Conversion of Uracil Nucleotides into Isotopically Labelled 5-Substituted Uracil Nucleotides: a Convenient Route to Thymine Nucleotides

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Unprotected uracil nucleotides and nucleosides are converted into 5-formyluracil derivatives using a palladium(II) coupling reaction followed by oxidation of the intermediate styryl derivative.

Recently Reese and Sanghvil[1] reported a convenient procedure for the conversion of 2'-deoxyuridine into thymidine using formaldehyde as the carbon source for the 5-methyl group. We report an alternative method starting with formaldehyde, that has the advantages of (i) selective introduction of carbon and/or hydrogen isotopes, (ii) generation of reactive 5-aldehyde and 5-hydroxymethyl intermediates, which are useful for further chemical modification, and (iii) application to nucleotides and polynucleotides.

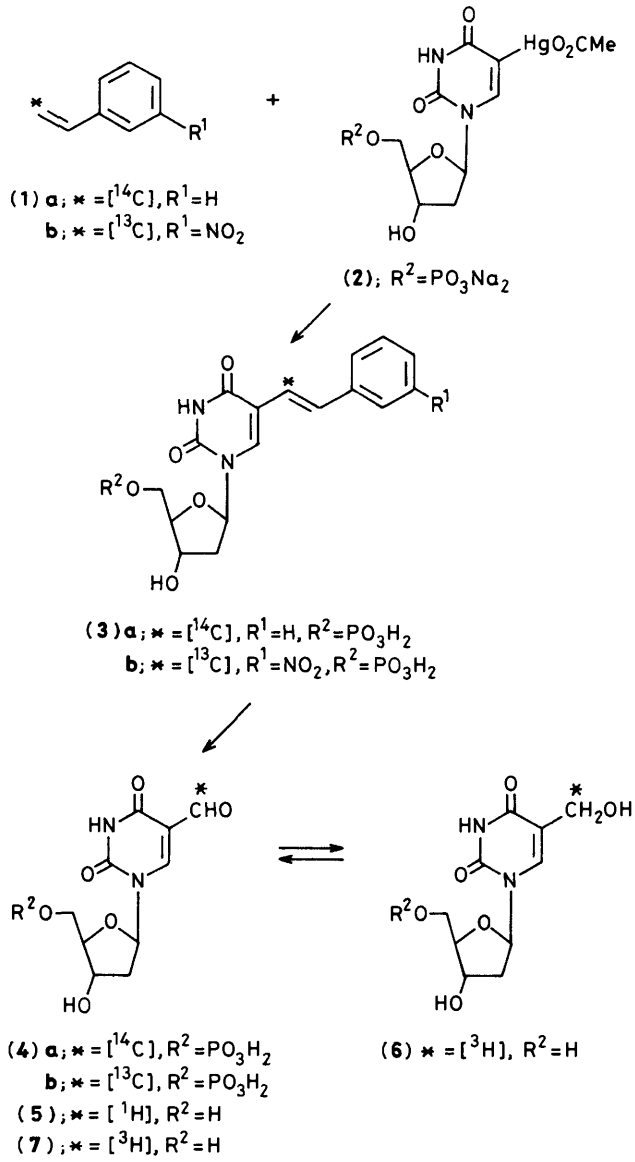
The labelled styrenes (**1**) were prepared by the addition of a saturated aqueous solution of sodium carbonate (2 equiv.) to an aqueous solution containing either [^{13}C]- or [^{14}C]-formaldehyde (1 equiv.) and the Wittig reagent (2 equiv.) prepared from either benzyl chloride or 3-nitrobenzyl chloride and triphenylphosphine.[2] After stirring for 3 h at 25 °C and filtration, a 60% yield of the styrenes (**1a**) or (**1b**) was isolated by extraction of the aqueous mixture three times with equal volumes of heptane. The dried heptane extract was evaporated carefully, and the residue of (**1**) was used without further purification.

An analytically pure† sample of the [^{13}C]styryl nucleotide (**3b**) was obtained in 30% yield using the following general procedure [(**3a**) was formed in 50% yield]. An aqueous solution (15 ml) containing the disodium salt of 2'-deoxyuridine 5'-phosphate (2.5 mmol) and mercury(II) acetate (4.4 mmol) was heated to 55 °C for 5 h.[3] The styrene (**1a**) or (**1b**) (3.2 mmol) in tetrahydrofuran (10 ml) and a 0.1 M solution of lithium tetrachloropalladate in methanol (28 ml) was added to the aqueous solution containing the 5-mercurioacetate derivative (**2**), and the mixture was heated overnight at 50 °C under an inert atmosphere.[4,5] The product (**3a**) or (**3b**) was isolated by resolution on DEAE-cellulose.[5]

Oxidation of the styryl derivatives (**3**) to the 5-formylnucleotides (**4**) was accomplished by stirring a mixture of (**3**) (1 equiv.), osmium tetroxide (1.5 mol. equiv.), sulphuric acid (14 mol. equiv.), and sodium metaperiodate (4 mol. equiv.) in 80% acetone–water for 4 h at 0 °C. After neutralization of the acid, concentration, and extraction with diethyl ether the aqueous solution was resolved on DEAE-cellulose to give either (**4a**) or (**4b**) in 75% yield.[6,7]

The introduction of hydrogen isotopes on the 5-substituent is illustrated by the following method. 5-Formyl-2'-deoxyuridine [(**5**) 0.08 mmol] in 5 mM phosphate buffer (pH 7) (1.5 ml) and low specific activity sodium [^{3}H]borohydride (0.1 mmol) was stirred for 1 h at 25 °C. After acidification with acetic acid, 5-[^{3}H]hydroxymethyl-2'-deoxyuridine[7,8] (**6**) was purified by paper chromatography. Reoxidation of (**6**) to give the [^{3}H]aldehyde (**7**) was accomplished by the method of Imai and Honjo;[9] oxygen was bubbled through a 50% acetic acid solution of (**6**) and freshly prepared platinum oxide for 72 h. The product (**7**) was isolated using either silica gel or paper chromatography.

† Spectral properties (u.v., n.m.r.), h.p.l.c., and C, H, and N analysis [(**3a**), (**3b**), (**4a**)] confirmed the structures of the products in this report.



The procedures described in this report are useful for the introduction of carbon and hydrogen isotopes on a one carbon unit substituted on C-5 of uracil nucleosides and nucleotides. As the available literature reports that the reduction of the hydroxymethyl derivative (**6**) to the corresponding thymine derivative[10] and the oxidation of the formyl compounds (**4a**) and (**4b**) to the corresponding carboxyuracil derivatives are reasonable,[8] the synthesis of uracil derivatives substituted at C-5 with a one carbon unit at any of the four oxidation states is readily achieved. Furthermore, it has been shown that the primary coupling reaction works well with ribonucleotides[5] and with polyribonucleotides.[2]

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References

1 C. B. Reese and Y. S. Sanghvi, *J. Chem. Soc, Chem. Commun.*, 1983, 877.
2 C. F. Bigge, K. E. Lizotte, J. S. Panek, and M. P. Mertes, *J. Carbohydr., Nucleosides, Nucleotides*, 1981, **8**, 295.
3 R. M. K. Dale, E. Martin, D. C. Livingston, and D. C. Ward, *Biochemistry*, 1975, **14**, 2447.
4 D. E. Bergstrom and J. L. Ruth, *J. Am. Chem. Soc.*, 1976, **98**, 1587.
5 C. F. Bigge, P. Kalaritis, J. R. Deck, and M. P. Mertes, *J. Am. Chem. Soc.*, 1980, **102**, 2033.
6 A. Kampf, C. J. Pillar, W. J. Woodford, and M. P. Mertes, *J. Med. Chem.*, 1976, **29**, 909.
7 M. P. Mertes and M. T. Shipchandler, *J. Heterocycl. Chem.*, 1971, **8**, 133.
8 R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, 1959, **81**, 2521.
9 K. Imai and M. Honjo, *Chem. Pharm. Bull. Jpn.*, 1965, **13**, 1.
10 G. L. Bubbar and V. S. Gupta, *Can. J. Chem.*, 1970, **48**, 3417.