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Note

An improved synthesis of 6-azathymidine

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The formation of 6-azathymidine [2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-6-methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione] (**5**) from 6-azathymine [6-methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione] (**1**) by a transdeoxyribosylation mechanism was observed in a bacterial subcellular system[1] in 1955. The biological activity of azapyrimidine nucleosides has been recently reviewed[2]. 6-Azathymidine (**5**) is a strong inhibitor of the synthesis of nucleic acids, and a much more potent antagonist of thymine and thymidine than is 6-azathymine[3,4] (**1**). Several enzymic preparations of 6-azathymidine (**5**) have been reported[1,5,6]. Furthermore, a chemical synthesis of this nucleoside, using the chloromercuric coupling method, has been described[7,8]. However, by either method, the final product was obtained as an amorphous solid.

It has been shown[9] that fusion of the protected sugar moiety with the trimethylsilyl-protected pyrimidine base results in a preponderance of the β-D anomer of the nucleoside. Shen *et al.*[10] have successfully applied this method in the synthesis of 2′-deoxy-5-(trifluoromethyl)-6-azauridine and reported that none of the unwanted isomers were isolated. This paper describes the synthesis of 6-azathymidine (**5**) by use of the trimethylsilyl ether-coupling method.

2-Deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-*erythro*-pentofuranosyl chloride (**3**), prepared according to the procedure of Ness[11], was fused with the trimethylsilyl derivative **2** of 6-azathymine (**1**). The β-D anomer **4** was obtained without chromatographic purification as white prisms in 86% yield. Removal of the *p*-nitrobenzoyl groups of **4** with ammonia in methanol for 12 h at room temperature afforded the free nucleoside **5**, which was isolated in 48% yield. The overall yield from **3** was 41%. Elementary analysis, u.v. spectrum, and ^{1}H-n.m.r. spectral data were consistent with the structure of **5**.

The route just described represents a marked improvement on the previous syntheses of 6-azathymidine. The yield for the coupling reaction has been increased, the number of steps reduced, and the final product was obtained as crystals for the

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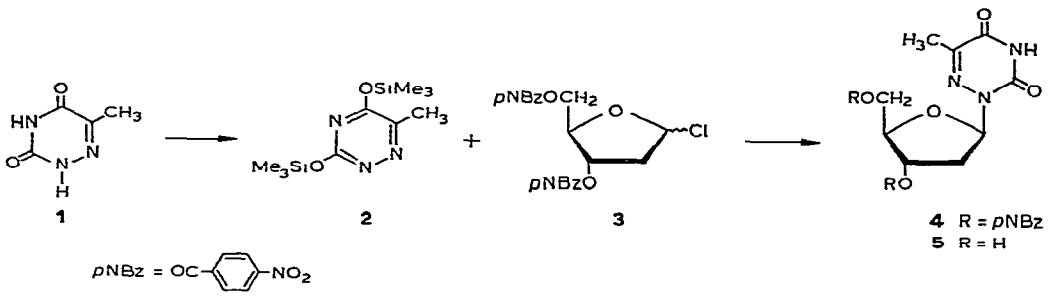

first time. The availability of crystalline **5** afforded preliminary X-ray diffraction studies by A. Banerjee (Max Planck Institute for Experimental Medicine, Göttingen, West Germany), which will be reported elsewhere.

EXPERIMENTAL

Melting points were measured with a Thomas–Hoover Unimelt apparatus and are not corrected. T.l.c. was performed on plastic films coated with Silica gel Merck F-254 (EM Laboratories Inc., Elmsford, NY 10523) with 1:4 (v/v) ethanol–chloroform as solvent system. Elementary analyses were performed by Baron Consulting Co., Orange, CT 06477. The u.v. spectra were recorded on a Beckman Model 25 spectrophotometer. The ^{1}H-n.m.r. spectra were obtained on a Bruker 270 HX spectrometer at a concentration of 30mM in Me_2SO-d_6 with Me_4Si as the internal reference. The spectral analysis is first-order, except for the strongly coupled H-5′ and H-5″, which are treated as the BC part of an ABC system. Chemical shifts (δ) are reported in p.p.m., and coupling constants (J) in Hz.

6-Methyl-3,5-bis(trimethylsiloxy)-1,2,4-triazine (**2**). — A mixture of 6-azathymine (**1**) (5.00 g, 39.4 mmol), hexamethyldisilazane (10 ml), and chlorotrimethylsilane (0.4 ml) was heated under reflux at 170° for 30 min under anhydrous conditions[10]. The mixture was then evaporated *in vacuo* to yield **2** as a crude oil that crystallized overnight at 3°, giving 6.57 g (61%) of **2**, m.p. 43°.

*2-(2-Deoxy-3,5-di-*O*-p-nitrobenzoyl-β-*D*-erythro-pentofuranosyl)-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione* (**4**). — A mixture of **2** (4.00 g, 14.7 mmol) and 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-*erythro*-pentofuranosyl chloride[11] (**3**) (3.00 g, 6.66 mmol) was fused for 30 min at 150°/25 mm Hg, and then cooled to room temperature[10]. The dark gum was dissolved in warm dichloromethane (25 ml), and then treated with methanol (5 ml) to cleave the silyl ether groups. The solution was kept overnight at 3° to yield crystalline **4** (3.12 g, 86%), m.p. 219–222°. Recrystallization from 1:2 (v/v) methanol–acetone gave pure **4**, m.p. 227–228°; $[\alpha]_D^{25}$ −66.5° (*c* 0.01, chloroform); R_F 0.78; λ_{max}^{EtOH} 261 nm (ε 32 700), λ_{min}^{EtOH} 224 nm (ε 11 500), $\lambda_{max}^{pH\ 1.5}$ 266 nm (ε 25 000), $\lambda_{max}^{pH\ 12.7}$ 267 nm (ε 26 900); n.m.r.: δ 9.03 (bs, 1 H, NH-3), 8.36, 8.34, 8.25, 8.20 (4 d, 8 H, J 8.82 Hz, 2 C_6H_4), 6.53 (dd, 1 H, $J_{1',2'}$ 5.88, $J_{1',2''}$ 6.62 Hz, H-1′), 5.74 (m, 1 H, $J_{3',2'}$ 5.88, $J_{3',2''}$ 4.41 Hz, H-3′), 4.65 (m, 1 H, H-4′), 4.57 (m, 2 H, H-5′), 2.90 (m, 1 H, $J_{2',2''}$ 13.97 Hz, H-2′), 2.58 (m, 1 H, H-2″), and 2.04 (s, 3 H, CH_3–5).

Anal. Calc. for $C_{23}H_{19}N_5O_{11}$: C, 51.02; H, 3.54; N, 12.94. Found: C, 50.95; H, 3.58; N, 12.98.

*2-(2-Deoxy-β-*D*-erythro-pentofuranosyl)-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione* (**5**). — A suspension of **4** (2.00 g, 3.70 mmol) in anhydrous methanol (300 ml) saturated with ammonia was stirred overnight in a stoppered flask at room temperature[12]. The solvent was removed under reduced pressure at 40°. The dry residue was dissolved in water (100 ml) and extracted with ether (4 × 60 ml) to remove the *p*-nitrobenzamide. The aqueous layer was evaporated *in vacuo* at 40° to a syrup that was then dissolved in ethanol (30 ml) and re-evaporated to dryness. The residue was redissolved in acetone and filtered. Colorless crystals formed on keeping the filtrate for 2 days at room temperature. The product was filtered off and washed with a small volume of cold acetone, to give pure **5** as colorless prisms (0.32 g, 35%), m.p. 153–154°; $[\alpha]_D^{25}$ −68.0° (*c* 0.01, water); R_F 0.37; λ_{max}^{EtOH} 267 nm (ε 7 750), λ_{min}^{EtOH} 236 nm (ε3 800), $\lambda_{max}^{pH\ 1.5}$ 264 nm (ε 6 550), $\lambda_{max}^{pH\ 12.7}$ 254 nm (ε 7 800) {lit.[8] $[\alpha]_D$ −76.7° (*c* 0.5, pyridine); $\lambda_{max}^{pH\ 1.9}$ 264 nm (log ε 3.78)}; n.m.r. δ 9.10 (bs, 1 H, NH-3), 6.31 (dd, 1 H, $J_{1',2'}$ 5.31, $J_{1',2''}$ 6.63 Hz, H-1'), 5.17 (bs, 1 H, OH-3'), 4.63 (bs, 1 H, OH-5'), 4.27 (dd, 1 H, $J_{3',2'}$ 5.75, $J_{3',2''}$ 4.42, $J_{3',4'}$ 4.86 Hz, H-3'), 3.70 (dd, 1 H, $J_{4',5'}$ 5.31, $J_{4',5''}$ 6.19 Hz, H-4'), 3.46 (dd, 1 H, $J_{5',5''}$ 11.50 Hz, H-5'), 3.34 (dd, 1 H, H-5''), 2.40 (m, 1 H, $J_{2',2''}$ 13.27 Hz, H-2'), 2.09 (s, 3 H, CH_3-5), 2.04 (m, 1 H, H-2''). A second crop (0.13 g) was obtained by keeping the concentrated mother liquor overnight at 3° (total yield 0.44 g, 48%).

Anal. Calc. for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.39; N, 17.28. Found: C, 44.29; H, 5.27; N, 16.96.

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