Synthetic Studies on Carbohydrate Antibiotics

Part V. Synthesis of 6-Acetamido-2,3,4-tri-O-benzyl-6-deoxy-D-glucopyranosyl Chloride and Its Use in Königs-Knorr Reaction

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Treatment of methyl tri-O-acetyl-6-azido-6-deoxy-D-glucopyranoside (II) with benzyl chloride afforded the corresponding tri-O-benzyl derivative. Reduction of the azido group with zinc-acetic acid or sodium amalgam gave 6-amino derivative. N-Acetylation, hydrolysis of the 1-O-methyl group, acetylation and treatment with dry hydrogen chloride in dioxane afforded the title compound (VIII). Königs-Knorr reaction of the chloride with (+)- and (-)-2-aminocyclohexanol produced the corresponding glycosides, one of which was proved to have the α -configuration.

In the preceding paper, we described the synthesis of α -D-glucosides of (+)- and (-)-*trans*-2-aminocyclohexanol by utilizing a benzylhalogenoglucose.¹⁾ As a logical extension of the synthetic study of some carbohydrate antibiotics—aminoglycosidic antibiotics, we have prepared a polybenzylhalogeno derivative of 6-amino-6-deoxy-D-glucose and tried its Königs-Knorr reaction with (+)- and (-)-*trans*-2-aminocyclohexanol.

As one of the polybenzylated halogeno derivatives of 6-amino-6-deoxy-D-glucose, 6-acetamido-2,3,4-tri-O-benzyl-6-deoxy-D-glucopyranosyl chloride (VIII) was prepared by the reaction sequence indicated in Fig. 1.²⁾

As the starting compound methyl 6-azido-6-deoxy- α -D-glucopyranoside (I) was prepared

according to Cramer's procedure,^{3,4)} the direct benzylation of 6-amino-derivative or N-protected compounds such as N-carboboxy, and N-dinitrophenyl derivatives was found to yield impure products which were hardly purified.

The reaction of the acetate (II) with benzyl chloride and powdered potassium hydroxide proceeded rapidly to give the corresponding benzyl derivative (III) in a good yield. The selective reduction of the azido group with elimination of O-benzyl group in the compound (III) was achieved with zinc-acetic acid or 3% sodium amalgam in methanol, although a perfect reduction occurred by catalytic hydrogenation with palladium on charcoal affording methyl 6-amino-6-deoxy-D-glucopyranoside. The selectively reduced compound (IV) was acetylated to give the acetamido derivative (V), which was partial

1) N. Kurihara, T. Ueno, S. Hashimoto and M. Nakajima, *This Journal*, **31**, 1343 (1967).

2) S. Umezawa, S. Koto, Y. Kato and T. Tsumura have reported recently a synthesis of another benzyl derivative of 6-amino-6-deoxy-D-glucose, and its use in α -glucosidation with cyclohexanol. (The 20th National Meeting of the Chemical Society of Japan, Tokyo, April 1967, Abstract III-596.)

3) F. Cramer, H. Otterbach and H. Springmar *Chem. Ber.*, **92**, 384 (1959).

4) E. Hardegger, G. Zanetti and K. Steiner, *Helv. Chim. Acta*, **46**, 282 (1963).

FIG. 1. Benzyl Halogeno Derivative of 6-Amino-6-deoxy-D-glucose. (Bzl = $\text{C}_6\text{H}_5\text{CH}_2$).

FIG. 2. NMR Spectra of the Glycoside from (-)-Aglycon.

hydrolyzed to the compound VI by gentle heating in the solution of acetic acid and 6N HCl (6:1, v/v). The compound VI was acetylated and the acetate (VII) was treated with 4% solution of hydrogen chloride in absolute dioxane to give crystalline 6-acetamido-2,3,4-tri-O-benzyl-6-deoxy-D-glucopyranosyl chloride (VIII). When VI was treated with thionyl chloride as in our preceding paper,¹¹ it gave an oxazoline like derivative (IX) as expected from Bergmann's and Winstein's works.⁵¹ Its infrared spectrum showed a strong absorption at 1690 cm^{-1} ($\text{C}=\text{N}$ stretching) and no absorption corresponding to an amide.

Königs-Knorr condensation of the chloride (VIII) with N-carbobenzoxy derivative of (+)- and (-)-*trans*-2-aminocyclohexanol was carried out essentially in the same condition as in the syntheses of glucosides in the preceding paper. In each case, we isolated a single product. The anomeric configuration of the product from (-)-aglycon was assigned as α , since it showed characteristic NMR signals for an anomeric proton around 5 p.p.m. from internal standard DSS. The spectra were taken

5) M. Bergmann and A. Miekely, *Z. Physiol. Chem.*, **140**, 128 (1924); S. Winstein and R. Boschan, *J. Am. Chem. Soc.*, **72**, 4669 (1950).

in the mixed solvents of *d*-chloroform and *d*₅-pyridine; the ratio of the two solvents being 1:2, 1:1 and 3:1. As shown in the Fig. 2, a doublet corresponding to the anomeric proton moved as a set among other signals. Its spacing was 3.5 c.p.s., which corresponded clearly to the α -configuration.⁶⁾

The product from (+)-aglycon had a large optical rotation and was thought to have also the α -configuration. A definite conclusion of configuration could not be drawn, since its anomeric proton signal was not clearly assigned in the NMR spectra.

EXPERIMENTAL

Melting points were uncorrected. IR spectra were recorded with a Shimadzu spectrophotometer AR 275. NMR spectra were recorded with a Varian A-60 spectrometer. Optical rotations were measured with a Yanagimoto photo-magnetic direct reading polarimeter, OR-20.

Methyl 6-Azido-2,3,4-tri-O-benzyl-6-deoxy- α -D-glucopyranoside (III)

A mixture of methyl 2,3,4-tri-O-acetyl-6-azido-6-deoxy- α -D-glucoside (II)³⁾ (9.5 g), benzyl chloride (100 g) and powdered potassium hydroxide (25 g) was stirred at 90~100°C (bath temp.) for 3 hours under anhydrous condition. The reaction was exothermic and immediately after the reaction started the inner temperature rised to 130~140°C. Insoluble materials were filtered off on Celite 545 pad, and washed with benzene. The filtrate and washings were combined and diluted with benzene to a total volume of 400 ml. The benzene solution was washed with water and dried over sodium sulfate and evaporated to a syrup. It was further heated at 90~110°/10⁻² mmHg to remove the by-products of high boiling point. The resulting syrupy residue was purified by passing in benzene solution through an alumina column. A colorless syrup was obtained. Yield 10.8 g (80%). $[\alpha]_D^{+53.1^\circ}$ (*c* 2.4, in chloroform). It showed a single spot on a thin layer chromatogram (Merck silica gel G, petroleum ether-methanol, 20:1). *Anal.* Found: C, 69.16; H, 6.38; N, 8.86. Calcd. for C₂₈H₃₁N₃O₅: C, 68.69; H, 6.38; N, 8.58%. IR spectrum (liquid film) showed absorptions at 2130 (azide),

ca. 1600, 740 and 700 cm⁻¹ (monosubstituted benzene). Around 1700 cm⁻¹, there are no absorptions corresponding to ester carbonyl.

Methyl 6-Acetamido-2,3,4-tri-O-benzyl- α -D-glucopyranoside (V)

1) Zinc-acetic acid reduction. The azide (III) (2 g) was dissolved in 90% aq. acetic acid (100 ml), and zinc powder (7.5 g) was added portionwise with stirring for 2 hours at 5°C. After the addition had been finished, the stirring was continued for an additional 1 hour. Insoluble materials were filtered off and the filtrate was concentrated to dryness giving a syrup which had no absorptions of azido group in its IR spectrum (IV). The syrup was mixed with acetic anhydride and pyridine; then was left to overnight. The mixture was evaporated off under a reduced pressure to a syrup. It was dissolved in benzene (100 ml) and the solution was washed with 2N NaOH, water and 2N HCl, and dried with Na₂SO₄. Concentration gave a syrup. After decolorization with active charcoal in ethanol, the syrup was triturated with ether giving fine needles of m. p. 150~151°C. Yield 1.3 g (63%). $[\alpha]_D^{-6^\circ}$ (*c* 2, in chloroform). *Anal.* Found: C, 71.53; H, 7.00; N, 3.02. Calcd. for C₃₀H₃₅NO₆: C, 71.26; H, 6.98; N, 2.77%.

2) Sodium amalgam reduction. The azide (III) (1.0 g) was dissolved in methanol (100 ml). Three % sodium amalgam (5 g) was added portionwise in 30 minutes to the solution with stirring. The stirring was continued 6 hours at room temperature. After separation of mercury, the methanol solution was dried up, and the residue was dissolved in benzene. The benzene solution was washed with water, dried with Na₂SO₄, and concentrated to give a syrup which showed no absorptions of azido group in its IR spectrum. It was acetylated in a usual manner, affording crystals identical with those obtained by zinc-acetic acid reduction in IR spectrum and in m. p. Yield 550 mg (54%), $[\alpha]_D^{-6^\circ}$ (in chloroform).

IR spectrum in Nujol showed absorptions at 3300 (NH), 1640, 1580 (amide I and II) and 760~740, 700 cm⁻¹ (monosubstituted benzene).

6-Acetamido-2,3,4-tri-O-benzyl-6-deoxy-D-glucose (VI)

The methyl glycoside derivative (V) (500 mg) was dissolved in a mixed solution of acetic acid (9 ml) and 6N HCl (1.5 ml). The mixture was heated in a water bath with occasional shaking at 60~70°C for 1 hour. After the reaction, the solution was decolorized with active charcoal and evaporated to a

6) J. M. van der Veen, *J. Org. Chem.*, **28**, 564 (1963).

symp. The syrup was dissolved in ethanol (5 ml), and water was gradually added until it showed a slight turbidity. Fine needles of m. p. 113~115°C were obtained by scratching the vessel's wall. Yield 350 mg (73%). M. p. 116~117°C after recrystallizations from ethanol-water. $[\alpha]_D^{20} + 10^\circ$ (c 1, in chloroform). *Anal.* Found: C, 70.77; H, 7.03; N, 2.99. Calcd. for $C_{29}H_{33}NO_6$: C, 70.85; H, 6.77; N, 2.85%. Its IR spectrum showed absorptions at 3500~3200 (OH and NH), 1600~1620, 1580 (amide), 760~720, and 700 cm^{-1} (monosubstituted benzene).

6-Acetamido-1-O-acetyl-2,3,4-tri-O-benzyl-6-deoxy-D-glucose (VII)

1-Hydroxy compound (VI) (1.3 g) was mixed with pyridine (10 ml) and acetic anhydride (10 ml) and allowed to stand overnight at room temperature. The solution was evaporated to a syrup, which was dissolved in chloroform. The chloroform solution was washed with 2 N NaOH, 2 N HCl and then water, and dried with Na_2SO_4 . Evaporation gave a syrup, which was crystallized from ethanol. Yield 700 mg, m. p. 151°C. From the mother liquor, an amorphous solid (450 mg) was obtained which showed the same IR spectrum with that of the crystals. The recrystallized sample melted at 152~153°C, and the total yield was 81%. *Anal.* Found: C, 69.95; H, 6.74. Calcd. for $C_{31}H_{35}NO_7$: C, 69.77; H, 6.61%. Its IR spectrum showed absorptions at 3300 (NH), 1760 (ester carbonyl), 1650, 1560 (amide), 750 and 700 cm^{-1} (monosubstituted benzene).

6-Acetamido-2,3,4-tri-O-benzyl-6-deoxy-D-glucopyranosyl Chloride (VIII)

1-O-Acetate (VII) (840 mg) was dissolved in 4% HCl in absolute dioxane (24 ml), and the resulting solution was kept at room temperature for 70 hours and then concentrated below 35°C to dryness. Toluene was added and evaporated repeatedly. The resulting amorphous residue gave crystals of m. p. 71~75°C (500 mg) (60%) by recrystallization from absolute ether. The crystals were positive in the Beilstein test and showed no ester-carbonyl absorption in the IR spectrum. It showed absorptions at 3300 (NH), 1660, 1560 (amide), 740 and 700 cm^{-1} (monosubstituted benzene).

The Reaction of 6-Acetamido-2,3,4-tri-O-benzyl-6-deoxy-D-glucose with Thionyl Chloride

The mixture of the compound VI (1.3 g) and thionyl chloride (5 ml) was kept at 70°C for 3 hours. Evaporation in vacuo, addition of toluene, and then further evaporation of the solvent in vacuo were

repeated in order to remove a trace of the reagent to give a syrup. The IR spectrum of this compound (IX) lacked NH-stretching and amide I and II absorptions, but showed the strong absorption of C=N stretching at 1690 cm^{-1} .

6-Acetamido-2,3,4-tri-O-benzyl-6-deoxy-D-glucopyranosyl (+)-N-Carbobenzoxy-trans-2-aminocyclohexanol

(+)-N-Carbobenzoxy-trans-2-aminocyclohexanol (650 mg) was added to absolute benzene with Drierite (12 g) and silver carbonate (5 g). The mixture was stirred overnight at room temperature in the dark. Then the benzene solution (40 ml) of the chloride (VIII) (1.88 g) was added along with silver perchlorate (200 mg), and the stirring was continued for 110 hours under the same condition as described previously. After the reaction, the insoluble materials were filtered off, and the residue was washed with chloroform. The filtrate and the washings were combined and washed with water, dried with Na_2SO_4 , and evaporated to dryness. Recrystallizations from ethanol of the residue gave crystals of m. p. 196°C, $[\alpha]_D^{20} + 111^\circ$ (c 0.8 in chloroform). Yield was 120 mg. *Anal.* Found: C, 71.12; H, 7.00. Calcd. for $C_{43}H_{50}N_2O_8$: C, 71.44; H, 6.97%. Its IR spectrum showed absorptions at 3300 (NH), 1700 (carbobenzoxamide I), 1670 (acetamide I), 1570 (amide II), 750 and 700 cm^{-1} (monosubstituted benzene).

6-Acetamido-2,3,4-tri-O-benzyl-6-deoxy- α -D-glucopyranosyl (-)-N-Carbobenzoxy-trans-2-aminocyclohexanol

According to the same procedure as described above, the benzene solution (40 ml) containing 6-acetamido-2,3,4-tri-O-benzyl-6-deoxy-D-glucopyranosyl chloride (VIII) (540 mg) was added with silver perchlorate (150 mg) to the stirred mixture of (-)-N-carbobenzoxy-trans-2-aminocyclohexanol (650 mg), Drierite (12 g) and silver carbonate (5 g) in benzene (40 ml). The stirring was continued for 96 hours at room temperature in the dark. After the reaction, the obtained syrup was recrystallized from ethanol to give crystals of m. p. 188°C, $[\alpha]_D^{20} + 1^\circ$ (c 1.66 in chloroform). The yield was 65 mg. *Anal.* Found: C, 71.81; H, 7.49. Calcd. for $C_{43}H_{50}N_2O_8$: C, 71.44; H, 6.97%. The IR spectrum showed absorptions at 3330 (NH), 1690 (carbobenzoxamide I), 1650~1660 (acetamide), 1550 (amide II), 740 and 700 cm^{-1} (monosubstituted benzene). Its NMR spectra showed H1-H2 coupling constant of 3.5 c. p. s. at 5.04~4.97 p. p. m. from DSS in mixed solvents of d_6 -pyridine and d -chloroform

(See the text and Fig. 2.).

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