PHOTOCHEMICAL SYNTHESIS OF $C_{(5)}$ ALKYL AND HETEROARYL SUBSTITUTED PYRIMIDINE NUCLEOTIDES

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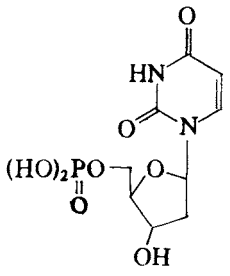
A simple and direct method for the synthesis of C-5 modified nucleosides is described. Photoirradiation of 2'-deoxyuridine 5'-phosphate (*I*), in the presence of haloheteroarenes afforded the C-5 heteroaryl substituted nucleotides. 5-(2-Hydroxyethyl)nucleotide also obtained from photocoupling of *I* with 2-iodoethanol. Photoirradiation of 5-iodo-2'-deoxyuridine 5'-phosphate (*II*), in the presence of methyl acrylate or acrylonitrile, gave 5-(2-methoxycarbonylethenyl) and 5-(2-cyanoethenyl) nucleotide, respectively.

Pyrimidine nucleotides substituted at the $C_{(5)}$ position constitute a class of biologically significant compounds. As analogues of 2'-deoxythymidine 5'-phosphate and potential inhibitors of thymidylate synthetase, they could be important in the clinical control of cancer growth and/or viral infection[1-5]. In the search for new compounds of this class, we have sought a direct synthetic route to 5-heteroaryl and 5-alkylsubstituted pyrimidine 5'-nucleotides without requiring the nucleoside bond synthesis or phosphorylation. Bergstrom's group has developed the palladium-catalyzed coupling reaction for the preparation of C-5 substituted nucleosides[6]. Mertes and coworkers[7] were successful in extending this reaction for the synthesis of 5-styrylnucleotides. Attempts to exploit this reaction for the synthesis of 5-aryl substituted nucleotides were not very promising. On the other hand, photochemical approaches to these compounds were more encouraging[7,8]. In this paper we wish to report the application of such a photochemical coupling reaction for the direct synthesis of 5-heteroaryl and 5-alkyl substituted 5'-nucleotides.

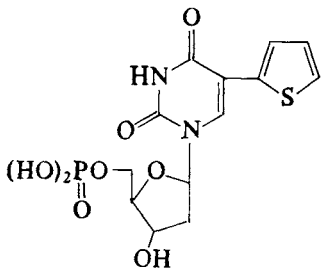
While in the previous photochemical reactions it was necessary to render the nucleotides soluble in a non-polar solvent[7], we overcame this solubility problem by using a mixed solvent system (30% aqueous acetonitrile). The use of such a solvent system eliminates the need for prior nucleotide derivatization, and subsequent hydrolytical removal of the protecting groups. We have already used a similar solvent system in our previous photochemical modification of nucleosides[9,10].

On irradiation of 2'-deoxyuridine 5'-phosphate (*I*) and 2-iodothiophene in deoxygenated aqueous acetonitrile at 254 nm 5-(2-thienyl)-2'-deoxyuridine 5'-phosphate (*II*) was obtained in 28% yield. Compound *II* was identified by its mass spectrum which gives the fragment of 5-(2-thienyl)uracil, arising from cleavage of the glycoside

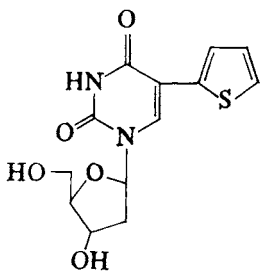
bond. The structure of *II* was further confirmed by phosphorylation of 5-(2-thienyl)-2-deoxyuridine[9] (*III*) according to the procedure of Sowa and Ouchi[10]. Nucleoside[9] *III* and other nucleosides[11,12] prepared by this photocoupling reaction were identified by mass, ultraviolet and NMR spectroscopy and compared to known standards[7] by HPLC. In all cases, only the 5-substituted nucleoside was obtained. No 6-isomer could be detected under these reaction conditions[9,11,12]. This regiospecificity was further confirmed by the phosphorylation of *III* to give the corresponding 5'-nucleotide, with mass spectrum and HPLC retention time identical with the data obtained from the above direct coupling reaction.



I



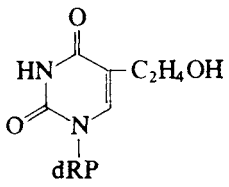
IIa



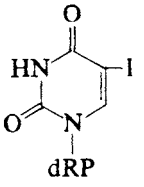
III

Similar results were obtained from the photochemical coupling reaction of *I* with other haloheteroarenes (2-iodofuran, 1-methyl-2-iodopyrrole, 3-iodothiophene) to afford the corresponding 5-(2-furyl)-, 5-(1-methyl-2-pyrryl)- and 5-(3-thienyl)-2-deoxyuridine 5-monophosphate, respectively.

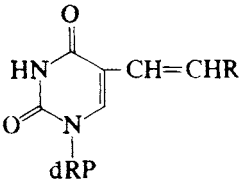
Photo-irradiation of *I* in presence of 2-iodoethanol at 254 nm afforded 5-(2-hydroxyethyl)-2'-deoxyuridine 5'-phosphate (*IV*).



IV



V



VIa, R = CN
VIb, R = $COOCH_3$

On the other hand, 5-iodo-2'-deoxyuridine 5'-phosphate (*V*), when irradiated in presence of methyl acrylate or acrylonitrile, afforded 5-(2-carbomethoxyethenyl)-2'-deoxyuridine 5'-phosphate (*VIa*), and 5-(2-cyanoethenyl)-2'-deoxyuridine 5'-phosphate (*VIb*), respectively.

Though the yield in the above reactions never exceeded 30%, the simplicity of this photochemical approach makes it more advantageous than the stepwise approaches for the preparation of C-5 modified uracil nucleotides. The overall yield in the latter cases would be approximately the same if not lower than the yield obtained by the present approach.

EXPERIMENTAL

IR spectra were measured on a Unicam S.P. 2006, and UV spectra on a Perkin–Elmer 554 recording spectrophotometer. ^{1}H NMR spectra were obtained on a Varian 56/60A, and mass spectra on a Varian CH5 mass spectrometer. C, H, N analyses were performed by Cairo University Microanalytical Center.

2'-Deoxyuridine, 2'-deoxyuridine 5'-phosphate, and 5-iodo-2'-deoxyuridine 5'-phosphate were purchased from Calbiochem. Rayonet Model RPR 100 photochemical reactor was a product of Southern New England Ultraviolet Co. HPLC analysis was performed using Partisil PXS 10/25 SAX (strong anion exchanger) column.

5-(2-Thienyl)-2-deoxyuridine 5-Phosphate (*IIa*)

Method A: A solution of disodium 2'-deoxyuridine 5'-monophosphate (500 mg, 1·42 mmol) and 2-iodothiophen (10 ml) in 100 ml of 25% water in acetonitrile was deoxygenated with argon, and irradiated at 254 nm for 24 h. The solvent was removed under reduced pressure, the residue was taken in 10 ml water, and extracted with diethyl ether (2 × 10 ml). The aqueous layer was separated on a DE-32 DEAE-cellulose column (2·6 × 40 cm) with a linear gradient of triethylammonium hydrogen carbonate buffer (0·01 to 0·3 mol l^{-1}, pH 7·5, total 600 ml). Fractions 40–45, which contained *II* as well as the starting salt as an impurity, were lyophilized and rechromatographed on DEAE cellulose (2·6 × 40 cm column) to give *II* (28% yield) as a single symmetrical peak on HPLC, (0·03 mol l^{-1} KH_2PO_4, pH 3·75, 2 ml min^{-1}), with retention time of 2·12 min. Paper chromatography, (1-butanol–acetic acid–water, 7 : 1 : 2), R_F 0·08. For C_{13}·$.H_{15}N_2SO_8P$ (390·2) calculated: 40·00% C, 3·85% H, 7·18% N; found: 39·72% C, 4·23% H, 6·85% N. Mass spectrum, *m/e* (rel. intensity): 194 (100, 5-thienyluracil), 163 (18), 142 (22), 125 (30). ^{1}H NMR spectrum (^2H_2O): δ 8·20 (s, 1 H, $C_{(6)}$—H), 7·48–7·34 (m, 3 H, heterocyclic), 6·18 (t, 1 H, J = 6·1 Hz, $C_{(1)}$—H). UV spectrum (H_2O): λ_{max} 262 nm (ε 10 300).

Method B: The procedure of Sowa and Ouchi[10] was followed for the phosphorylation of *III*. A 10 ml stock solution of the phosphorylating reagent was made at 0°C by slowly dropping (2·07 ml, 0·026 mol) of pyridine into a stirred solution of $POCl_3$ (2·15 ml, 0·024 mol) and water (0·27 ml, 0·0149 mol), followed by the addition of 55 ml of acetonitrile. The solid nucleoside (93 mg, 0·3 mmol) was added to 0·55 ml of the reagent solution. The mixture stirred at 5°C for 5 h. Ice-water (1 ml) was added, stirring continued for an additional hour at 5°C. The mixture was chromatographed on DE-32 DEAE-cellulose as indicated in the previous procedure. HPLC analysis of the product co-injected with the compound obtained by method *A*, gave a single symmetrical peak; the mass spectrum has the same fragmentation pattern as that of the product prepared by method *A*.

5(2-Furyl)-2'-deoxyuridine 5'-Phosphate (*IIb*)

It was prepared according to method *A*, using 2-iodofuran in 22% yield. For $C_{13}H_{15}N_2O_9P$ (374·2) calculated: 41·71% C, 4·01% H, 7·49% N; found: 41·34% C, 4·28% H, 7·13% N. Mass

spectrum, *m/e* (rel. intensity): 178 (100, heterocyclic base), 158 (16), 125 (30), 123 (20). ^{1}H NMR spectrum (^2H_2O): δ 7·8 (s, 1 H, $C_{(6)}$—H), 6·40—6·32 (m, 3 H, heterocyclic), 6·12 (t, 1 H, J = = 7 Hz, $C_{(1)}$—H). HPLC retention time 2·08 min. R_F value 0·09. UV spectrum (H_2O): λ_{max} 229 nm (ε 9 490).

5-(1-Methylpyrrol-2-yl)-2′-deoxyuridine 5′-Phosphate (*IIc*)

It was prepared in 18% yield according to method *A*, using 1-methyl-2-iodopyrrol. For $C_{14}H_{18}N_3$·.O_8P (387·2) calculated: 43·41% C, 4·65% H, 10·85% N; found: 43·25% C, 4·89% H, 10·98% N. Mass spectrum, *m/e* (rel. intensity): 191 (100, heterocyclic base,) 162 (34), 132 (18), 125 (42). ^{1}H NMR spectrum (^2H_2O): δ 7·54 (s, 1 H, $C_{(6)}$—H), 7·12 (m, 1 H, heterocyclic), 6·92 (m, 2 H, heterocyclic), 3·28 (s, 3 H, methyl), 6·32 (t, 1 H, J = 6·8 Hz, $C_{(1)}$—H). HPLC retention time 2·16 min. R_F value 0·1. UV spectrum (H_2O): λ_{max} 213 nm (ε 9 700).

5-(3-Thienyl)-2′-deoxyuridine 5′-Phosphate (*IId*)

It was prepared according to method *A* using 3-bromothiophene in 26% yield. For $C_{13}H_{15}N_2SO_8P$ (390·2) calculated: 40·00% C, 3·85% H, 7·18% N; found: 39·67% C, 4·19% H, 7·02% N. Mass spectrum, *m/e* (rel. intensity): 194 (100, heterocyclic base), 162 (10), 160 (14), 142 (30), 125 (18). ^{1}H NMR spectrum (^2H_2O): δ 8·2 (s, 1 H, $C_{(6)}$—H), 7·26 (m, 3 H, heterocyclic), 6·42 (t, 1 H, J = 6·3 Hz, $C_{(1)}$—H). HPLC retention time 2·10 min. R_F value 0·085. UV spectrum (H_2O): λ_{max} 238 nm (ε 11 010).

5-(2-Hydroxyethyl)-2′-deoxyuridine 5′-Phosphate (*IV*)

A solution of disodium 2′-deoxyuridine 5′-monophosphate (500 mg, 1·42 mmol) and 2-iodoethanol (10 ml) in 100 ml of 25% water in acetonitrile was deoxygenated and irradiated at 254 nm for 24 h. Removal of the solvent and purification as described above afforded (*IV*) in 24% yield. For $C_{11}H_{17}N_2O_9P$ (352·2) calculated: 37·50% C, 4·83% H, 7·95% N; found: 37·16% C, 5·09% H, 7·64% N. Mass spectrum, *m/e* (rel. intensity): 155 (100, 5-(2-hydroxyethyl)uracil), 143 (22), 140 (18), 127 (34). ^{1}H NMR spectrum (^2H_2O): δ 7·78 (s, 1 H, $C_{(6)}$—H), 6·36 (t, 1 H, J = 6·4 Hz, $C_{(1)}$—H), 3·48 (m, 2 H, CH_2OH), 2·52 (t, 2 H, J = 3·0 Hz, CH_2CH_2OH). HPLC retention time 2·26 min. R_F value 0·14. UV spectrum (H_2O): λ_{max} 300 nm (ε 14 900).

5-(2-Methoxycarbonylethenyl)-2′-deoxyuridine 5′-Phosphate (*VIa*)

A solution of 5-iodo-2′-deoxyuridine 5′-phosphate (*VI*) (100 mg, 0·21 mmol) and methyl acrylate (3 ml) in 60 ml of 25% aqueous acetonitrile was deoxygenated and irradiated at 254 nm for 24 h. Removal of the solvent and work-up as described above afforded *VIa* in 17% yield. For $C_{13}H_{17}$·.$N_2O_{10}P$ (392·2) calculated: 39·80% C, 4·34% H, 7·14% N; found: 40·17% C, 4·65% H, 6·78% N. Mass spectrum, *m/e* (rel. intensity): 195 (100, heterocyclic base), 162 (42), 136 (24), 125 (14). HPLC retention time 2·32 min. R_F value 0·17. UV spectrum (H_2O): λ_{max} 284 nm (ε 9 800).

5-(2-Cyanoethenyl)-2′-deoxyuridine 5′-Phosphate (*VIb*)

This reaction was performed as described for the previous compound, with *V* (100 mg, 1·42 mmol) and acrylonitrile (3 ml). Nucleotide *VIb* was obtained in 14% yield. For $C_{12}H_{14}N_3O_8P$ (359·2) calculated: 40·11% C, 3·90% H, 11·70% N; found: 40·36% C, 4·21% H, 11·31% N. Mass spectrum, *m/e* (rel. intensity): 162 (100, heterocyclic base), 136 (20), 135 (22), 125 (32). HPLC retention time 2·38 min. R_F value 0·19. UV spectrum (H_2O): λ_{max} 278 nm (ε 12 820).

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