggesting that the equatorial C-3 hydroxyl group at C-3 was more reactive[8] than the axial one at C-2. Treatment of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**8**) with 1.2 equivalents of benzoyl chloride in pyridine at −10 to −5° gave only the 3-benzoate **9**, as colorless crystals in 70% yield. Neither the 2,3-dibenzoate nor the 2-benzoate was formed. The structure of **9** was confirmed by an n.m.r. double-resonance study (Fig. 2). The presence of an OH-proton signal (δ 2.60) indicated that the product was a monobenzoate. Irradiation at δ 4.27 collapsed the anomeric resonance at δ 4.60 to a singlet, a quartet at δ 5.85 to a doublet, and the hydroxyl resonance to a singlet. Therefore, the quartet at δ 5.85 was assigned to H-3. The fact that the signal of H-3 was observed at such a low field indicated that C-3 hydroxyl group was benzoylated. The H-3 signal was observed at lower field (δ 5.85) in C_6D_6 than in $CDCl_3$ (δ 5.65), and this difference is attributed to a solvent effect and carbonyl-group anisotropy.

Treatment of **9** with *p*-nitrophenoxycarbonyl chloride in pyridine gave methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-(*p*-nitrophenoxycarbonyl)-α-D-mannopyranoside (**10**) in 93% yield. The i.r. spectrum of the product showed the presence of carbonate, ester carbonyl, and $-NO_2$ groups.

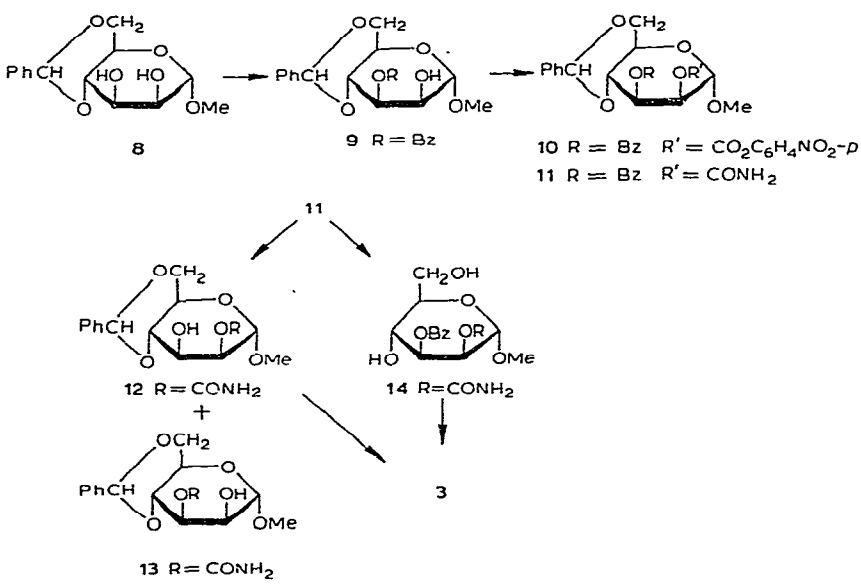

Chart 2.

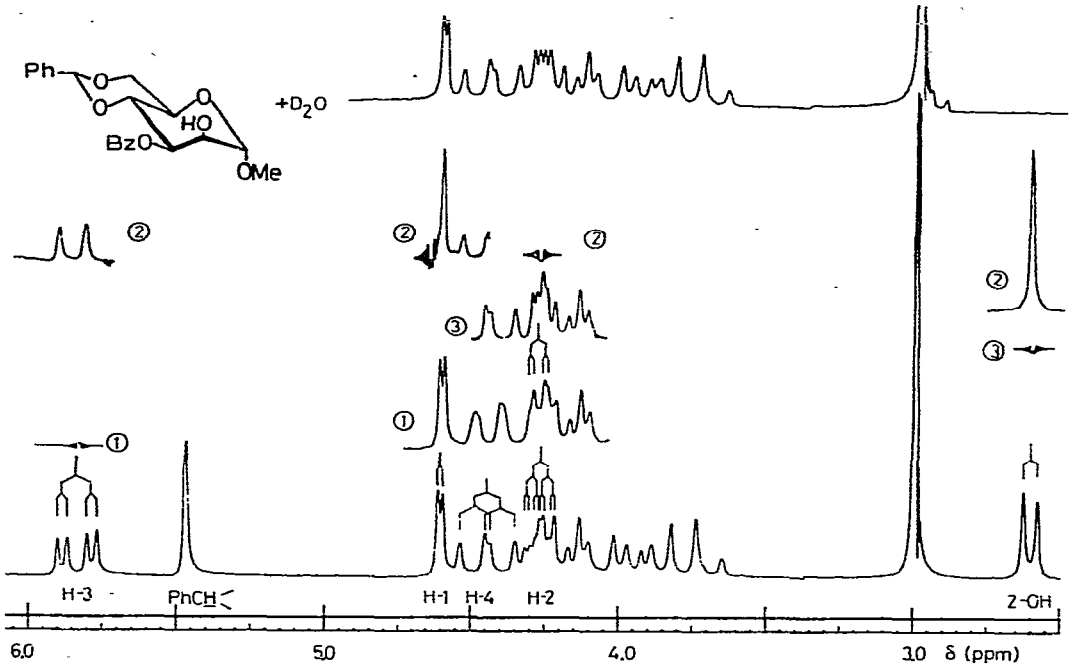

Fig. 2. Partial n.m.r. spectrum of methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**9**) at 100 MHz in C_6D_6.

Treatment of **10** with methanolic ammonia gave methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-carbamoyl-α-D-mannopyranoside (**11**) in 82% yield, as colorless crystals whose i.r. spectrum showed the presence of a carbamoyl group.

Two alternative routes were examined for removing the protective groups from **11** to give **3**. One is alkaline removal of the benzoyl group, followed by catalytic hydrogenolysis of the benzylidene group. The other involves initial catalytic hydrogenolysis, followed by treatment with alkali.

When **11** was suspended in methanol and treated with methanolic sodium methoxide, unfavorable side-reactions, such as migration and removal of the carbamoyl group, were observed to occur before all of the benzoyl groups had been removed. This behavior might be a consequence of the poor solubility of **11** in methanol.

On the other hand, when **11** was subjected to hydrogenolysis over palladium-on-charcoal in the presence of acetic acid, methyl 3-*O*-benzoyl-2-*O*-carbamoyl-α-D-mannopyranoside (**14**) was obtained in 97% yield. Compound **14** could then be treated with sodium methoxide to give **3** in quantitative yield. The n.m.r. spectrum of **3** in D_2O (Fig. 3) showed the H-2 resonance at δ 5.23 (quartet).

Compound **3** was not differentiated from **2** by t.l.c. on cellulose or silica gel, or by g.l.c. Neither were the acetates (**6** and **4**) separated by t.l.c. on silica gel, but, in contrast, the benzoates (**4** and **5**) could be so resolved. The R_F values were 0.33 for **5**

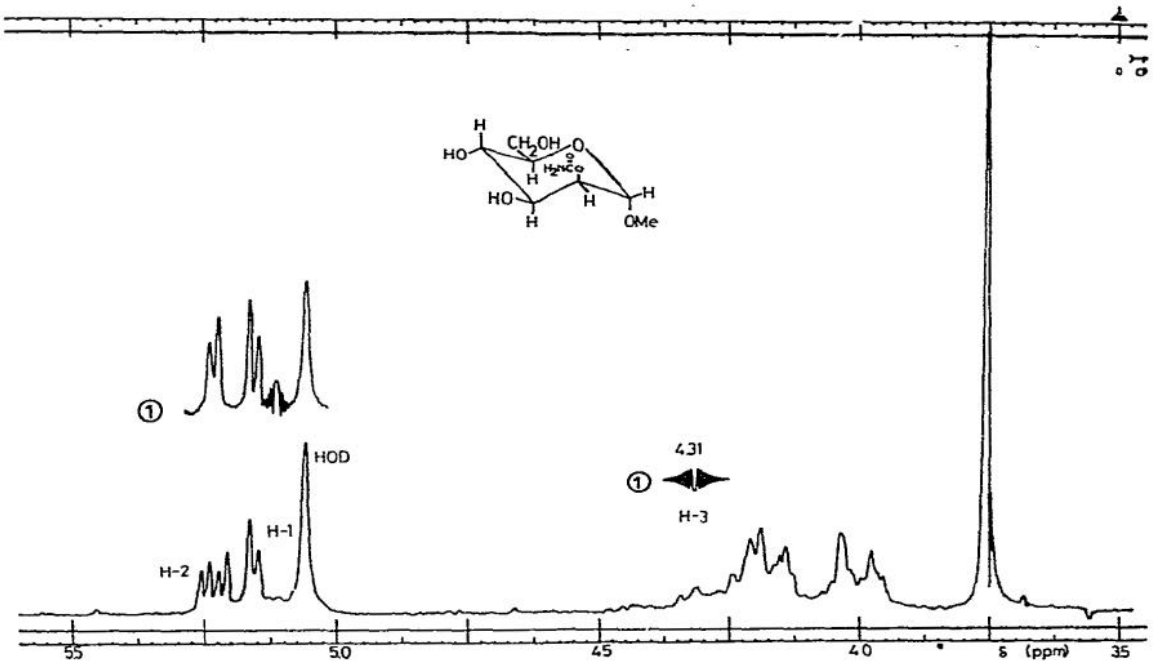

Fig. 3. N.m.r. spectrum of methyl 2-*O*-carbamoyl-α-D-mannopyranoside (3) at 100 MHz in D_2O.

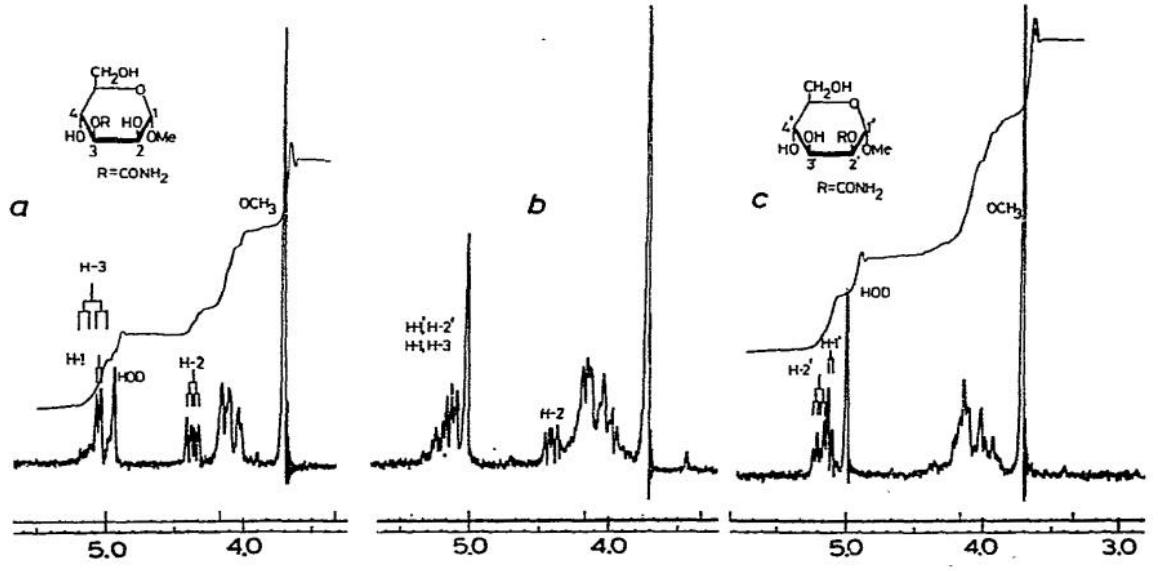

Fig. 4. N.m.r. spectrum of compound 2(*a*), 3(*c*) and their alkali-treated products (*b*) at 60 MHz in D_2O with Me_4Si as external reference.

and 0.22 for **7** when 7:1 benzene–ethyl acetate was used as a developing solvent. This information was very useful in a subsequently study of iso-bleomycin.

Migration of the carbamoyl group. — The n.m.r. spectra of methyl 2-*O*- and 3-*O*-carbamoyl-α-D-mannopyranosides (**3** and **2**) are shown in Fig. 4 (*a* and *c*). The H-2 signal of the 3-carbamate **2** appeared as a quartet at δ 4.4, whereas no signal was observed at this region in the spectrum of the 2-carbamate **3**. The percentage of **2** and **3** in the mixture may be calculated by the following equation:

$$\text{Percentage of } \mathbf{2} = \frac{I_{\delta 4.4}}{I_{\mathrm{OMe}} \times \frac{1}{3}} \times 100(\%)$$

$$\text{Percentage of } \mathbf{3} = 100 - \text{percentage of } \mathbf{2}$$

where $I_{\delta 4.4}$ is the integrated area of the signal at δ 4.4 in the spectrum of the mixture, and I_{OMe} is that of the methoxyl-group signal, which appeared as a singlet at δ 3.7 in the mixture.

In order to investigate the migration of the carbamoyl groups, 30 mg of **2** and **3** were individually dissolved in a solution composed of 0.25 ml of triethylamine, 4.0 ml of ethanol, and 1.6 ml of water, and the solutions were kept overnight at 20°. The solvents were evaporated and the products analyzed by n.m.r. spectroscopy. Both samples gave almost the same spectra (Fig. 4, *b*). This result indicates that the equilibrium between **2** and **3** is established under these conditions. The ratio of **2** and **3** at the equilibrium point was estimated as 74:26.

After completion of his study, iso-bleomycin A_2 was prepared by treatment of bleomycin A_2 with alkali. The presence of a 2-*O*-carbamoyl-D-mannose moiety in iso-bleomycin A_2 was confirmed by isolation of the benzoate (**7**). This work will be published in the Journal of Antibiotics.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-1 melting-point apparatus. Optical rotations were measured with a Carl Zeiss Photoelectric Precision Polarimeter. I.r. spectra were determined with KBr discs on a Hitachi EPI-1 spectrometer. N.m.r. spectra were recorded on Varian A60D and HA-100D spectrometers with tetramethylsilane as the internal or external (D_2O) standard; chemical shifts are given as δ values. N.m.r. data are given only for the protons indicated.

Methyl 2,3-O-carbonyl-α-D-mannopyranoside (**1**). — This compound was prepared according to the method of Hough and Priddle[6] in 77% yield from methyl α-D-mannopyranoside, which had been obtained in 93% yield by refluxing D-mannose in methanol with Amberlyst-15.

Methyl 3-O-carbamoyl-α-D-mannopyranoside (**2**), *and its 2,4,6-triacetate and 2,4,6-tribenzoate.* — A solution of methyl 2,3-*O*-carbonyl-α-D-mannopyranoside (851 mg) in 60 ml of liquid ammonia was kept at room temperature and then

evaporated to give 860 mg of crude methyl 3-*O*-carbamoyl-α-D-mannopyranoside as a hygroscopic solid having no distinct m.p.; $[\alpha]_D^{22}$ +49° (*c* 1.0, methanol). It was chromatographically homogeneous in all systems used.

Acetylation of the crude **2** (450 mg) gave 320 mg of the crystalline acetate (**4**), which was recrystallized from isopropyl alcohol; m.p. 142.5°, $[\alpha]_D^{23}$ +35.8° (*c* 1.1, chloroform); *m/e*: 332 (M−31), [**4** isolated from bleomycin A_2: m.p. 142.0°, $[\alpha]_D^{22}$ +35.7° (*c* 1.0, chloroform)].

Benzoylation of the crude **2** (270 mg) with benzoyl chloride (2 ml) in pyridine (10 ml) gave 377 mg of methyl 2,4,6-tri-*O*-benzoyl-3-*O*-carbamoyl-α-D-mannopyranoside (**5**) (glass) and 32 mg of the crystalline 2-carbamate isomer (**7**) after column chromatography on silica gel (7:1 benzene–ethyl acetate).

Treatment of **5** with a catalytic amount of sodium methoxide in methanol gave pure methyl 3-*O*-carbamoyl-α-D-mannopyranoside (**2**) in quantitative yield as a hygroscopic glass; $[\alpha]_D^{20}$ +50° (*c* 1.0, ethanol), λ_{max} 1610, 1710 cm^{-1} ($\overset{O}{\overset{\|}{OCNH_2}}$); n.m.r.: ($D_2O$, 100 MHz, sodium 4,4-dimethyl-4-silapentane-1-sulfonate as the standard) δ 4.76, doublet ($J_{1,2}$ 1.8 Hz, H-1), 4.06, quartet ($J_{2,3}$ 3.3 Hz, H-2), 4.78, doublet ($J_{3,4}$ 8.0 Hz, H-3).

Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-carbamoyl-α-D-mannopyranoside (**5**); glass $[\alpha]_D^{21}$ −19.5° (*c* 0.9, chloroform).

Anal. Calc. for $C_{29}H_{27}NO_{10}$: C, 63.38; H, 4.95; N, 2.55. Found: C, 63.36; H, 4.86; N, 2.16.

Methyl 3,4,6-tri-*O*-benzoyl-2-*O*-carbamoyl-α-D-mannopyranoside (**7**), m.p. 189.5°, $[\alpha]_D^{20}$ −16.0° (*c* 1.0, chloroform); *Anal.* Found: C, 63.47; H, 4.87; N, 2.64.

Methyl 3-O-benzoyl-4,6-O-benzylidene-α-D-mannopyranoside (**9**). — To a solution of 5.71 g of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside[9] (**8**) in pyridine (200 ml), was added 3.2 g (1.13 equiv.) of benzoyl chloride at −20°. The reaction mixture was kept for 3 h at −20° and then for 30 h at 5°. It was then poured into ice–water and the product was extracted twice with 100 ml of chloroform. The chloroform layer was dried, and evaporated to dryness to give 8.0 g of a syrup, which was purified on a column of silica gel with a 8:1 benzene–ethyl acetate as eluant. The major fraction was evaporated and the residue crystallized from hexane to give 5.5 g (71%) of **9**, m.p. 131.8°, $[\alpha]_D^{25}$ −26.5° (*c* 0.94, chloroform); ν_{max}^{KBr} 3500 (OH) and 1710 cm^{-1} (benzoate).

Anal. Calc. for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74. Found: C, 65.23; H, 5.74.

Methyl 3-O-benzoyl-4,6-O-benzylidene-2-O-(p-nitrophenyloxycarbonyl)-α-D-mannopyranoside (**10**). — To a solution of 1.16 g of **9** in 60 ml of pyridine was added *p*-nitrophenyloxycarbonyl chloride (860 mg) and the reaction mixture was kept overnight at room temperature. Benzene (50 ml) was then added and the solution was washed with 0.5M sulfuric acid (5 × 20 ml), M sodium hydrogen carbonate (5 × 20 ml), and finally with water. The benzene solution was dried and evaporated to give 1.53 g (93%) of pale-yellow, crystalline **10**, which was recrystallized from isopropyl alcohol;

m.p. 178.5°, $[\alpha]_D^{27}$ −38.1° (*c* 1.2, chloroform); ν_{max}^{KBr} 1736 (–OC–) and 1780 cm^{-1} (–OC–O–); n.m.r.: (C_6D_6, 100 MHz) δ 4.65, doublet ($J_{1,2}$ 1.3 Hz, H-1), 5.67, quartet ($J_{2,3}$ 3.0 Hz, H-2), 6.04, doublet of doublets ($J_{3,4}$ 11.0 Hz, H-3), 4.42, doublet of doublets ($J_{4,5}$ 9.5 Hz, H-4).

Anal Calc. for $C_{28}H_{25}NO_{11}$: C, 61.09; H, 4.69; N, 2.87. Found: C, 60.98; H, 4.57; N, 2.54.

Methyl 3-O-benzoyl-4,6-O-benzylidene-2-O-carbamoyl-α-D-mannopyranoside (**11**). — To a solution of 1.1 g of **10** in 15 ml of dichloromethane was added 15 ml of 4.4M methanolic ammonia and the solution was kept at room temperature. After 6 h, the solvent was evaporated. The residue was dissolved in 100 ml of dichloromethane and washed with M sodium carbonate (4 × 50 ml), and water. After evaporation, 750 mg (82%) of colorless, crystalline **11** was obtained, m.p. 248° (dec.) (recrystallized from isopropyl alcohol), $[\alpha]_D^{27}$ −52.3° (*c* 1.1, chloroform); ν_{max}^{KBr} 1740 (–OC–) and 1610, 1700 cm^{-1} (–OCNH$_2$).

Anal. Calc. for $C_{22}H_{23}NO_8$: C, 61.38; H, 5.44; N, 3.63. Found: C, 61.53; H, 5.40; N, 3.26.

Methyl 4,6-O-benzylidene-2-O-carbamoyl-α-D-mannopyranoside (**12**). — To a solution of 84 mg of **11** in methanol (20 ml) was added 0.1 ml of methanolic sodium methoxide (110 mg of Na in 10 ml of methanol). After 20 h, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin. After removal of the resin, the solvent was evaporated. The residual syrup was purified on a column of silica gel, with 20:1 benzene–ethanol as an eluant, to give 64 mg (66%) of **12**, m.p. 186.8°, $[\alpha]_D^{20}$ −4° (*c* 2.0, methanol); n.m.r. (methanol-d_4, 100 MHz): δ 4.70, doublet ($J_{1,2}$ 1.4 Hz, H-1), 4.91, quartet ($J_{2,3}$ 3.1 Hz, H-2), 4.03 (overlapped, H-3).

Anal. Calc. for $C_{15}H_{19}NO_7$: C, 55.38; H, 5.89; N, 4.31. Found: C, 55.34; H, 5.64; N, 4.37.

When the reaction was continued for 40 h, a small amount of methyl 4,6-*O*-benzylidene-3-*O*-carbamoyl-α-D-mannopyranoside (**13**) was isolated as a syrup; n.m.r. (methanol-d_4, 100 MHz): δ 4.68, doublet ($J_{1,2}$ 1.5 Hz, H-1), 4.09, quartet ($J_{2,3}$ 3.3 Hz, H-2), 5.03, doublet of doublets ($J_{3,4}$ 10.0 Hz, H-3).

Methyl 3-O-benzoyl-2-O-carbamoyl-α-D-mannopyranoside (**14**). — Compound **11** (650 mg) was reduced with hydrogen and 300 mg of palladium-on-charcoal in 60 ml of 1:1 acetic acid–methanol. The catalyst was filtered off, and the filtrate was evaporated. The residue was dissolved in water and evaporated. The evaporation was repeated until colorless crystals of **14** appeared. The crystals (500 mg, 97%) were recrystallized from isopropyl alcohol–isopropyl ether; m.p. 150.5°, $[\alpha]_D^{20}$ +2.6° (*c* 1.0, methanol); ν_{max}^{KBr} 1600, 1725 cm^{-1} (–OC–NH$_2$, OC–); n.m.r. (methanol-d_4,

100 MHz): δ 4.65, doublet ($J_{1,2}$ 1.5 Hz, H-1), 5.12, doublet of doublets ($J_{2,3}$ 3.3 Hz, H-2), 5.32, doublet of doublets ($J_{3,4}$ 9.7 Hz, H-3).

Anal. Calc. for $C_{15}H_{19}NO_8$: C, 52.78; H, 5.61; N, 4.10. Found: C, 53.23; H, 5.77; N, 4.00.

Methyl 2-O-carbamoyl-α-D-mannopyranoside (**3**), *its acetate* **6**, *and benzoate* **7**. — Compound **3** was obtained, in quantitative yield, by catalytic hydrogenation of **12** over palladium-on-charcoal or by treatment of **14** with sodium methoxide, as a hygroscopic solid, $[\alpha]_D^{21}$ +36.5° (*c* 1.1, ethanol); ν_{max}^{KBr} 1610 and 1720 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-NH_2$). The acetate **6** was a colorless glass, $[\alpha]_D^{21}$ +23° (*c* 1.0 chloroform), *m/e* 332 (M−31). The benzoate **7** formed colorless crystals from isopropyl alcohol; m.p. 189.5, $[\alpha]^{20}$ −16.0° (*c* 1.0, chloroform).

Anal. Calc. for $C_{29}H_{27}NO_{10}$: C, 63.38; H, 4.95; N, 2.55. Found: C, 63.47; H, 4.87; N, 2.64.

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