Synthesis of a Photoproduct from Ultraviolet-irradiation of Bacterial Spores: 5,6-Dihydro-5-(α-thyminyl)thymine

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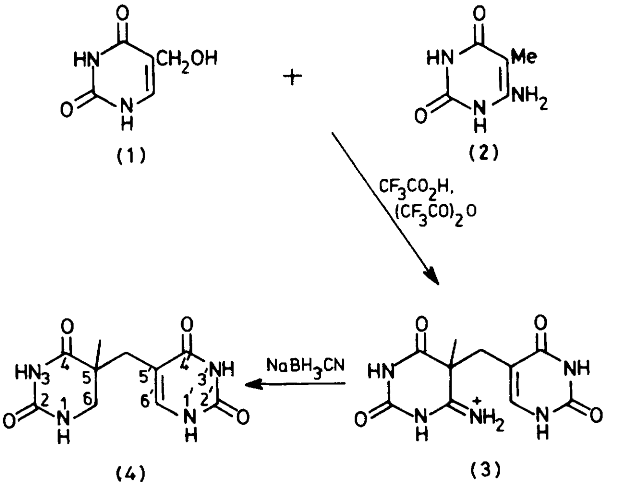
Summary 5-Hydroxymethyluracil and 6-aminothymine were coupled in trifluoroacetic acid to give 5,6-dihydro-6-imino-5-(α-thyminyl)thymine which was reduced by sodium cyanotrihydridoborate to 5,6-dihydro-5-(α-thyminyl)thymine.

The principal photoproduct formed on u.v.-irradiation of bacterial spores[1] or DNA in a dehydrated state[2] has been assigned the structure 5,6-dihydro-5-(α-thyminyl)thymine (**4**) on the basis of spectroscopic and chromatographic evidence.[2] We have devised a synthetic route to 5,6-dihydro-5-(α-thyminyl)thymine which requires only two steps from readily available starting materials.

Electrophilic substitution at the C-5 position of 6-aminouracil and related derivatives can often be achieved without difficulty. In particular numerous examples of acylation or carbonyl group condensations at the C-5 carbon are known.[3] Consequently we undertook the synthesis of 5,6-dihydro-5-(α-thyminyl)thymine reasoning that the desired carbon backbone with all of the necessary functionality could be fabricated in a single chemical operation, namely, electrophilic substitution at C-5 of 6-aminothymine by the α-thyminyl cation. Also ready generation of the α-thyminyl cation and subsequent coupling with phenols and related activated aromatic compounds *via* electrophilic substitution is a well established reaction which proceeds in high yield.[4]



5-Hydroxymethyluracil[5] (**1**) and excess of $(CF_3CO)_2O$ dissolved in CF_3CO_2H gave an intermediate which we postulate to be 5-trifluoroacetoxymethyluracil on the basis of the shift of the methylene proton resonance from δ 4·67 (s, 2H) to 5·20 (s, 2H). Addition of 1 equiv. of 6-aminothymine[6] (**2**) to this solution followed by refluxing for 4 h resulted in a shift of the methylene proton resonance from δ 5·20 to 3·51. On the basis of the ^1H n.m.r. spectrum [in CF_3CO_2H, resonances were observed at δ 2·01 (3H, s), 3·51 (2H, br s), and 7·80 (1H, br s)] and the fact that it is rapidly hydrolysed to 5-methyl-5-(α-thyminyl)barbituric acid, this intermediate is presumed to be protonated 5,6-dihydro-6-imino-5-(α-thyminyl)thymine (**3**). Yields as high as 83% could be achieved by rigorously excluding water. Removal of CF_3CO_2H *in vacuo* gave (**3**) as a white powder which was used directly in all subsequent synthetic transformations without purification.

Slow addition of aqueous $NaBH_3CN$ over 30 min to (**3**) while maintaining pH 2—4 by periodic addition of 10% HCl resulted in reduction of the amidinium function at C-6. Removal of water *in vacuo* left a residue which was taken up in methanol and filtered. The methanol-insoluble material was recrystallized from ethanol–water to give 5,6-dihydro-5-(α-thyminyl)thymine (**4**) in 38% yield: ^1H n.m.r. (CF_3CO_2H) δ 1·42 (3H, s, Me), 2·77 (1H, d, *J* 14 Hz), 3·12 (1H, d, *J* 14 Hz), 3·57 (2H, br s, 6-CH_2), 7·52 (1H, br s, 1-H), and 7·77 (1H, d, *J* 5 Hz, 6′-H); ^{13}C n.m.r. [$(CD_3)_2SO$] (p.p.m. relative to internal Me_4Si) 174·91 (C-4), 164·65 (C-4′), 153·23 (C-2), 151·05 (C-2′), 140·32 (C-6′), 106·86 (C-5′), 45·28 (C-6), 40·95 (C-5), 30·03 (–CH_2–), and 20·01 (Me); *m/e* 252 (M^+), 179, 151, and 127; i.r. (KBr) 3190 (N–H) and 1660–1750 cm^{-1} (C=O); u.v. (H_2O, pH 6) λ_{max} 265 nm (ε 8000).

The ^1H n.m.r. assignments were confirmed by reducing intermediate (**3**) with $NaBD_3CN$ to give (**4**) deuteriated at C-6 which showed the two doublets at δ 2·77 and 3·12 but no signal at δ 3·57.

A sample of authentic 'spore photoproduct' was obtained from u.v.-irradiated dry DNA following the procedure of Varghese.[2] The product isolated from DNA and synthetic 5,6-dihydro-5-(α-thyminyl)thymine were subjected to t.l.c. on silica gel. Both had identical R_f values in four different solvent systems. Furthermore, spectral (^1H n.m.r., i.r., u.v., mass) and analytical data for the synthetic material were found to be virtually identical to those data published[2] for the photoproduct isolated from u.v.-irradiated dry DNA, thus establishing the identity of 'spore photoproduct' as 5,6-dihydro-5-(α-thyminyl)thymine (**4**).

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