Sequence Dependent Photochemistry of Di(deoxynucleoside) Phosphates containing 4-Thiouracil

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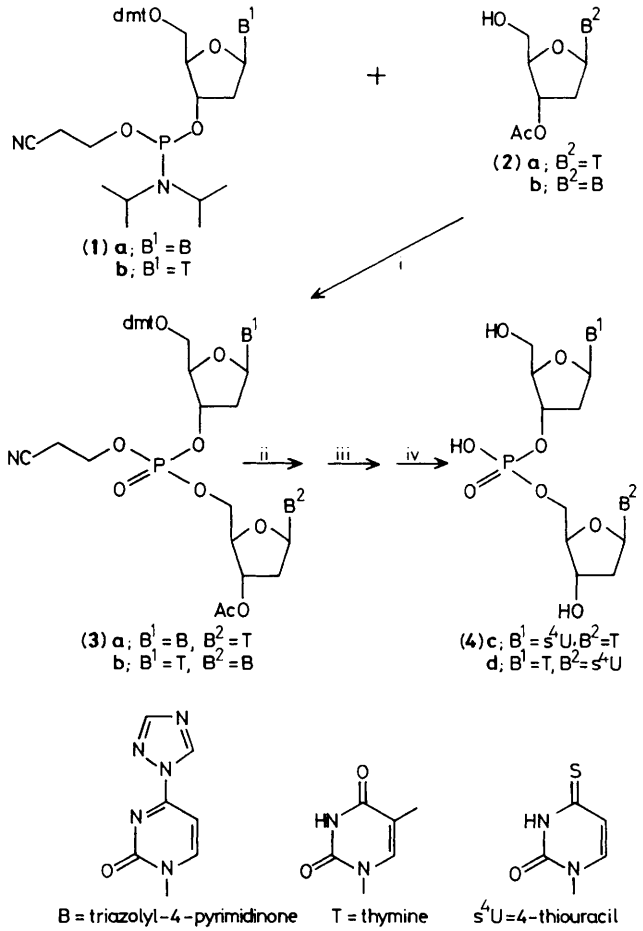
The photochemistry of (dideoxynucleoside) phosphates (**4c**) and (**4d**) is strictly sequence dependent.

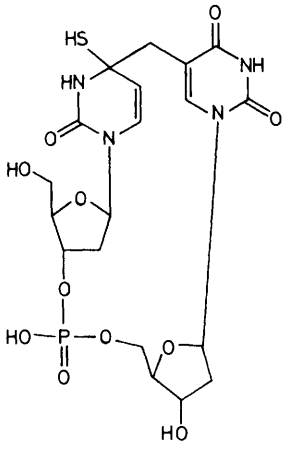
The development of chemical and photochemical probes has proven to be quite valuable for deciphering the spatial relationships within nucleic acid assemblies (particularly RNAs) and nucleoprotein complexes.[1] The advantages are evident when such tools for structural investigations can be activated *in vivo* as well as *in vitro*. Also, indisputable information can be extracted if the photochemical pathways which lead to probe cross-linking are precisely known.

In this respect the design of 4-thiouracil (s^4U), a close analogue of uracil, holds great promise as a photochemical probe for nucleic acids. It can be activated photoselectively in RNAs and its photochemistry is known to produce two main types of reactions (presumably *via* its triplet state[2]). In one case it undergoes [2 + 2] cycloadditions with alkenic compounds[3] and in the other case there are coupling reactions with derivatives having hydroxy[4] or amino[5] functional groups, *via* hydrogen abstraction and radical recombination. These reactions can be observed in biological systems. Thus, upon selective irradiation of s^4U in tRNA, present at position 8 of 70% of *Escherichia coli* tRNAs, a crosslinking reaction occurs with a cytosine at position 13.[6] This reaction reveals a salient feature of tRNA three-dimensional structure[7] and causes remarkable biological effects.[8] Moreover, 4-thiouridine can be used as a uridine analogue to incorporate s^4U in the RNA of prokariotic and eukariotic cells. In the case of *E. coli, in vitro* photoactivation of purified ribosomes has triggered the efficient formation of RNA–RNA and RNA–protein cross-links.[9]

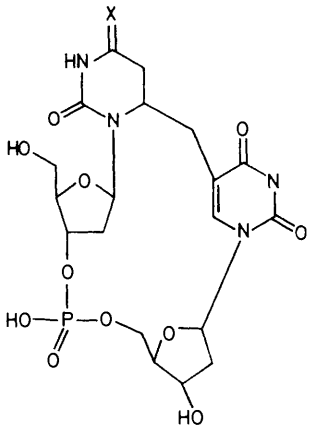
Before the full scope of the potential uses of s^4U as a photoaffinity probe can be realized, it is necessary to examine its photophysics and photochemical reactions in simple systems. Short oligonucleotides are ideal for these studies



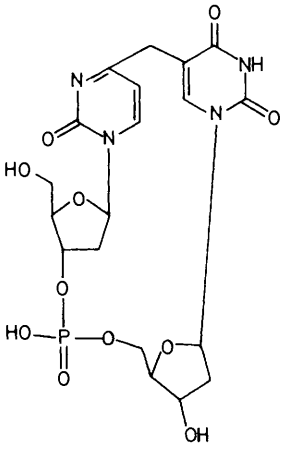
Scheme 1. *Reagents*: i, tetrazole, MeCN then I_2, tetrahydrofuran–pyridine–H_2O; ii, H_2S, Pr_2NH, CH_2Cl_2; iii, NH_4OH–pyridine; iv, 80% AcOH. Purification by cellulose preparative t.l.c.



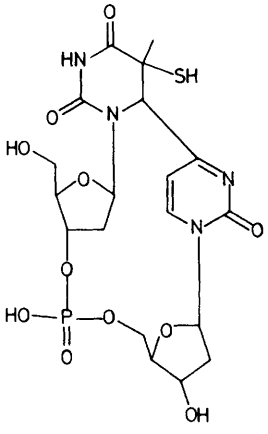
(5)



(6); X = S
(8); X = O



(7)



(9)

since they have simple well defined sequences and high resolution atomic level information can be obtained. Here we report the unexpected behaviour of s^4U in 5′-*O*-(2′-deoxy-4-thiouridylyl)thymidine (**4c**) and 2′-deoxy-5′-*O*-thymidylyl-4-thiouridine (**4d**), which were found to undergo strictly sequence dependent photoreactions.[10]

The two dimers were prepared[11] as indicated in Scheme 1 by application of 4-(triazolyl)pyrimidin-2-one chemistry pioneered by Sung,[12] which has been illustrated by various syntheses of base modified oligonucleotides.[13] Here the phosphoramidite intermediate (**1a**) was found appropriate for the preparation of (**3c**) [or (**3b**)]. Displacement of the triazolyl group in (**3a**) [or (**3b**)] by the hydrosulphide ion produced derivative (**3c**) [or (**3d**)]. Usual deprotection manipulations gave the dideoxynucleotides (**4c**) and (**4d**), which were completely characterized by their spectral data [fast atom bombardment (f.a.b.) m.s., u.v., and n.m.r. spectroscopy].

Irradiation† of (**4c**) (s^4UpT) resulted in its rapid disappearance, which was accompanied by the formation of two photoproducts, presumably (**5**) and (**6**); they were too unstable to be isolated. The resultant end products, after separation by successive preparative cellulose t.l.c. and RP 18 reverse phase h.p.l.c., were assigned structures (**7**) and (**8**). The f.a.b.m.s. and u.v. spectroscopic data of compounds (**7**) and (**8**) agree with the proposed structures. The ^1H and ^{13}C n.m.r. spectra indicated the absence of a methyl signal, which suggests that a methylene radical has been generated by hydrogen abstraction from the methyl at the C-5 position of the thymine moiety. Radical recombination at the C-4 and C-6 positions of the 4-thiouracil residue gave (**5**) and (**6**), respectively.

In the case of compound (**5**), hydrogen sulphide elimination led to (**7**), while the alkali unstable 5,6-dihydro-4-thiouracil derivative (**6**) was transformed into the corresponding 5,6-dihydrouracil (**8**).‡ Evidence from n.m.r. spectroscopy supports the proposed structures. The ^1H n.m.r. spectrum of (**7**) exhibits three alkenic proton signals. Two are mutually coupled and the third is a broad singlet which shows allylic coupling with the methylene protons located on the carbon bridging the two pyrimidines (long range COSY).[15] By contrast, the ^1H n.m.r. spectrum of (**8**) is characterized by the presence of only one alkenic signal. The other spectral data can be interpretated on the basis of a 5,6-dihydrouracil system which is substituted at position C-6.

† Broad band u.v. light (335—360 nm), super pressure HBO 350 W lamp. Quantum yield for s^4UpT photolysis: $\phi = 3.6 \pm 0.2 \times 10^{-2}$. For Tps^{4}U, ϕ is three to four times lower. Purification of photoproducts by preparative cellulose t.l.c. (n-propanol: water: conc. ammonia, 55 : 35 : 10) gave compounds (**7**), (**8**) and (**9**) whose respective yields were 37, 31 and 45%.

‡ For cellulose t.l.c. a solvent system containing ammonia was used; under these conditions 5,6-dihydro-4-thiouracil is transformed into unstable 5,6-dihydrocytosine.[14]

Upon irradiation[14] of (**4d**) (Tps^{4}U) a single compound (**9**) was observed. F.a.b.m.s. data indicate that compound (**9**) has the same molecular weight as the parent molecule. The u.v. spectrum of (**9**) was characteristic of a 4-substituted pyrimidin-2-one chromophore, suggesting the formation of a 6–4 bond between thymine and 4-thiouracil. Such a bipyrimidine coupling product recently has been isolated after short wavelength irradiation of 5′-*O*-thymidylylthymidine, which produced a compound labelled T6pT4.[16] Indeed, the n.m.r. spectroscopic data of (**9**) and those reported for T6pT4 show a great number of indicative similarities. Compared to compound (**4d**) the H-6 signal of s^{4}U is shifted downfield by 0.48 p.p.m., and the singlet due to the H-6 proton of thymine in (**4d**) appears as a sharp singlet at δ 5.15. This is in agreement with the observations made in the case of T6pT4. Clearly compound (**9**) arose from [2 + 2] cycloaddition and ring opening of the resulting thietane.

In conclusion, the two closely related di(deoxynucleoside) phosphates (**4c**) and (**4d**) differ drastically in their photochemical behaviour. Interestingly, compound (**7**) represents the first example of a photochemical reaction product resulting from a radical addition at position C-4 of 4-thiouracil. It is obvious that the photochemistry of (**4c**) and (**4d**) is governed by the conformational transitions of their phosphodiester backbone, which manifests a strict sequence effect. To date, such sharp differences cannot be rationalized on the basis of the n.m.r. spectroscopic studies of the starting products. Also, molecular modelling[17] investigations of the various possible reaction products do not reveal any significant differences in stability which might favour one pathway over the other for both dimers. Analysis of a number of related molecules and their photochemical reactions may provide a better understanding of their conformational behaviour and reactivity in solution. Such studies are essential for further optimizing the development of s^{4}U as an intrinsic photochemical probe for biomolecules.

Received, 15th March 1989; Com. 9/01122F

References

1 J. Welsh and C. R. Cantor, *Trends Biochem. Sci.*, 1984, **10**, 505; R. Starzyk, *ibid.*, 1985, **11**.
2 A. Favre, *Photochem. Photobiol.*, 1974, **19**, 15; S. J. Midler and D. S. Kliger, *J. Am. Chem. Soc.*, 1985, **107**, 7365.
3 P. Jouin and J.-L. Fourrey, *Tetrahedron Lett.*, 1975, 1329 and references cited therein.
4 J.-L. Fourrey and P. Jouin, *Tetrahedron Lett.*, 1973, 3225.
5 J.-L. Fourrey and P. Jouin, *Tetrahedron Lett.*, 1976, 297.
6 A. Favre, M. Yaniv, and A. M. Michelson, *J. Mol. Biol.*, 1971, **58**, 367; D. S. Carre, G. Thomas, and A. Favre, *Biochimie*, 1974, **56**, 1089.
7 Y. Lemaigre Dubreuil, L. Kaba, B. Hajnsdorf, and A. Favre, *Biochemistry*, 1986, **25**, 5726 and references cited therein.
8 Growth delay photoprotection: J. Jagger, *Photochem. Photobiol.*, 1975, **22**, 67; M.-O. Blondel and A. Favre, *Biochem. Biophys. Res. Commun.*, 1988, **150**, 979. Antiphotomutagenic effect: A. Caldeira de Araujo and A. Favre, *EMBO J.*, 1986, **5**, 175.
9 A. Expert-Bezancon, R. Bezerra, E. Hajnsorf, Y. Lemaigre Dubreuil, and A. Favre, *Photochem. Photobiophys., Suppl.*, 1987, 33.
10 Interestingly 4-thiouridine was shown to interact photochemically with thymine residues of DNA: A. Ito, F. T. T. Robb, and J. G. Peak, *Photochem. Photobiol.*, 1988, **47**, 231.
11 For a preparation of dimers containing s^{4}U see; J. H. van Boom, F. M. J. Burgers, J. den Hartog, and G. van der Marel, *Recl. Trav. Chim. Pays-Bas*, 1976, **95**, 108.
12 W. L. Sung, *J. Org. Chem.*, 1982, **47**, 3623; *J. Chem. Soc., Chem. Commun.*, 1982, 522.
13 T. Huynh-Dinh, N. Duchange, M. M. Zakin, A. Lemarchand, and J. Igolen, *Proc. Natl. Acad. Sci. U.S.A.*, 1985, **82**, 7510; T. R. Webb and M. D. Matteuci, *Nucl. Acids. Res.*, 1986, **14**, 7661; S. Pochet, T. Huynh-Dinh, and J. Igolen, *Tetrahedron*, 1987, **43**, 3481.
14 V. Skaric, B. Gaspert, M. Hohnjec, and G. Lacan, *J. Chem. Soc., Perkin Trans. 1*, 1974, 267.
15 A. Bax and R. Freeman, *J. Magn. Reson.*, 1981, **44**, 542.
16 R. E. Rycyna and J. L. Alderfer, *Nucleic Acids Res.*, 1985, **13**, 58; J.-S. Taylor, D. S. Garett, and M. P. Cohrs, *Biochemistry*, 1988, **28**, 7206.
17 W. C. Still, N. G. J. Richards, W. C. Guida, M. Lipton, G. Liskamp, G. Chang, and T. Hendrickson, Macromodel V 1.5, Department of Chemistry, Columbia University, New York 10027, U.S.A.