Direct Glycosylation of 1,3,5-Triazinones. A New Approach to the Synthesis of the Nucleoside Antibiotic 5-Azacytidine (4-Amino-1-β-D-ribofuranosyl-1,3,5-triazin-2-one) and Related Derivatives[1]

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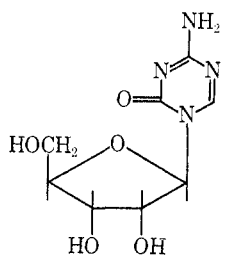
The first instance of direct glycosylation of the 1,3,5-triazine ring has been described. The synthesis of the nucleoside antibiotic 5-azacytidine (4-amino-1-β-D-ribofuranosyl-1,3,5-triazin-2-one, I) has been achieved in 34% yield by treatment of the trimethylsilyl derivative of 4-amino-1,3,5-triazin-2-one (5-azacytosine) with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide in acetonitrile, followed by deblocking with methanolic ammonia. Similar treatment of the trimethylsilyl derivative of 5-azacytosine with 3,5-di-*O*-acetyl-2-deoxyribofuranosyl chloride resulted in the α and β anomers of 2′-deoxy-5-azacytidine, which were clearly distinguished by pmr. In a similar manner, 1-(β-D-ribofuranosyl)cyanuric acid (V) and 1-β-D-ribofuranosyl-3-methylcyanuric acid (VI) were prepared from cyanuric acid and 1-methylcyanuric acid, respectively. Attempts to prepare 4-amino-1-β-D-arabinofuranosyl-1,3,5-triazin-2-one (5-azaarabinofuranosylcytosine) were unsuccessful because 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-1,3,5-triazin-2-one (VII) could not be deblocked without concomitant destruction of the triazine ring. The nucleoside derivatives of the 1,3,5-triazine ring present some interesting nucleosides for future biochemical and biophysical studies.

5-Azacytidine (4-amino-1-β-D-ribofuranosyl-1,3,5-triazin-2-one, I) has been isolated from *Streptoverticillium ladakanus*.[2,3] This antibiotic inhibits gram-negative bacteria and is active against T-4 lymphoma and L-1210 leukemia in mice.[2] The identity of I was



I

established[3] by comparison with authentic 5-azacytidine prepared by a lengthy procedure involving ring closure of 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4-methylisobiuret.[4] The remarkable biological activity of I against experimental leukemia[5-9] has resulted in the selection of 5-azacytidine for clinical trial against leukemia in human subjects.[8,9] 5-Azacytidine inhibits protein synthesis[10] and is incorporated into both RNA and DNA.[11]

Recent studies in this laboratory utilizing various trimethylsilyl pyrimidines in a direct glycosylation procedure has succeeded where other methods have failed.[12-14] The application of this study to the s-triazine ring has now resulted in the direct attachment of the D-ribofuranose moiety to the 1,3,5-triazine ring system. 5-Azacytosine[15] was treated with hexamethyldisilazane in a manner similar to that previously employed for 4-amino-6-pyrimidone.[13] The resulting trimethylsilyl derivative (II) was dissolved in acetonitrile and treated with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide.[16] The crude blocked nucleoside was isolated and treated with methanolic ammonia to give crude I, yield 34%. Recrystallization gave 5-azacytidine, yield 11%, mp 231–233° dec. Rigorous comparison of this product with 5-azacytidine isolated from cultures of *S. ladakanus* proved the samples to be identical.

Utilization of this procedure for the synthesis of 5-aza-2′-deoxycytidine (III) was also successful. Syrupy 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose[17] was converted into 3,5-di-*O*-acetyl-2-deoxy-D-ribofuranosyl chloride and allowed to react with an excess of the trimethylsilyl derivative of 5-azacytosine in acetonitrile. After 7 days at room temperature the reaction mixture was treated as for the preparation of I and the product was purified *via* column chromatography on silica gel to give a mixture of anomers of 1-(3,5-di-*O*-acetyl-2-deoxy-D-ribofuranosyl)-5-azacytosine. This mixture was treated with ethanolic ammonia to remove the acetyl groups. The resulting α and β anomers were separated by a combination of fractional crystallization and preparative layer chromatography on silica gel to give pure 1-(2-deoxy-α-D-ribofuranosyl)-5-azacytosine (IV) and 2′-deoxy-5-azacytidine [4-amino-1-(2-deoxy-β-D-ribofuranosyl)-1,3,5-triazin-2-one, III]. Assignment of the β configuration to III was made by comparison of the pmr signals observed for the anomeric protons[17] of III and the corresponding α anomer, IV. It should be noted that a lengthy synthesis of 5-aza-2′-deoxycytidine has been reported[18] in a preliminary communication *via* 1-(3,5-di-*O*-*p*-toluyl-2-deoxy-D-ribofuranosyl)-4-methylisobiuret. However, no yield was

(1) Supported by Research Grants CA 08109-02 and CA 08109-03 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

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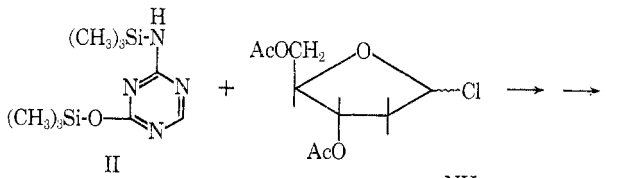
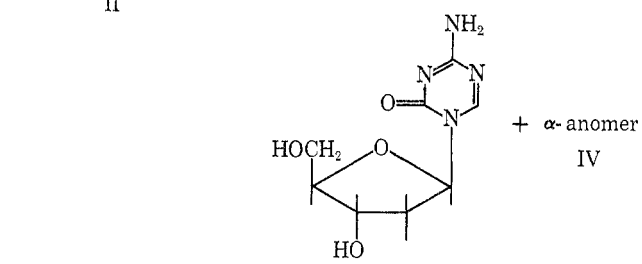
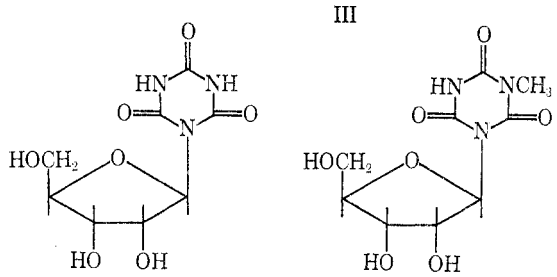
given and the authors did not distinguish between the possible two anomers, III and IV.



II

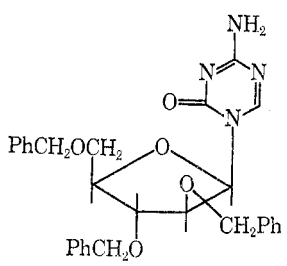


III + α-anomer IV



V

VI



VII

In an attempt to prepare 1-β-D-arabinofuranosyl-5-azacytosine, 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride[19] and the trimethylsilyl derivative of 5-azacytosine were dissolved in dichloromethane and kept at room temperature for 11 days to give a 47% yield of 1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-5-azacytosine (VII). Catalytic debenzylation of VII with palladium and hydrogen resulted in destruction of the triazine ring. A fusion reaction of 2,3,5-tri-*O*-acetyl-D-arabinofuranosyl chloride with the trimethylsilyl derivative of 5-azacytosine gave only the α anomer, 1-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-5-azacytosine.

Treatment of 2,4,6-tris(trimethylsilyloxy)-1,3,5-triazine, prepared from cyanuric acid, with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide gave a 43% yield, after deblocking of 1-(β-D-ribofuranosyl)cyanuric acid (V). This molecule is of particular interest since it is symmetrical. There is essentially no *anti* form to this nucleoside. The structural resemblance to uridine is, however, indeed striking. Such a nucleoside should be of considerable theoretical interest to both biochemists and biophysical chemists, since it has been postulated that certain enzymes *prefer* either the *syn* or *anti* conformation of pyrimidine nucleosides.[20] Further studies on this nucleoside are in progress in our laboratories. For comparative purposes, the compound 1-(β-D-ribofuranosyl)-3-methylcyanuric acid (VI) was similarly prepared from 1-methylcyanuric acid[21] in 39% yield.

It would appear that the silylation procedure of nucleoside synthesis is generally applicable even to ring systems such as 1,3,5-triazine, which have not previously been alkylated by other methods of nucleoside synthesis.

Experimental Section

Melting points were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected. Specific rotations were measured in a 1-dm tube with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Proton magnetic resonance (pmr) spectra were measured with appropriate internal standards of tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate with a Varian Model A-60 nmr spectrometer. Ultraviolet spectra were determined with a Beckman Model DK-2 spectrometer. Infrared spectra were determined with a Beckman Model IR-5 spectrophotometer. Detection of components on SilicAR 7 GF (Mallinckrodt) and alumina HF 254 (Brinkmann) was by ultraviolet light. Alumina used in columns was obtained from Merck & Co. (suitable for chromatographic absorption). Silica gel was purchased from J. T. Baker Chemical Co. (suitable for chromatographic use). Solvent proportions were by volume. Evaporations were performed under diminished pressure at 35° with a Buchi Rotovapor.

Trimethylsilyl derivatives of various *s*-triazines were prepared using the general procedure of Wittenburg.[17] The *s*-triazines were heated under reflux in an excess of hexamethyldisilazane with a catalytic quantity of ammonium sulfate under anhydrous conditions until complete solution was achieved. The excess hexamethyldisilazane was removed by distillation under diminished pressure and the residue (oil or crystalline solid) was used directly without further purification.

5-Azacytidine (I).—To the trimethylsilyl derivative of 5-azacytosine (prepared from 10 g of 5-azacytosine[15]) was added 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide (prepared from 20 g of tetra-*O*-acetyl-D-ribofuranose[16]) in dry acetonitrile (180 ml). After initial stirring, the solution was left at room temperature for 3 days. The solution was evaporated to a syrup. Sodium bicarbonate, water, and ethanol were added. The mixture was evaporated to dryness. Coevaporation with absolute ethanol removed the last traces of water. The residue was extracted with chloroform (Celite used) and the extract was evaporated to dryness. The residue was extracted once more with chloroform and the solvent was removed to give 24.5 g of a foam. To this material was added methanolic ammonia solution (150 ml of methanol saturated at 0° with ammonia). The vessel was sealed and the solution was left at room temperature for 2 hr and then at 5° overnight. The mixture was evaporated to near dryness. To the residue was added methanol and the solid was collected, yield 5.2 g (34%), mp 192–209°. This material was dissolved in warm water and the solution was decolorized with charcoal. Evaporation gave crystals of 5-azacytidine, yield 1.75 g (11%), mp 231–233°. Recrystallization from aqueous ethanol (charcoal) gave pure 5-azacytidine (I), mp 235–237° dec, $[\alpha]^{26}D$ +22.4° (*c* 1.00, water).

Anal. Calcd for $C_8H_{12}N_4O_5$: C, 39.34; H, 4.95; N, 22.94. Found: C, 39.14; H, 4.99; N, 22.91.

A mixture melting point with authentic material[3] showed no depression. The $[\alpha]^{26}D$ (*c* 1, water) value recorded by us for authentic material was +26.6°.[4] The ir, uv, and pmr spectra were identical with those of authentic material.[2,3] The product was shown to be homogeneous by tlc on SilicAR 7GF with ethyl acetate–methanol (4:1) as solvent and it had the same R_f as a marker of authentic material.[3]

1-(3,5-Di-*O*-acetyl-2-deoxy-α,β-D-ribofuranosyl)-5-azacytosine.—Syrupy 2-deoxy-1,5-tri-*O*-acetyl-D-ribofuranose[17] (21 g) was dissolved in dry ether (400 ml) containing acetyl chloride (30 ml) and the solution was saturated with hydrogen chloride at 0° for 1 hr. The sealed solution was left at 0° for 1 day. The solution was evaporated to a syrup which was coevaporated with toluene

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to give a dark-colored, crude glycosyl halide. This product was dissolved in acetonitrile (200 ml) and transferred to the trimethylsilyl derivative of 5-azacytosine (from 15 g of 5-azacytosine). The sealed mixture was stirred at room temperature for 4 days. The mixture was evaporated to a syrup and sodium bicarbonate and ethanol were added. The mixture was evaporated to dryness and the residue was extracted with chloroform. The extract was evaporated to *ca.* 200 ml and applied to a column (40 × 5.0 cm) of silica gel prepacked in chloroform. The column was eluted with chloroform and 200-ml fractions were collected. At fraction 10 the solvent was changed to chloroform–ethyl acetate (9:1), at fraction 20 to ethyl acetate, and at fraction 31 to ethyl acetate–methanol (19:1). Fractions 33–39 were pooled and evaporated to a semicrystalline material. This mixture was extracted with chloroform and the extract was applied to a column (37 × 3.2 cm) of silica gel prepacked in chloroform. Elution was started with chloroform and 200-ml fractions were collected. The solvent was changed to chloroform–ethyl acetate (4:1) at fraction 8 and to ethyl acetate at fraction 12. Fractions 14–25 were evaporated to a small volume, whereupon crystallization occurred. Ether was added to give 2.42 g of white crystals, mp 162–165°.

Anal. Calcd for $C_{12}H_{16}N_4O_6$: C, 46.15; H, 5.16; N, 17.94. Found: C, 45.90; H, 5.07; N, 17.85.

This product, when examined by tlc on silicAR 7GF with acetone as developer, showed two very closely moving components (typical of anomers): a major, slower moving component and a minor, faster moving component. Attempts at fractional crystallization failed. The mixture exhibited the following spectral data: pmr (DMSO-d_6) δ 1.99 (s) and 2.11 (s, 6, OAc), 2.20–3.10 (m, 2, 2′ H), 4.07–4.36 ("s" at 4.15, "s" at 4.22, and "s" at 4.31, 2, 5′ CH_2OAc), 4.70–5.00 (m, 1, 4′ H), 5.08–5.42 (m, 1, 3′ H), 6.12 (rough q, 1, 1′ H), 7.55 (s) and 7.63 (s, 2, 4 NH_2), and 8.40 (s, 1, 6 H); λ_{max}^{KBr} 1740 cm^{-1} (OAc).

2′-Deoxy-5-azacytidine (III) and the α Anomer (IV).—To a solution of ammonia-saturated (at 0°) ethanol (200 ml) was added 2.76 g of the mixture of 1-(3,5-di-*O*-acetyl-2-deoxy-α,β-D-ribofuranosyl)-5-azacytosine, and the sealed mixture was stirred at 5° for 2 hr to achieve solution. The solution was maintained at −15° for 5 days and then evaporated at 25° to a syrup which was heated at 60° under oil pump vacuum to remove acetamide. A 100-mg portion of the residue (A) was applied to the short edge of a silicAR 7GF plate (2 × 200 × 400 mm) and the plate was developed several times with ethyl acetate–methanol (4:1). Two closely moving zones were observed, one major (α anomer), slower moving zone and a minor, very slightly faster moving (β anomer) zone. Extraction of the smaller zone with methanol and solvent removal gave a minute quantity of crystalline residue (crude β anomer). The remaining crude syrupy mixture of anomers (A) was dissolved in warm methanol and seeded with this material to give white needles of III, yield 0.10 g, mp 189–191°. The mother liquor and washings (ethanol and ether) deposited white prisms (1.05 g) of α anomer contaminated with a faint trace of β anomer. Recrystallization of the β anomer from methanol–2-propanol gave pure product, 2′-deoxy-5-azacytidine (III): mp 191–193°; $[\alpha]^{28}D$ +63.8° (*c* 1.00, water); λ_{max}^{KBr} 1600–1710 cm^{-1} [5-azacytosine absorptions, in the region 1200–4000 cm^{-1} the spectrum was very similar to that of 5-azacytidine (I)]; $\lambda_{max}^{pH\ 1}$ 253 mμ, $\lambda_{max}^{pH\ 4}$ 239 (ε 8200), and $\lambda_{max}^{pH\ 11}$ 253 sh (2300); pmr (D_2O) δ 2.36–2.67 (m, 2, 2′ H), 3.80–3.94 ("d" centered at 3.80 "*J*" = 2.0 cps, "s" at 3.88, 2,5′ CH_2OH), 6.29 (t, 1, *W* = 13.0 cps, $J_{1',2'}$ = 6.5 cps, 1′ H), and 8.58 (s, 1, 6 H).

Anal. Calcd for $C_8H_{12}N_4O_4$: C, 42.10; H, 5.30; N, 24.55. Found: C, 41.81; H, 5.15; N, 24.52.

Recrystallization of the crude α anomer (IV) from methanol–2-propanol gave 0.81 g of pure product: mp 177–179°; $[\alpha]^{29}D$ −40.8° (*c* 1.0, water); ν_{max}^{KBr} 1600–1660 cm^{-1} (5-azacytosine absorptions); $\lambda_{max}^{pH\ 1}$ 253 mμ, $\lambda_{max}^{pH\ 4}$ 239 (ε 8200), and $\lambda_{max}^{pH\ 11}$ 253 sh (2700); pmr (D_2O) δ 2.00–3.12 (m, 2, 2′H), 3.58–3.81 ("s" centered at 3.69 and "s" centered at 3.75, 2, 5 CH_2OH), 4.30–4.65 (m, 2, 3′, and 4′ H), 4.86 (solvent), 6.16 (q, 1, *W* = 9.0 cps, "*J*" = 2.0, 7.0 cps, 1′ H), and 8.48 (s, 1, 6 H).

Anal. Calcd for $C_8H_{12}N_4O_4$: C, 42.10; H, 5.30; N, 24.55. Found: C, 41.86; H, 5.15; N, 24.55.

The various mother liquors were evaporated and applied (*ca.* 100 mg/plate) to the short edge of silicAR 7GF plates (2 × 200 × 400 mm). The plates were developed several times with ethyl acetate–methanol (4:1) until the zones corresponding to the anomers were separated. The zones were excised and extracted with methanol. Solvent removal and crystallization from methanol–ethanol gave an additional 0.12 g of β anomer (III), mp 191–193°, and 0.40 g of α anomer (IV), mp 177–179°.

In a subsequent experiment the procedure was modified as follows. To a solution of ammonia-saturated (at 0°) ethanol (130 ml) was added 1.70 g of 1-(3,5-di-*O*-acetyl-2-deoxy-α,β-D-ribofuranosyl)-5-azacytosine and the sealed mixture was stirred at 5° for 7 hr. The solution was stored at −15° for a further 6 days. The solution was evaporated below 25° to a syrup. This material was dissolved in methanol and the solution was decolorized with charcoal. The solution was evaporated to smaller volume and then coevaporated with ethanol to give 0.77 g of white crystals, mp 175–177° (A), containing largely α anomer. The mother liquor, richer in the β anomer, was evaporated and applied (*ca.* 100 mg/plate) to the short edges of 5 silicAR 7GF plates (2 × 200 × 400 mm). The plates were developed several times with ethyl acetate–methanol (4:1) and the faster moving of the two barely separated zones was excised and extracted with methanol. Solvent removal and crystallization of the residue from methanol–ethanol gave 56.7 mg (4%) of 2′-deoxy-5-azacytidine (III), mp 193–194°. The crystalline material (A) was also subjected to a similar separation to give 33.6 mg (3%) of β anomer (III), mp 191–192°. The slower moving zone was treated similarly to give a total yield of 0.65 g (52%) of α anomer (IV), mp 181–182°.

1-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-5-azacytosine (VII).—To the trimethylsilyl derivative of 5-azacytosine (prepared from 7.5 g of 5-azacytosine) was added 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride [prepared from 15.0 g of 2,3,5-tri-*O*-benzyl-1-*p*-nitrobenzoyl-D-arabinofuranose[19] in dry dichloromethane (125 ml)] and the resulting solution was protected from moisture and left at room temperature for 11 days. The solution was evaporated to dryness and the residue was treated with sodium bicarbonate, water, and ethanol. The mixture was evaporated to dryness and the residue was coevaporated with ethanol. The residue was extracted with chloroform and the chloroform extract was evaporated to dryness. The residue was extracted once more with chloroform and the extract was applied to a column (40 × 3.3 cm) of silica gel prepacked in chloroform. Fractions (200 ml each) were collected and the fractionation was monitored by tlc on SilicAR 7GF with chloroform–ethyl acetate (4:1) as developer. Elution was started with chloroform. At fraction 39 the eluting solvent was changed to chloroform–ethyl acetate (9:1). Fractions 8–43, which contained a single nucleosidic component, were evaporated to dryness. The residue was crystallized from ethanol–ether to yield 6.39 g (47%) of white crystals, mp 141–143°. Recrystallization from ethanol gave pure VII: mp 142–143°; pmr ($CDCl_3$) δ 3.60 (d, 2, "*J*" = 5.5 cps, 5′ CH_2OH), 3.93–4.32 (m, 3, 2′, 3′, and 4′ H), 4.38 (s, 2, $PhCH_2$), 4.48 (s, 2, $PhCH_2$), 4.51 (s, 2, $PhCH_2$), 6.31 (d, 1, *J* = 4.0 cps, 1′ H), 7.06–7.47 (m, 15, PhH), 7.69 (broad s, 2, 4 NH_2), and 8.26 (s, 1, 6 H).

Anal. Calcd for $C_{29}H_{30}N_4O_5$: C, 67.69; H, 5.88; N, 10.89. Found: C, 67.60; H, 5.82; N, 11.10.

1-(β-D-Ribofuranosyl)cyanuric Acid (V).—To the trimethylsilyl derivative of cyanuric acid (from 15 g of cyanuric acid) was added 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (from 40 g of 1-*O*-acetyl-2-3,5-tri-*O*-benzoyl-β-D-ribofuranose) in dry acetonitrile (250 ml). After sealing and initial stirring, the solution was left at room temperature for 8 days. The solvent was evaporated to a syrup and absolute ethanol was added to the residue. The mixture was evaporated to dryness and extracted (Celite filtration) with chloroform. The solvent was removed and the residue, redissolved in chloroform, was applied to a column (48.5 × 5.0 cm) of silica gel prepacked in chloroform. Fractions (200 ml each) were collected and the fractionation was monitored by tlc on silicAR 7 GF with ethyl acetate–chloroform (3:7) as developer. At fraction 16 the solvent was changed to chloroform–ethyl acetate (9:1) and at fraction 25 to chloroform–ethyl acetate (4:1). Fractions 14–34 were pooled and evaporated, yielding 22.60 g of a dry syrup. A portion (16.0 g) was dissolved in methanol (250 ml) saturated at (0°) with ammonia and left in a pressure vessel for 4 days. The solution was filtered and the filtrate was evaporated to smaller volume, whereupon crystallization of the product occurred. The mixture was coevaporated with absolute ethanol to yield 6.25 g (43%) of V, mp 222–223° dec. Recrystallization from water–ethanol gave pure material: mp 229–230° dec; $[\alpha]^{26}D$ 24.3° (*c* 1, water); ν_{max}^{KBr} 1680 and 1770 cm^{-1} (C=O of heterocycle); pmr (D_2O) δ 3.57–4.23 (m, 3, 4′ H and 5′ CH_2OH, 5 CH_2OH as "s" at 3.85), 4.38 (t, 1, *W*

= 12.0 cps, $J_{3',2'}$ = 6.0 cps, 3' H), 4.55–4.85 (m, solvent and 2' H), and 6.22 (d, 1, $J_{1',2'}$ = 3.5 cps, 1' H).

Anal. Calcd for $C_8H_{11}N_3O_7$: C, 36.79; H, 4.25; N, 16.09. Found: C, 36.48; H, 3.92; N, 15.85.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)cyanuric Acid.—The remaining portion of the crude benzoate (above, 6.60 g) was crystallized from chloroform–ethyl acetate–heptane to yield 4.44 g of white crystals, mp 211–21°. The product was dissolved in a mixture of methanol and ethyl acetate and the solution was decolorized. The solution was evaporated to small volume and heptane was added. Pure product was deposited as white needles: mp 211–213°; ν_{max}^{KBr} 1655 and 1770 cm^{-1} (C=O of heterocycle, and benzoate); pmr (DMSO-d_6) δ 4.50–5.00 [m, 3, 4' H overlapping 5' CH_2OH (s) centered at 4.75], 6.13–6.47 (m, 2, 2' and 3' H), 6.57 (s, 1, $J_{1',2'}$ < 1 cps, 1' H), 7.22–8.26 (m, 15, benzoate), and 11.94 (s, 2, NH).

Anal. Calcd for $C_{29}H_{23}N_3O_{10}$: C, 60.73; H, 4.04; N, 7.33. Found: C, 60.91; H, 4.18; N, 7.18.

1-(β-b-Ribofuranosyl)-3-methylcyanuric Acid (VI).—To the trimethylsilyl derivative of 1-methylcyanuric acid (prepared from 5 g of 1-methylcyanuric acid[21]) was added 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (prepared from 10 g of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose) in dry acetonitrile (150 ml). The sealed mixture was stirred initially and then left at room temperature for 2 weeks. The solution was evaporated to a syrup and the syrup was treated with absolute ethanol (50 ml). The mixture was evaporated to dryness and the residue was extracted with chloroform. Evaporation provided a syrup which was redissolved in chloroform, and the solution was applied to a column (40 × 3.3 cm) of silica gel prepacked in chloroform. The fractionation was monitored by tlc on SilicAR 7 GF with chloroform–ethyl acetate (7:3) as developer. Fractions of 200 ml each were collected up to fraction 4. Fractions 5 and 6 were of 100-ml volume. Fractions 7–12 were again of 200-ml volume. Fractions 6–12 were pooled and evaporated to give 8.37 g of a white, homogeneous foam: pmr ($CDCl_3$) δ 3.23 (s, 3, N_3CH_3), 4.50–4.88 (m, 3, 4' H and 5' CH_2OH), 6.05–6.30 (m, 2, 2' and 3' H), 6.48 (s, 1, $J_{1',2'}$ < 1 cps, 1' H), 7.10–7.60 (m, 9, benzoate), and 7.77 (m, 6, benzoate). This material was dissolved in ammonia-saturated (at 0°) methanol (100 ml) and left at room temperature for 4 days in a sealed vessel. The solution was filtered through Celite and the filtrate was evaporated to a syrup. This syrup was dissolved in a mixture of chloroform and water. The aqueous solution was further extracted three times with chloroform and then evaporated to dryness. The residue was coevaporated with absolute ethanol and the residue was stirred in ether (200 ml) for several days. The resulting white powder (3.40 g) was collected and crystallized from methanol–2-propanol, yield 2.05 g (39%), mp 144–146°. This material was dissolved in methanol and the solution was decolorized with activated carbon. After solvent removal the syrup was crystallized from methanol–2-propanol: mp 144–146°; $[\alpha]^{31}D$ −21.5° (*c* 1, water); ν_{max}^{KBr} 1680 and 1720 cm^{-1} (C=O of heterocycle); pmr (D_2O) δ 3.26 (s, 3, N_3 CH_3), 3.80–4.15 [m, 3, 5' CH_2OH (s) at 3.83 overlapped by 4' H], 4.40 (t, 1, $J_{3',2'}$ = 12.3 cps, 3' H), 4.53–5.00 (m, 2' H and solvent), and 6.11 (d, 1, $J_{1',2'}$ = 3.5 cps, 1' H).

Anal. Calcd for $C_9H_{13}N_3O_7$: C, 39.27; H, 4.76; N, 15.27. Found: C, 39.19; H, 4.82; N, 15.39.

1-(2,3,5-Tri-*O*-acetyl-α-D-arabinofuranosyl)-5-azacytosine.—To a solution of sodium (0.5 g) in anhydrous methanol (500 ml) was added methyl 2,3,5-tri-*O*-benzoyl-D-arabinofuranoside[22] (84 g) and the solution was heated under reflux for 45 min. To the stirred cooled solution was added portionwise Dowex 50 (H^+, X4, 200–400 mesh) until the solution was neutral. The resin was filtered off and washed with methanol. The filtrate and washings were evaporated to a syrup. The syrup was dissolved in chloroform and extracted with chloroform several times. The aqueous layer was evaporated to a syrup and the syrup was dried by coevaporation with ethanol and then with dry pyridine. The dry syrup was treated overnight with acetic anhydride (200 ml)–pyridine (200 ml). The solution was poured onto ice and the mixture was extracted with chloroform. The chloroform extract was washed consecutively with water, ice-cold 2 *N* hydrochloric acid, water-saturated sodium bicarbonate solution, and water. The dried ($MgSO_4$) solution was evaporated to give syrupy methyl 2,3,5-tri-*O*-acetyl-D-arabinofuranoside.

(22) H. G. Fletcher, Jr., in "Methods in Carbohydrate Chemistry," Vol. 2, M. L. Wolfrom and R. L. Whistler, Ed., Academic Press Inc., New York, N. Y., 1963, p 228.

This syrup was dissolved in a mixture of acetic anhydride (150 ml) and acetic acid (550 ml). Concentrated sulfuric acid (35 ml) was added dropwise to the ice-cold solution and the solution was left at room temperature overnight. The solution was poured onto ice and the mixture was extracted with chloroform. The chloroform extract was stirred with excess saturated sodium bicarbonate overnight at 5°. The extract was washed with water and dried ($MgSO_4$). Solvent removal afforded 64 g of 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose as an oil.

Dry hydrogen chloride gas was bubbled through an ice-cold solution of 53 g of the above syrup in ether (1 l.) containing acetyl chloride (100 ml) until the solution was saturated (*ca.* 1 hr). The solution was sealed and maintained at 0° for 6 days. The solution was evaporated and the residue was coevaporated with toluene.

This syrup was dissolved in toluene (150–200 ml) and transferred to the trimethylsilyl derivative of 5-azacytosine (prepared from 25 g of 5-azacytosine). An aspirator vacuum was applied to the magnetically stirred solution and the temperature was quickly raised to 195° using an oil bath. The temperature was maintained at 195° for 25 min. Ethanol and sodium bicarbonate were added to the residue. The mixture was evaporated to dryness and the residue was extracted with chloroform (Celite). The chloroform extract was evaporated to smaller volume and applied to a column (69 × 4.0 m) of silica gel prepacked in chloroform. Fractions (200 ml each) were collected and the fractionation was monitored by tlc on silicAR 7 GF with ethyl acetate–methanol (9:1) as developer. At fraction 31 the eluting solvent was changed to chloroform–ethyl acetate (9:1), at fraction 41 to chloroform–ethyl acetate (7:3), at fraction 46 to ethyl acetate, and at fraction 64 to ethyl acetate–methanol (19:1). Fractions 44–74 were collected and evaporated to a syrup which was crystallized from ethyl acetate–ether, yield 2.77 g, mp 165–168°. The mother liquor was evaporated and the residue was dissolved in chloroform. Silica gel was added and the mixture was evaporated to give a free-running powder. This material was added to a dry column of silica gel (43.5 × 4.0 cm) so that the total column size was 66.0 × 4.0 cm. Elution was started with chloroform and 200-ml fractions were collected. At fraction 5 the solvent was changed to chloroform–ethyl acetate (9:1), at fraction 9 to chloroform–ethyl acetate (7:3), at fraction 13 to ethyl acetate (1:1), at fraction 21 to ethyl acetate–chloroform (7:3), at fraction 35 to ethyl acetate, and at fraction 60 to ethyl acetate–methanol (98:2). Fractions 36–66 were evaporated to a syrup which was crystallized as above to give 1.80 g, mp 163–165°.

The mother liquor was evaporated to a syrup and dissolved in chloroform. Silica gel was added and the mixture was evaporated to give a free-running powder. This material was added to a dry column (25 × 3.3 cm) of silica gel so that the total column size was 44.0 × 3.3 cm. The elution was started with chloroform and 200-ml fractions were collected. At fraction 6 the solvent was changed to chloroform–ethyl acetate (9:1), at fraction 11 to chloroform–ethyl acetate (7:3), at fraction 15 to chloroform–ethyl acetate (3:7), and at fraction 26 to ethyl acetate. Fractions 22–30 were evaporated and crystallized as above to give 0.65 g, mp 170–171°. The various crystalline materials were combined and dissolved in chloroform. The solution was decolorized and evaporated to a syrup, which was crystallized from ethyl acetate–ether to give 4.85 g (8%), mp 166–168°. A further crystallization gave pure product: mp 167–168°; pmr ($CDCl_3$) δ 2.10 (s, 3, Ac), 2.18 (s, 6, Ac), 4.33 ("d", 2, "*J*" = 5.5 cps, 5' CH_2OH), 4.61–4.91 (m, 1, 4 H), 5.20–5.38 (m, 1, 3' H), 5.62–5.80 (m, 1, 2' H), 5.95 (d, 1, $J_{1',2}$ = 2.5 cps, 1' H), 6.73 (s, 2, 4 NH_2), and 8.21 (s, 1, 6 H).

Anal. Calcd for $C_{14}H_{18}N_4O_8$: C, 45.40; H, 4.90; N, 15.13. Found: C, 45.14; H, 4.74; N, 15.04.

1-(2,3-Isopropylidene-β-D-ribofuranosyl)cyanuric Acid.—1-(β-D-Ribofuranosyl)cyanuric acid (6.25 g) was dissolved in a mixture of dimethylformamide (20 ml) and dimethoxypropane (18 ml) containing 15 drops of a solution of 4 *M* hydrogen chloride in dioxane. The sealed solution was left at room temperature for 3 days. Sodium bicarbonate (5 g) was added and the mixture was stirred for 2 hours. The solution was filtered through Celite and the filter was washed with 1-butanol. The filtrate was evaporated to dryness under oil pump vacuum. The residue was dissolved in ethanol (100 ml) containing glacial acetic acid (5 ml). The solution was heated on a steam bath for 5 min and then left overnight at room temperature. The solvent was removed and the residue was coevaporated with toluene. The residue was dissolved in chloroform and silica gel (40 g) was added. The

mixture was then evaporated to give a free-running powder. This material was added to a dry column of silica gel (41 × 3.3 cm) so that the final size was 61 × 3.3 cm. The column was eluted with chloroform and 100-ml fractions were collected. At fraction 11 the solvent was changed to chloroform–methanol (9:1). Fractions 15–20, which were homogeneous as judged by tlc on SilicAR 7GF with ethyl acetate developer (detection with sulfuric acid), were evaporated to dryness. The syrupy residue was crystallized from ethanol–heptane to yield 6.36 g (88%) of white product, mp 179–181°. This material was recrystallized from ethanol–heptane to give pure product: mp 180–181°; λ_{max}^{KBr} 1680–1800 cm^{-1} (C=O of cyanuric acid); pmr (DMSO-d_6) δ 1.32 (s, 3, CCH_3), 1.52 (s, 3, CCH_3), 3.54 ("d," 2, "J" = 6.5 cps, 5′ CH_2OH), 3.80–4.18 (m, 1, 4′ H), 4.54–4.93 (m, 2, 3′ H and 5′ CH_2OH), 5.17 (d, 1, $J_{2',3'}$ = 6.0 cps, 2′ H), 6.18 (s, 1, $J_{1',2'}$ <1 cps, 1′ H), and 11.84 (broad, s, 2, NH).

Anal. Calcd for $C_{11}H_{15}N_3O_7$: C, 43.85; H, 5.02; N, 13.95. Found: C, 44.21; H, 5.45; N, 14.20.

1-(2,3-*O*-Isopropylidene-5-methylsulfonyl-β-D-ribofuranosyl)-cyanuric Acid.—To a stirred solution of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)cyanuric acid (6.32 g) in dry pyridine (50 ml) at 0° was added dropwise methylsulfonyl chloride (1.80 ml) and the resulting solution was sealed and stored at 0° for 36 hr. Absolute ethanol (a few drops) was added and the solution was left overnight at 0°. The solution was evaporated to dryness and the residue was coevaporated with toluene. The dried (oil pump vacuum) residue was dissolved in methanol and silica gel was added. The mixture was evaporated to give a free-running powder which was added to a column (51 × 3.5 cm) of silica gel so that the final dimensions were 72 × 3.5 cm. Elution was started with chloroform. Fractions (200 ml each) were collected and the fractionation was monitored by tlc on SilicAR 7GF with ethyl acetate–chloroform (7:3) as developer (detection by sulfuric acid). At fraction 9 the solvent was changed to chloroform–ethyl acetate (4:1) and at fraction 14 to ethyl acetate. Fractions 16–19, which were of 100-ml volume and which contained a single component, were pooled and evaporated to a foam. Crystallization from ethyl acetate–heptane yielded 7.06 g (89%) of white crystals, mp 194–196°. These crystals were dissolved in methanol and the solution was decolorized. After solvent removal, the product was crystallized from ethanol–heptane to give pure material: mp 195–197°; ν_{max}^{KBr} 1710–1760 cm^{-1}; pmr (DMSO-d_6) 1.33 (s, 3, CCH_3), 1.52 (s, 3, CCH_3), 3.20 (s, 1, 5′ CH_3SO_2), 4.10–4.60 [m, 3, 5′ CH_2O (s) at 4.36 overlapped by 4′ H], 4.74–4.98 (m, 1, 3′ H), 5.21 (d, 1, $J_{2',3'}$ = 7.0 cps, 2′ H), 6.14 (s, 1, $J_{1',2'}$ <1 cps, 1′ H), and 11.66 (s, 2, NH).

Anal. Calcd for $C_{12}H_{17}N_3O_9S$: C, 37.98; H, 4.52; N, 11.08. Found: C, 37.88; H, 4.42; N, 11.04.

Registry No.—I, 320-67-2; III, 2353-33-5; IV, 22432-95-7; V, 22432-96-8; VI, 22432-97-9; VII, 22432-98-0; 1-(3,5-di-*O*-acetyl-2-deoxy-α,β-D-ribofuranosyl)-5-azacytosine, 22432-93-5; 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)cyanuric acid, 22432-99-1; 1-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-5-azacytosine, 22433-00-7; 1-(2,3-isopropylidene-β-D-ribofuranosyl)cyanuric acid, 22433-01-8; 1-(2,3-*O*-isopropylidene-5-methylsulfonyl-β-D-ribofuranosyl)cyanuric acid, 22433-02-9.

Synthesis of 21-Hydroxymethylprogesterone

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The synthesis of 21-hydroxymethylprogesterone was accomplished by two pathways, from progesterone and from 3β-acetoxy-5-pregnen-20-one. The preferred method involved the formylation and subsequent borohydride reduction of the 3-monoketal of progesterone. This diol was subsequently tritylated, oxidized, and hydrolyzed to yield 21-hydroxymethylprogesterone.

The C-17 side chains of the progestational and adrenocortical steroid hormones may be compared with the lowest members of the deoxy sugar and sugar series, respectively. Elongation of these side chains by addition of hydroxymethyl groups would yield homologs of the steroid-substituted carbohydrates. The higher hydroxymethyl homologs of progesterone and cortisol would have side chains which may be pictured as 1-substituted deoxy ketoses and 1-substituted ketoses, respectively. We wish to report the synthesis of 21-hydroxymethylprogesterone (**7a**), our initial objective in these studies.

A simple, direct method has been reported for the synthesis of 21-hydroxymethylcortisol by condensation of cortisol with formaldehyde.[2] When we attempted this method with pregnenolone and formaldehyde, we recovered only starting steroid. Our further studies with this method will be the subject of a separate paper. We did not obtain monohydroxymethylation in the desired position.

Very few primary aliphatic α-unsubstituted β-hydroxy ketones have been reported in the literature.[3] We presumed that 21-hydroxymethylprogesterone would be quite labile and that synthesis by indirect methods would be very sensitive to manipulations involved in protecting the other functional groups in the molecule. This did not prove to be the case.

The addition of the hydroxymethyl group on C-21 was accomplished by condensation of the 17β-acetyl group of pregnenolone acetate (**1**) with formate ester[4] followed by reduction with borohydride to the triol **2a** in the reaction medium (Scheme I). A number of routes were considered in order to utilize this condensation reaction for the synthesis of 21-hydroxymethylprogesterone. That the formate condensation occurs on C-21 has been demonstrated by Hirai, *et al.*,[5] as well as from evidence below.

One approach was to form the 20,21a-acetonide derivative[6] of the triol **2a** in order to oxidize selectively the Δ^5-3β-hydroxyl to the Δ^4-3-ketone by the Oppenauer method. Hydrolysis of the acetonide **4** yielded the diol ketone **5a**. The overall yield of this method to this point was so low that we turned to other approaches. An attempt to shortcut this pathway by tritylation of

(1) (a) Senior Postdoctoral Fellow, 1965–1967, supported by Grant 5 TU-MH6418, National Institutes of Health.

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(3) See, *e.g.*, T. White and R. N. Howard, *J. Chem. Soc.*, 25 (1943), and the patent literature for 1-hydroxybutan-3-one.

(4) L. Ruzicka, U. S. Patent 2,398,861 (1946); W. Bockmühl, G. Ehrhart, and H. Ruschig, German Patent 871,451 (1953); N. J. Doorenbos and L. Milewich, *J. Org. Chem.*, **31**, 3193 (1966).

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(6) M. Tanabe and B. Bigley, *J. Amer. Chem. Soc.*, **83**, 756 (1961); A. Hampton, J. C. Fratantoni, P. M. Carroll, and S. Wang, *ibid.*, **87**, 5481 (1965).