The Synthesis of Nucleosides derived from 5-Ethynyluracil and 5-Ethynylcytosine

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5-Ethynyluridine and 2'-deoxy-5-ethynyluridine have been synthesised by condensation of the trimethylsilyl derivative of 5-ethynyluracil with the appropriate blocked sugar derivatives and removal of the blocking groups. The α-anomer of 2'-deoxy-5-ethynyluridine was also obtained. 2,4-Dichloro-5-(1-chlorovinyl)pyrimidine upon treatment with ammonia gave a mixture of 2-amino-4-chloro-5-(1-chlorovinyl)pyrimidine and 4-amino-2-chloro-5-(1-chlorovinyl)pyrimidine. The latter upon treatment with potassium hydroxide in aqueous dioxan gave 5-ethynylcytosine. Condensation of the trimethylsilyl derivative of 5-ethynylcytosine with appropriate protected sugar derivatives and removal of the protecting groups gave 5-ethynylcytidine, 2'-deoxy-5-ethynylcytidine, and its α-anomer.

NUMEROUS 5-substituted pyrimidine nucleosides have been synthesised and some of them, usually 2'-deoxyribonucleosides, show antiviral and anticancer activities.[1,2] Recently we have described the synthesis of 5-ethynyluracil [3] and Bobek and his co-workers have briefly reported the synthesis of the same compound and 2'-deoxy-5-ethynyluridine. The latter shows activity against leukaemia cells.[4-6,†] Because of their interesting biological activities, we have synthesised a number of 5-substituted pyrimidine nucleosides. This paper gives details of the synthesis of 5-ethynyluridine, 2'-deoxy-5-ethynyluridine, 5-ethynylcytosine, 5-ethynylcytidine, 2'-deoxy-5-ethynylcytidine, and the α-anomers of the 2'-deoxyribonucleosides. A brief preliminary account of part of this work has already been published.[7]

The most widely used method for the synthesis of pyrimidine nucleosides is to condense a protected sugar derivative with the trimethylsilyl derivative of the pyrimidine. The latter is obtained by the action of hexamethyldisilazane and/or trimethylchlorosilane on the pyrimidine. Often ammonium sulphate is also added. In our case, using 5-ethynyluracil, (1; R = -C≡CH, R' = H) the silylation conditions were critical. Addition of ammonium sulphate caused reaction with the ethynyl group. This became evident upon the addition of water when 30% of the compound had been converted into 5-acetyluracil (1; R = Ac, R' = H. The best procedure was to treat 5-ethynyluracil with hexamethyldisilazane and a small amount of trimethylchlorosilane. The product was isolated by distillation under reduced pressure and shown by n.m.r. spectroscopy to be a bistrimethylsilyl derivative (2). Bobek and Bloch [6] have reported that during the transformation of 5-formyluracil into 5-ethynyluracil, migration of a trimethylsilyl protecting group to the terminal acetylene function occurred. Our results show that a similar derivative is not formed upon trimethylsilylation of 5-ethynyluracil.

The trimethylsilyl derivative of 5-ethynyluracil was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose under the conditions described by Niedballa and Vorbruggen,[8] to give 5-ethynyl-2',3',5'-tri-*O*-benzoyluridine (1; R = C≡CH; R' = 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl) in 75% yield. The n.m.r. spectrum showed that the compound was a β-nucleoside. Removal of the benzoyl groups with sodium methoxide in methanol gave 5-ethynyluridine (1; R = C≡CH; R' = β-D-ribofuranosyl).

† *Note added in proof:* During the course of this work Dr. Bobek made available to us a communication in which the synthesis of 5-ethynyluridine and 2'-deoxy-5-ethynyluridine by a similar procedure to ours was described.

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[2] Y. C. Cheng, J. P. Neenan, B. Goz, D. C. Ward, and W. H. Prusoff, *Ann. New York Acad. Sci.*, 1975, **255**, 332.
[3] P. J. Barr, A. S. Jones, and R. T. Walker, *Nucleic Acids Research*, 1976, **3**, 2845.
[4] (*a*) J. Perman, R. A. Sharma, and M. Bobek, *Tetrahedron Letters*, 1976, 2427; (*b*) M. Bobek and A. Bloch, Amer. Chem. Soc. Symposium, San Francisco, 1976.
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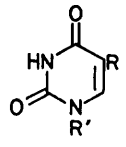
[6] M. Bobek and A. Bloch, *Amer. Chem. Soc. Abs.*, 1976, 172, CARB, 35.
[7] A. S. Jones, P. Serafinowski, and R. T. Walker, *Tetrahedron Letters*, 1977, 2459.
[8] U. Niedballa and H. Vorbruggen, *J. Org. Chem.*, 1974, **39**, 3654.

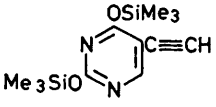
The synthesis of 2′-deoxyribonucleosides by similar methods gives a mixture of α- and β-anomers. By a suitable choice of conditions high yields of the required β-anomer have been obtained, but as there is no procedure which is generally applicable, a large number of methods, which differ from each other with regard to solvent and type and amount of catalyst, have been used. The best conditions for the synthesis of 2′-deoxy-5-ethynyluridine were to condense the trimethylsilyl derivative of 5-ethynyluracil with 1-chloro-2-deoxy-3,5-di-*p*-toluoyl-α-D-*erythro*-pentofuranose in 1,2-dichloroethane in the presence of 0.01 molecular proportions of mercury(II) bromide. This gave a yield of 67% blocked deoxyribonucleosides the β : α ratio of which (as measured by n.m.r.) was 1.45. Both the α- and β-anomers [1; R = C≡CH; R′ = α- and β- (respectively) 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl] were isolated and characterised. Removal of the *p*-toluoyl groups in the usual way gave 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-5-ethynyluracil (1; R = C≡CH, R′ = 2-deoxy-α-D-*erythro*-pentofuranosyl) and 2′-deoxy-5-ethynyluridine (1; R = −C≡CH, R′ = 2-deoxy-β-D-*erythro*-pentofuranosyl).

The o.r.d. spectrum of the latter gave a positive Cotton effect very similar to that of thymidine whereas the former gave a negative Cotton effect. This confirmed the assignment of the structure based on n.m.r. spectroscopy. The structure of 2′-deoxy-5-ethynyluridine has also been determined by *X*-ray crystallography.[9]

It appears, therefore, that direct condensation of 5-ethynyluracil with the appropriate sugar derivative is a satisfactory route of synthesis, so that to proceed *via* the 5-(2,2-dibromovinyl) derivative [4a] offers no advantages, particularly in view of the ease of synthesis of 5-ethynyluracil.[3]



(1)



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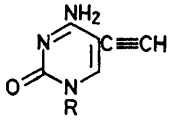

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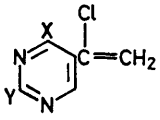

(4)

In order to obtain 5-ethynylcytosine (3; R = H), 2,4-dichloro-5-(1-chlorovinyl)pyrimidine (4; X = Y = Cl) [3] was treated with ethanolic ammonia to give a mixture of 2-amino-4-chloro-5-(1-chlorovinyl)pyrimidine (4; X = Cl; Y = NH_2) and 4-amino-2-chloro-5-(1-chlorovinyl)-pyrimidine (4; X = NH_2; Y = Cl). The structure of these compounds was assigned on the basis of the chemical shifts of the H-6 protons (8.38 and 8.18 p.p.m. respectively) which was consistent with the values quoted elsewhere for similar compounds.[10] The 4-amino-2-chloro-compound was converted into 5-ethynylcytosine (3; R = H) by treatment with potassium hydroxide in boiling aqueous dioxan. The structure of this compound was established by comparison of its u.v. spectra with those of 5-ethylcytosine,[11] its n.m.r. spectrum, and by the fact that the compound was also obtained by the action of ammonia on 4-ethoxy-5-ethynyl-2(1*H*)-pyrimidone.[3]

5-Ethynylcytosine was readily converted into its trimethylsilyl derivative by the procedure described above. This compound was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of tin(IV) chloride to give 2′,3′-5′-tri-*O*-benzoyl-5-ethynylcytidine (3; R = 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl). The n.m.r. spectrum showed that the compound was a β-nucleoside.[12] This was readily converted into 5-ethynylcytidine (3; R = β-D-ribofuranosyl) in the usual way.

The trimethylsilyl derivative of 5-ethynylcytosine was condensed with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose in the presence of tin(IV) chloride to give a mixture of 2′-deoxy-5-ethynyl-3′,5′-di-*O*-*p*-toluoylcytidine and its α-anomer. Separation of the blocked anomers could be achieved with difficulty by column chromatography on silica gel but the best methods of obtaining the pure anomers was to remove impurities by column chromatography and then to separate the anomers by fractional crystallisation. The structures of these compounds were assigned by means of n.m.r. spectroscopy by comparison with the results obtained by Kulikowski and Shugar for 2′-deoxy-5-ethylcytidine derivatives.[10] Thus the α-anomer gave for the anomeric proton a quartet centred at δ 6.30; the β-anomer also gave a quartet (centred at δ 6.38), but this merged so that the signal was little different from the pseudotriplet usually obtained for β-anomers.[13] When the *p*-toluoyl protecting groups were removed, the unblocked β-anomer gave a typical pseudotriplet. The *p*-toluoyl protecting groups were removed from the blocked nucleosides to give 2′-deoxy-5-ethynylcytidine (3; R = 2-deoxy-β-D-*erythro*-pentofuranosyl) and 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-5-ethynylcytosine (3; R = 2-deoxy-α-D-*erythro*-pentofuranosyl) respectively.

An interesting point arises in connection with the n.m.r. spectra of 5-ethynylcytidine and 2′-deoxy-5-ethynylcytidine. The signals arising from the protons of the exocyclic amino-group appear as two singlets with

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[11] T. D. Kulikowski and D. Shugar, *Acta Biochim. Polon.*, 1971, **18**, 209.

[12] M. W. Winkley and R. K. Robins, *J. Chem. Soc.* (*C*), 1969, 791.

[13] M. J. Robins and R. K. Robins, *J. Amer. Chem. Soc.*, 1965, **87**, 4934.

δ values of 6.78 and 7.66 in both cases. Cytidine and 2′-deoxycytidine each gave a singlet at δ 7.06 p.p.m. however. The α-anomer of 2′-deoxy-5-ethynylcytidine gives a singlet at δ 7.04 but this integrates for only one proton. The other proton probably gives a signal in the same region as those given by the hydroxy-groups. It appears, therefore, that, in the case of the 5-ethynyl derivatives, the two protons of the exocyclic amino-group are in different environments. The most probable explanation of this phenomenon is that hydrogen bonding occurs between one proton of the amino-group and the ethynyl group, the latter acting as the hydrogen acceptor. Evidence from i.r. spectroscopy has been obtained for the existence of a similar type of hydrogen bonding in the case of certain compounds containing both hydroxy and ethynyl groups, *e.g.* 2-ethynylphenol.[14-16]

The compounds described above are being subjected to biological tests. Preliminary results indicate that the 2′-deoxy-5-ethynylribonucleosides show antiviral activity. Detailed results have been published elsewhere.[17]

EXPERIMENTAL

Trimethylsilyl Derivative of 5-Ethynyluracil.—5-Ethynyluracil (1g) was suspended in hexamethyldisilazane (HMDS) (10 ml), trimethylchlorosilane (0.2 ml) was added and the mixture boiled under reflux for 18 h with rigorous exclusion of moisture. The HMDS was then removed by evaporation *in vacuo* and the residue subjected to Kugelrohr high-vacuum distillation [8] at *ca.* 120 °C (0.2 mmHg) to give the *bistrimethylsilyl derivative* of 5-ethynyluracil (1.8 g, 87% yield), δ(CCl_4), 0.33, 0.39, (18 H, 2 s, Me_3Si- on 2-*O*- and 4-*O*-), 3.40 (1 H, s, ≡CH), and 8.34 (1 H, s, H-6) ($SiMe_4$ external standard).

2′,3′,5′-*Tri*-O-*benzoyl*-5-*ethynyluridine*.—To a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.52 g, 5.0 mmol) and the bistrimethylsilyl derivative of 5-ethynyluracil (1.57 g, 5.6 mmol in 1,2-dichloroethane (70 ml) was added a solution of tin(IV) chloride (0.5 ml) in 1,2-dichloroethane (50 ml). The solution was kept at room temperature for 4 h and then poured into an excess of saturated aqueous sodium hydrogen carbonate and the mixture extracted with 1,2-dichloroethane. The extract was dried (Na_2SO_4) and evaporated to dryness. The residue was crystallised from benzene to give almost pure product (2.05 g). An additional quantity of material (137 mg) was obtained from the mother liquors by chromatography on silica gel [Keiselgel 0.05—0.2 mm (70—325 mesh ASTM) type 7734, E. Merck, Darmstadt] with benzene–ethyl acetate (4 : 1) as eluant [total yield, 75%]. An analytically pure sample of 2′,3′,5′-tri-O-*benzoyl*-5-*ethynyluridine* was obtained by chromatography on silica gel, m.p. 202—204 °C (decomp.) (Found: C, 66.1; H, 4.2; N, 4.8. $C_{32}H_{24}N_2O_9$ requires C, 66.2; H, 4.16; N, 4.82%); δ($CDCl_3$) 3.03 (1 H, s, ≡CH), and 6.25 (1 H, d, J = 7 Hz, H-1′).

5-*Ethynyluridine*.—The above-mentioned compound (290 mg) was suspended in methanol (25 ml), sodium (12 mg) was added, and the mixture kept at 20 °C for 4 h. The solution was neutralised by careful addition of Dowex 50 resin (H^+ form) and the resin filtered off and washed with methanol–water (2 : 1). The combined filtrate and washings were evaporated to dryness and the residue (120 mg, 92% yield) crystallised from methanol to give 5-*ethynyluridine* (Found: C, 49.1; H, 4.2; N, 10.1. $C_{11}H_{12}N_2O_6$ requires C, 49.3; H, 4.5; N, 10.4%); δ[$(CD_3)_2SO$] 3.90 (1 H, s, ≡CH), 5.62 (1 H, d, J = 7 Hz, H-1′), and 8.28 (1 H, s, H-6); $\lambda_{max.}$ 230 (ε 9 380) and 290 nm (10 700); $\lambda_{max.}$ 228 (ε 9 280) and 286 nm (10 500); $\lambda_{min.}$ 253 nm at pH 7; $\lambda_{max.}$ 288 nm (ε 8 610); $\lambda_{min.}$ 260 nm at pH 12.

2′-*Deoxy*-5-*ethynyl*-3′,5′-*di*-O-p-*toluoyluridine and its* α-*Anomer*.—The bistrimethylsilyl derivative of 5-ethynyluracil (12 g, 43 mmol) was dissolved in dry 1,2-dichloroethane (50 ml) and added to a solution of 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (15.5 g, 40 mmol) and mercury(II) bromide (145 mg, 0.40 mmol) in 1,2-dichloroethane (450 ml). The mixture was stirred at 23 °C for 23 h and then evaporated under reduced pressure to a white foam. This was dissolved in benzene and applied to a column of silica gel (400 g). The column was eluted first with benzene (2 l) to remove impurities and then with benzene–ethyl acetate (7 : 3) until all the nucleoside material had been eluted. The benzene–ethyl acetate solution was evaporated to dryness to give a white solid (13.1 g, 67% yield) which n.m.r. spectroscopy showed to be a mixture of α- and β-anomers (1 : 1.45). This solid was dissolved in the minimum quantity of boiling methanol and the solution set aside at room temperature for 18 h. The resulting microcrystalline needles were filtered off to give 2′-*deoxy*-5-*ethynyl*-3′,5′-*di*-O-p-*toluoyluridine* (5 g, 26% yield), m.p, 202—203 °C (Found: C, 65.9; H, 4.9; N, 5.4. $C_{27}H_{24}N_2O_7$ requires C, 66.4; H, 5.0; N, 5.7%); δ($CDCl_3$) 3.09 (1 H, s, ≡CH), 6.45 (1 H, t, H-1′, J = 7 Hz), $\lambda_{max.}$ 240 (ε 30 800) and 285 nm (ε 10 900); $\lambda_{min.}$ 266 nm (ε 8 200) in ethanol.

The methanol filtrate was concentrated and the solution cooled to 4 °C. The crystalline solid which separated with time was filtered off to give 5-*ethynyl*-1-(2-*deoxy*-3,5-*di*-O-p-*toluoyl*-α-D-(erythro-*pentofuranosyl*)*uracil* (4.9 g, 25% yield), m.p. 161—162 °C (Found: C, 66.2; H, 5.05; N, 5.7. $C_{27}H_{24}N_2O_7$ requires C, 66.4; H, 5.0; N, 5.7%), δ($CDCl_3$) 3.20 (1 H, s, ≡CH) and 6.45 (1 H, d, H-1′); $\lambda_{max.}$ 240 (ε 37 300) and 285 nm (12 400); $\lambda_{min.}$ 267 nm (ε 9 200) in ethanol.

2′-*Deoxy*-5-*ethynyluridine and its* α-*Anomer*.—The *p*-toluoyl protecting groups were removed from the two compounds described above by a similar procedure to that used to obtain 5-ethynyluridine. The two nucleosides were obtained in *ca.* 96% yield. 2′-Deoxy-5-ethynyluridine was obtained upon crystallisation from methanol–water as colourless needles, m.p. 197—199 °C (decomp.) (Found: C, 52.3; H, 5.1; N, 10.8. Calc. for $C_{11}H_{12}N_2O_5$: C, 52.4; H, 4.8; N, 11.1%), δ[$(CD_3)_2SO$] 4.11 (1 H, s, ≡CH), 6.23 (1 H, t, H-1′, J = 7 Hz), and 8.44 (1 H, s, H-6); $\lambda_{max.}$ 228 (ε 9 960) and 289 nm (11 460); $\lambda_{min.}$ 252 nm (ε 2 520) in ethanol. 1-(2-*Deoxy*-α-D-erythro-*pentofuranosyl*)-5-*ethynyluracil* was obtained by crystallisation from methanol, m.p. 207—208 °C (decomp.) (Found: C, 52.2; H, 4.7; N, 11.0. $C_{11}H_{12}N_2O_5$ requires C, 52.4; H, 4.8; N, 11.1%), δ[$(CD_3)_2SO$] 4.09 (1 H, s, ≡CH), 6.20 (1 H, dd, H-1′, J_1 = 9 Hz, J_2 = 2

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[16] B. Jordanov, A. Jovtscheif, S. Spassov, B. Bagloev, and M. Agova, *Tetrahedron*, 1964, **20**, 903.
[17] R. T. Walker, P. J. Barr, E. De Clercq, J. Descamps, A. S Jones, and P. Serafinowski, Nucleic Acids Research, Special Publication No. 4, 1978.

Hz), 8.33 (1 H, s, H-6); $\lambda_{max.}$ 230 and 287 nm; $\lambda_{min.}$ 254 nm in ethanol.

4-Amino-2-chloro-5-(1-chlorovinyl)pyrimidine.—To a solution of 2,4-dichloro-5-(1-chlorovinyl)pyrimidine (13.5 g) in ether at 0 °C (65 ml) there was added dropwise a solution of ammonia in ethanol (saturated at 0 °C) (100 ml), the temperature being maintained at 0 °C. After being kept at 0 °C for 2 h the temperature of the solution was then raised to 20 °C. After 18 h at this temperature, the white precipitate which had formed was filtered off and dissolved in boiling chloroform. Insoluble material was filtered off from the chloroform solution and the filtrate evaporated to dryness to give a mixture of crude 4-amino-2-chloro-5-(1-chlorovinyl)pyrimidine and 2-amino-4-chloro-5-(1-chlorovinyl)pyrimidine (8.13 g). By concentrating the filtrate from the original reaction solution and working up in a similar manner an additional amount (2.83 g) of material was obtained. The ratio of 4-amino-derivative to 2-amino-derivative was 3.7 : 1 (measured by n.m.r. spectroscopy). Crystallisation of this crude mixture from ethanol gave *4-amino-2-chloro-5-(1-chlorovinyl)pyrimidine* (4.7 g, 39%), m.p. 178—179 °C (decomp.) (Found: C, 37.8; H, 2.8; Cl, 37.3; N, 22.3. $C_6H_5Cl_2N_3$ requires C, 37.9; H, 2.65; Cl, 37.3; N, 22.1%), δ[$(CD_3)_2SO$–D_2O] 5.85 (2 H, m, $=CH_2$) and 8.18 (1 H, s, H-6). The compound was homogeneous by t.l.c. in several solvent systems.

2-Amino-4-chloro-5-(1-chlorovinyl)pyrimidine.— Mother liquors from the isolation of the aforementioned compound were evaporated to dryness and the residue fractionated on a column of silica gel using chloroform–ethanol (19 : 1) as eluant. The first compound eluted was 4-amino-2-chloro-5-(1-chlorovinyl)pyrimidine, then a mixture of the two isomers and finally pure *2-amino-4-chloro-5-(1-chlorovinyl)-pyrimidine* which was obtained crystalline from chloroform (yield 12%), m.p. 159—160 °C (decomp.) (Found: C, 38.1; H, 2.8; Cl, 37.6; N, 21.8. $C_6H_5Cl_2N_3$ requires C, 37.9; H, 2.65; Cl, 37.3; N, 22.1%), δ[$(CD_3)_2SO$–D_2O] 5.85 (2 H, m, $=CH_2$) and 8.38 (1 H, s, H-6).

5-Ethynylcytosine.—(*a*) 4-Ethoxy-5-ethynyl-2(1*H*)-pyrimidone [3] (82 mg) was suspended in ethanol, saturated with ammonia at 0 °C, and kept in a sealed tube at 15—20 °C for 19 h. The white precipitate which had formed was filtered off and washed with ethanol to give *5-ethynylcytosine* (13 mg) (Found: C, 53.1; H, 4.0; N, 30.9. $C_6H_5N_3O$ requires C, 53.3; H, 3.7; N, 31.1%); δ[$(CD_3)_2SO$–D_2O] 4.35 (1 H, s, ≡CH) and 7.88 (1 H, s, H-6); $\lambda_{max.}$ 238 (ε 15 900) and 301 nm (10 600); $\lambda_{min.}$ 263 nm (ε 2 900) at pH 1; $\lambda_{max.}$ 235 (ε 17 600) and 288 nm (7 700); $\lambda_{min.}$ 265 nm (ε 4 590) at pH 7; $\lambda_{max.}$ 256 (ε 16 600) and 303 nm (11 500); $\lambda_{min.}$ 277 nm (ε 5 810) at pH 12. The compound was homogeneous upon t.l.c. in chloroform–methanol (17 : 3) (R_F 0.27) and chloroform–methanol (7 : 3) (R_F 0.54).

(*b*) 4-Amino-2-chloro-5-(1-chlorovinyl)pyrimidine (1.19 g) was suspended in dioxan (12.5 ml) and 2M-aqueous potassium hydroxide (12.5 ml) was added. The solution was boiled under reflux for 1.5 h and then neutralised with M-hydrochloric acid; the mixture was then set aside at *ca.* 4 °C for 18 h. The resulting precipitate was filtered off to give pure *5-ethynylcytosine* (469 mg) which was identical to the compound obtained by procedure (*a*). An additional 123 mg of this compound was obtained from the aqueous dioxan solution (total yield 70%).

2′,3′,5′-Tri-O-benzoyl-5-ethynylcytidine.— 5-Ethynylcytosine (592 mg) was suspended in HMDS (6 ml), trimethylchlorosilane (0.1 ml) was added and the mixture boiled under reflux for 14 h with rigorous exclusion of moisture. The HMDS was then distilled off under reduced pressure and the residual oil submitted to Kugelrohr high-vacuum distillation at 110 °C (0.1 mmHg) to give the trimethylsilyl derivative of 5-ethynylcytosine (1.1 g) as a colourless oil. This was dissolved in 1,2-dichloroethane (18 ml) and added to a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.82 g) in 1,2-dichloroethane and tin(IV) chloride (0.6 ml) in 1,2-dichloroethane (36 ml) added. This reaction mixture was kept at room temperature for 2 h and then poured into an excess of saturated aqueous sodium hydrogen carbonate solution. The organic layer was separated, the aqueous layer extracted with more 1,2-dichloroethane and the combined organic layer and extracts dried (Na_2SO_4) and evaporated to dryness to give the crude product in 90% yield. This was crystallised from ethanol to give pure *2′,3′,5′-tri-O-benzoyl-5-ethynylcytidine*, m.p. 203—205 °C (decomp.) (Found: C, 66.1; H, 4.6; N, 7.1. $C_{32}H_{25}N_3O_8$ requires C, 66.3; H, 4.35; N, 7.25%), δ[$(CD_3)_2SO$] 2.95 (1 H, s, ≡CH) and 6.16 (1 H, d, H-1′, J = 7 Hz).

5-Ethynylcytidine.—The benzoyl blocking groups were removed from the compound mentioned above (290 mg) by the procedure previously described to give *5-ethynylcytidine* (126 mg, 94% yield) which was obtained crystalline from methanol (Found: C, 49.7; H, 5.2; N, 16.0. $C_{11}H_{13}N_3O_5$ requires C, 49.4; H, 4.9; N, 15.7%); δ[$(CD_3)_2SO$–D_2O] 4.30 (1 H, s, ≡CH), 5.76 (1 H, d, H-1′), 6.78 and 7.66 (2 H, 2 s, NH_2), and 8.36 (1 H, s, H-6); $\lambda_{max.}$ 236 and 302 nm; $\lambda_{min.}$ 261 nm at pH 1; $\lambda_{max.}$ 235 and 292 nm; $\lambda_{min.}$ 264 nm at pH 7; $\lambda_{max.}$ 237 and 296 nm; $\lambda_{min.}$ 267 nm at pH 12.

2′-Deoxy-5-ethynyl-3′,5′-di-O-p-toluoylcytidine and its α-Anomer.—To 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (1.55 g, 4.0 mmol) and the trimethylsilyl derivative of 5-ethynylcytosine (1.40 g, 5.0 mmol) in dichloromethane (50 ml), cooled to 0 °C, tin(IV) chloride (0.1 ml, 0.86 mmol) in dichloromethane was added dropwise. The clear solution which was obtained after a few minutes was kept at 0 °C for 8 h and then at 20 °C for 18 h. The reaction mixture was poured into an excess of saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane. The dichloromethane solution was dried (Na_2SO_4) and evaporated to dryness to give a solid residue (2.32 g). This was fractionated on a silica-gel column; unchanged sugar and other impurities were eluted with chloroform and the nucleoside derivatives with chloroform–propan-2-ol (19 : 1) to give a mixture of anomers (1.67 g, 85% yield, α : β ratio 6 : 5 as determined by n.m.r.). Crystallisation of the mixture from methanol gave *2′-deoxy-5-ethynyl-3′,5′-di-O-p-toluoylcytidine* (485 mg, 25%), m.p. 154.5—156 °C (Found: C, 64.1; H, 5.6; N, 8.1. $C_{27}H_{25}N_3O_6,H_2O$ requires C, 64.2; H, 5.4; N, 8.3%), δ($CDCl_3$) 3.22 (1 H, s, ≡CH) and 6.38 (1 H, q, H-1′). The mother liquors were concentrated to dryness and the residue crystallised from ethanol to give 1-(*2-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl*)-*5-ethynylcytosine* (567 mg, 29%), m.p. 95—115 °C (decomp.) (Found: C, 66.3; H, 5.1; N, 8.4. $C_{27}H_{25}N_3O_6$ requires C, 66.5; H, 5.2; N, 8.6%); δ($CDCl_3$) 3.30 (1 H, s, ≡CH) and 6.30 (1 H, q, H-1′). The following solvent systems were used to separate blocked nucleosides from other impurities by t.l.c. on silica gel: toluene–acetic acid–water (5 : 5 : 2, upper phase) and chloroform–propan-2-ol (19 : 1) (R_F of both anomers, 0.24 and 0.45 respectively). The anomers could be separated by t.l.c. in either toluene–dioxan–water (5 : 5 : 1, upper phase) or benzene–acetone (7 : 3). The latter solvent could

be used to separate the anomers on a silica-gel column and this provided an alternative procedure for obtaining them on a preparative scale.

2′-Deoxy-5-ethynylcytidine and its α-Anomer.—The *p*-toluoyl blocking groups were removed from the blocked nucleosides by the usual procedure to give *2′-deoxy-5-ethynylcytidine* in 93% yield, m.p. 155 °C (Found: C, 52.7; H, 5.1; N, 16.7. $C_{11}H_{13}N_3O_4$ requires C, 52.6; H, 5.2; N, 6.7%), δ[$(CD_3)_2SO$] 4.30 (1 H, s, ≡CH), 6.10 (1 H, t, H-1′), 6.78 and 7.66 (2 H, 2 s, NH_2), and 8.24 (1 H, s, H-6); $\lambda_{max.}$ 238 (ε 13 700) and 307 nm (ε 9 700); $\lambda_{min.}$ 264 (ε 2 200) at pH 2; $\lambda_{max.}$ 236 (ε 15 900) and 295 nm (9 500); $\lambda_{min.}$ 265 nm (ε 4 440) at pH 6; $\lambda_{max.}$ 236 (ε 15 600) and 296 nm (9 500); $\lambda_{min.}$ 266 nm (ε 4 630) at pH 12 and 1-(*2-deoxy-α-*D*-erythro-pentofuranosyl)-5-ethynylcytosine* in 95% yield. The product which was obtained crystalline from ethanol decomposed at 150 °C (Found: C, 52.4; H, 5.2; N, 16.4. $C_{11}H_{13}N_3O_4$ requires C, 52.6; H, 5.2; N, 16.7%), δ[$(CD_3)_2SO$] 4.32 (1 H, s, ≡CH), 6.02 (1 H, q, H-1′), 7.04br (1 H, s, 1 proton of NH_2), and 8.06 (1 H, s, H-6); $\lambda_{max.}$ 240 and 308 nm; $\lambda_{min.}$ 266 nm at pH 2; $\lambda_{max.}$ 236 and 294 nm; $\lambda_{min.}$ 266 nm at pH 6; $\lambda_{max.}$ 236 and 294 nm; $\lambda_{min.}$ 266 nm at pH 12.

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