Note

Nucleosides

Part LXXII. Synthetic studies on nucleoside antibiotics. 7. An improved synthesis of C-substance*

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(Received May 24th, 1971; accepted for publication, August 6th, 1971)

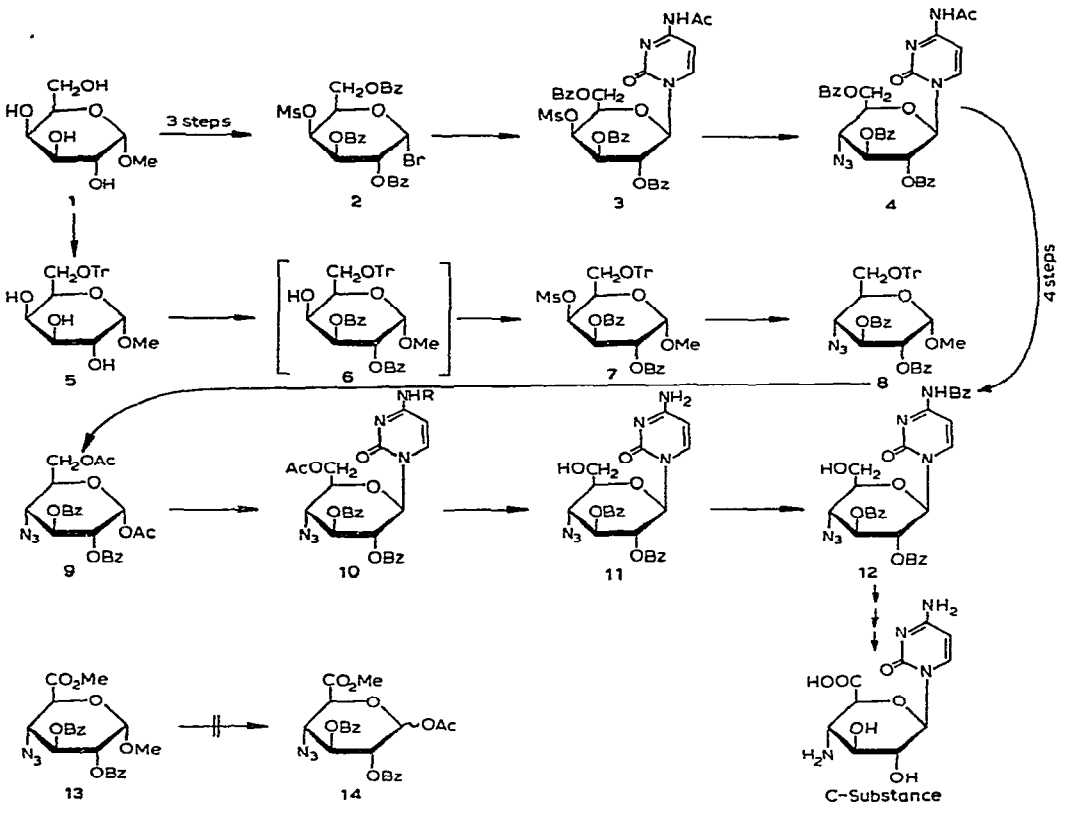
In a previous report[1] we described the total synthesis, starting from methyl α-D-galactopyranoside (**1**), of C-substance, the nucleoside moiety of the antibiotic Gougerotin. Since Gougerotin is not available in large quantities[2], we have developed an alternate and more efficient route to C-substance as part of our program directed toward the synthesis of this antibiotic and analogs thereof.

The previous procedure[1] for the 12-step preparation of C-substance from **1** shows the following disadvantages: (i) the condensation of the 4-*O*-(methylsulfonyl)-glycosyl bromide **2** (obtained in 3 steps from **1**) with *N*-acetylcytosine by the mercuric cyanide–nitromethane procedure to afford **3** was wasteful because it required two equivalents of the glycosyl bromide **2**; (ii) the preparation of the azide **4** from **3** on a large scale was achieved at best in only ~50% yield; (iii) the conversion of **4** into **12** (involving saponification, tritylation, benzoylation, and detritylation) was complicated due to overbenzoylation of the cytosine nucleus. The reaction sequence described herein overcomes these difficulties.

Tritylation of **1** afforded the 6-*O*-trityl derivative **5** which was selectively benzoylated to give the 2,3-dibenzoate **6**. The latter compound, without purification, was methylsulfonylated to give **7** (77% overall yield from **5**). The crystalline azide **8** was obtained in 83% yield by treatment of **7** with sodium azide in hexamethylphosphoric triamide. Acetolysis of **8** gave crystalline 1,6-di-*O*-acetyl-4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-D-glucose (**9**) in 66% yield.

Previous experiments[3] had indicated that the formation of a glycosyl halide from **9** would not be a promising approach. Smith and Brown[4] had shown that treatment of azido derivatives with hydrobromic acid–acetic acid led to conversion of the azido into an amino function. However, condensation of **9** with an equimolar amount of bis(trimethylsilyl)-N^4-acylcytosine in the presence of stannic chloride, according to the modified procedure of Niedballa and Vorbrüggen[5], gave the protected nucleoside **10** (R = acetyl or benzoyl) in 86% yield. De-*O*-acetylation and de-*N*-

*This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service (Grant No. CA-08748).



acylation to give **11** in almost quantitative yield was accomplished by treatment of **10** with methanolic triethylamine. Selective *N*-benzoylation[6] of **11** gave **12** in good yield when **11** was heated at reflux with benzoic anhydride in ethanol. Attempts to convert **10** into **12** directly by the procedure of Rammler and Khorana[7] (sodium methoxide in methanol) or by the use of triethylamine in methanol were not successful because de-*N*-acylation began to occur before de-*O*-acetylation was completed. The oxidation of the nucleoside **12** to C-substance by the use of chromic anhydride in a pyridine–acetic acid mixture containing a small amount of water has been described[1].

The overall yield of nucleoside **12** from **1** by this alternative approach is 2.5 times greater than that reported previously via the **2**→**3**→**4** route. The alternative synthesis also has the advantages of greater ease of handling for large scale preparations, fewer steps, and the absence of deleterious side-products.

Obviously it would be to great advantage to make use of a uronate (such as **14**) for condensation with a bis(trimethylsilyl)-N^4-acylcytosine. The necessary precursor to **14**, the glycosyluronate **13**, had been reported[3] from our laboratory. However, attempts to acetolyze **13** to give **14** were consistently unsuccessful. The reaction was accompanied by the formation of a mixture of many products (as shown by t.l.c. and n.m.r.) in which the desired product, at best, was only a minor component.

EXPERIMENTAL

General. — Melting points were determined with a Thomas–Hoover capillary apparatus and are corrected. Thin-layer chromatography (t.l.c.) was performed on microscope slides coated with silica gel GF_{254} (Merck). Compounds were visualized under u.v. light or by spraying with a 20% solution of sulfuric acid in ethanol followed by heating on a hot plate. N.m.r. spectra were obtained on a Varian A-60 spectrometer using tetramethylsilane as internal standard. I.r. spectra were determined on a Perkin–Elmer model 137B spectrometer. U.v. spectra were obtained on a Cary model 15 spectrometer. Evaporations were performed *in vacuo*. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Methyl 6-O-trityl-α-D-galactopyranoside (**5**). — A solution of methyl α-D-galactopyranoside (monohydrate, dried in a high vacuum at 80° for 2 days, Pfanstiehl Laboratories, Inc., Waukegan, Illinois, U. S. A.) (50 g, 0.26 mole) and chlorotriphenylmethane (74 g, 0.265 mole) in pyridine (750 ml, distilled in the presence of barium oxide) was stirred at room temperature for 24 h. The pyridine was evaporated and the resulting thin syrup was dissolved in dichloromethane (*ca.* 1 liter). The solution was washed three times with a cold saturated solution of sodium hydrogen carbonate (250 ml) and finally with water (500 ml). The organic layer was dried (sodium sulfate) and, after filtration, evaporated. The syrupy residue was diluted several times with toluene and the solution evaporated to eliminate residual pyridine, and the residual toluene was removed by addition and evaporation of ethanol. The syrup was dissolved in a minimum amount of hot ethyl acetate, and the solution was cooled, seeded, and refrigerated. The white precipitate was triturated with cold anhydrous ether until fully dispersed, and then filtered off, washed with cold ether and dried. Further amounts of product were obtained from the mother liquor (total yield, *ca.* 83 g, 74%). The analytical sample was recrystallized from ethyl acetate; it sinters at 89° with indefinite m.p., $[\alpha]_D^{24}$ +68° (*c* 1.18, chloroform).

Anal. Calc. for $C_{26}H_{28}O_6$: C, 71.54; H, 6.47: Found: C, 71.69; H, 6.50.

Methyl 2,3-di-O-benzoyl-6-O-trityl-α-D-galactopyranoside (**6**). — A solution of 97 g (0.222 mole) of **5** in dry pyridine (1 liter) was stirred and cooled to 10°. Benzoyl chloride (55 ml, 0.47 mole) was added dropwise at such a rate that the temperature remained at *ca.* 10°. The reaction mixture was kept at room temperature for 12 h. The precipitated pyridine hydrochloride was removed by filtration, and the filtrate was evaporated to give a heavy syrup which was dissolved in dichloromethane (1.5 l). The solution was washed with cold saturated sodium hydrogen carbonate (1 liter), twice with an equal volume of water, and dried (sodium sulfate). Evaporation gave a syrup which was treated repeatedly with benzene (to remove pyridine) and finally with ethanol. The syrup was dried for several hours under high vacuum at ~70° to yield a glass, $[\alpha]_D^{24}$ +98° (*c* 3.48, chloroform) which was used directly for conversion into **7**.

Methyl 2,3-di-O-benzoyl-4-O-(methylsulfonyl)-6-O-trityl-α-D-galactoside (**7**). — A solution of crude **6** in pyridine (600 ml) was stirred and treated slowly with methane-

sulfonyl chloride (51 ml, 0.66 mole). Stirring was continued overnight at room temperature. The reaction mixture was poured into an equal volume of dichloromethane and filtered to remove black, insoluble material. The filtrate was washed sequentially with ice–water, cold saturated sodium hydrogen carbonate solution, twice with cold water, and dried (sodium sulfate). After evaporation, the remaining pyridine was removed by repeated additions of toluene, followed by evaporation. The residual syrup was dried under high vacuum for 1 h at 50° and dissolved in hot methanol. The cooled solution was seeded and gradually a granular precipitate was obtained (121 g, 77%), m.p. 106–115°. A sample was recrystallized from ethanol, m.p. 108–115° to a glass which gradually liquified at ~130°; n.m.r. datum (chloroform-*d*): δ 2.77 (s, S-Me).

This product was contaminated with some triphenylmethanol and was used directly in the next step.

*Methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-6-*O*-trityl-α-*D*-glucopyranoside* (**8**). — A suspension of **7** (25 g, 35 mmoles) and sodium azide (11 g, 0.17 mole) in hexamethylphosphoramide (100 ml, dried over Linde sieves No. 13X) was stirred magnetically and heated for 20 h at 80°. The reaction mixture was poured into well-stirred cold water. The precipitate was removed by filtration, triturated thoroughly with water to remove the hexamethylphosphoramide, and dried in open air. (The undried crude material could be used directly in the acetolysis reaction after dissolution in dichloromethane, drying the solution with sodium sulfate, and evaporation to give a syrup). Crystallization from hot ethanol containing about 1% ethyl acetate gave 13.8 g. An additional 5.6 g (overall yield 83%) was obtained by concentration of the mother liquor. An aliquot was recrystallized from ethanol, m.p. 170–171° with slight sintering at 166°, $[\alpha]_D^{24}$ +117° (*c* 1.68, chloroform); i.r. datum: λ_{max}^{KBr} 4.7 μm (N_3); lit.[3]: m.p. 164–165°, 160–162°, $[\alpha]_D^{26}$ +114°; n.m.r. datum (chloroform-*d*): complete disappearance of the peak at δ 2.77 (S-Me).

Anal. Calc. for $C_{40}H_{35}N_3O_7$: C, 71.74; H, 5.28; N, 6.27. Found: C, 71.77; H, 5.18; N, 6.07.

*1,6-Di-*O*-acetyl-4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-*D*-glucopyranose* (**9**). — To a vigorously stirred solution of **8** (23.4 g, 35 mmoles) in glacial acetic acid (100 ml) was added acetic anhydride (50 ml), followed by sulfuric acid (5 ml), and the reaction mixture was stirred for 2 days at room temperature. Progress of the acetolysis reaction was followed by t.l.c. (15:1 dichloromethane–methanol). When the starting material persisted, the reaction mixture was heated for 1–2 h at 60°. After completion of the reaction, the mixture was partitioned between dichloromethane and ice–water. The organic layer was washed twice with equal volumes of a saturated solution of sodium hydrogen carbonate, once with water, and dried (sodium sulfate). After removal of the drying agent, the filtrate was evaporated to give a syrup. The acetic acid was removed by addition of benzene followed by evaporation. Methanol was added and the resulting precipitate was filtered off and dried (11.5 g, 66%). An aliquot of the crude solid, recrystallized from methanol, yielded needles, m.p. 152–153°, $[\alpha]_D^{24}$ +175° (*c* 2.11, chloroform); n.m.r. data (chloroform-*d*): disappearance of the signals at

δ 3.37 (OMe) and 7.27–7.68 (trityl) and appearance of a new signal at δ 2.13 (6 H, OAc at C-1 and C-6). The α-configuration was demonstrated by the low-field doublet (δ 6.51, $J_{1,2}$ 4.0 Hz).

Anal. Calc. for $C_{24}H_{23}N_3O_9$: C, 57.95; H, 4.66; N, 8.45. Found: C, 58.28; H, 4.69; N, 8.40.

1-(6-O-Acetyl-4-azido-2,3-di-O-benzoyl-4-deoxy-β-D-glucopyranosyl)-N⁴-acetylcytosine (**10**, $R = Ac$). — Bis(trimethylsilyl)-N^4-acetylcytosine was prepared in benzene solution essentially as previously described[8]. The crude, oily product was not distilled but was dissolved in a known volume of dry 1,2-dichloroethane and stored in the refrigerator. This stock solution was assayed by introducing 1 ml into 50% ethanol and stirring for 10 min. The recovered *N*-acetylcytosine was filtered off, dried, and weighed. The amount of silylated base calculated to be in the stock solution averaged 80–90%.

To a magnetically-stirred solution of **9** (2.82 g, 5.7 mmoles) in sieve-dried 1,2-dichloroethane (240 ml) was added tin tetrachloride (2 ml, 17 mmoles), and the mixture was stirred in a tightly-stoppered flask for 10 min. The amount of bis(trimethylsilyl)-N^4-acetylcytosine stock solution calculated to contain 1.5 g (5 mmoles) was added rapidly, and the reaction mixture was stirred overnight at room temperature and then was placed for 1 h in a bath at 50°. The dark solution was diluted with an equal volume of dichloromethane and added slowly to a stirred, matching volume of saturated sodium hydrogen carbonate. When the frothing ceased, the suspension was filtered through a Celite pad which was washed thoroughly with dichloromethane. The filtrate was washed once with water and was dried (sodium sulfate). After removal of the drying agent, the filtrate was evaporated to give a glass which on trituration with acetone gave a solid (2.53 g, 86%), m.p. 243–245° (eff). An aliquot, recrystallized from ethanol–ethyl acetate, yielded rosettes of needles, m.p. 247–249° (eff); u.v. data: λ_{max}^{EtOH} 212, 233, s 255, 282.5, and 298 nm (ε_{max} 23,720; 32,590; 17,570; 6,680; and 6,810, respectively); λ_{min}^{EtOH} 218, 278, and 287.5 nm (ε_{min} 22,700; 6,320; and 6,240, respectively).

Anal. Calc. for $C_{28}H_{26}N_6O_9$: C, 56.95; H, 4.44; N, 14.23. Found: C, 57.22; H, 4.47; N, 14.18.

1-(6-O-Acetyl-4-azido-2,3-di-O-benzoyl-4-deoxy-β-D-glucopyranosyl)-N⁴-benzoylcytosine (**10**, $R = Bz$). — Bis(trimethylsilyl)-N^4-benzoylcytosine was prepared by treatment of *N*-benzoylcytosine (9.4 g, 0.08 mole) with trimethylsilyl chloride (22.7 g, 0.21 mole) and triethylamine (28 ml, 0.204 mole) in benzene; a stock solution containing 96% of the theoretical quantity of the silylated base was obtained.

Condensation of **9** with bis(trimethylsilyl)-N^4-benzoylcytosine was carried out as described in the preceding section. Recrystallization of the crude product from ethanol gave micaceous platelets (76%), sintering at *ca.* 160° and slowly liquifying into a viscous glass at *ca.* 190°; the n.m.r. spectrum (chloroform-*d*) showed this product to be a monoalcoholate. A solvent-free analytical sample was obtained by drying in a high vacuum for 3 h at 100°. The melting point of the dried material was unchanged. U.v. data: λ_{max}^{EtOH} 232, 262, and 303–305 nm (ε_{max} 34,300; 28,690; and 9,420,

respectively); λ_{min}^{EtOH} 213, 247, and 292.5 nm (ε_{min} 22,390; 21,500; and 8,960, respectively).

Anal. Calc. for $C_{33}H_{28}N_6O_9$: C, 60.73; H, 4.32; N, 12.88. Found: C, 60.71; H, 4.29; N, 12.71.

1-(4-Azido-2,3-di-O-benzoyl-4-deoxy-β-D-glucopyranosyl)cytosine (**11**). — A suspension of **10** (24.3 g, 40 mmoles) in methanol (2400 ml) containing triethylamine (11 ml, 80 mmoles) was stirred overnight at room temperature. The clear solution was evaporated, and the residue triturated with ether and filtered off. The glassy product, dried for 1 h at 100° in a high vacuum, gave 17 g (88%) as the hemihydrate (n.m.r. in dimethyl sulfoxide-d_6). An aliquot, recrystallized from acetone, yielded needles, browning at 211°, liquifying at 200–225°, and effervescing at 226°; u.v. data: λ_{max}^{EtOH} 232 and 265–270 nm (ε_{max} 35,960 and 11,270, respectively); λ_{min}^{EtOH} 213 and 260 nm (ε_{min} 17,310 and 11,000, respectively).

Anal. Calc. for $C_{24}H_{22}N_6O_7 \cdot 0.5\,H_2O$: C, 55.92; H, 4.50; N, 16.30. Found: C, 56.03; H, 4.37; N, 16.70.

1-(4-Azido-2,3-di-O-benzoyl-4-deoxy-β-D-glucopyranosyl)-N⁴-benzoylcytosine (**12**). — A solution of **11** (6.5 g, 12.8 mmoles) and benzoic anhydride (6.5 g) in ethanol (650 ml) was heated at reflux for 5 h with additional amounts (6.5 g) of benzoic anhydride being introduced after every hour until a total of 26 g was used. The filtrate was evaporated to one quarter of its volume, and the cooled solution was poured into 2 l of petroleum ether (30–60°) and stirred to complete precipitation. The solid was filtered off and triturated with dry ether to afford 6.77 g (86%) of **12**. An aliquot, recrystallized from ethanol, yielded needles, browning at 228–230°, sintering at 230–232°, and effervescing at 234°; [lit.[1]: m.p. 229–230° with effervescence]. T.l.c. (50:1 chloroform–methanol) demonstrated the identity of this material with an authentic sample prepared by a different route[1].

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