SYNTHESIS AND PHOTOCHEMISTRY OF OLIGONUCLEOTIDES CONTAINING THIOSUBSTITUTED NUCLEOBASES. MODEL PHOTOREACTIONS FOR DNA PHOTOLESIONS.

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A convenient method for the preparation of oligonucleotides containing thiosubstituted nucleobases at selected positions was devised. It uses the standard phosphoramidite or H-phosphonate derivatives of the corresponding thionucleosides, where the thioamide function has been protected by S-pivaloyloxymethylation. This protection methodology is fully compatible with the current synthetic procedures of oligonucleotide construction and does not require any protocol modification. A specific affinity gel electrophoresis technique has been employed for their complete purification. Compared to the normal oligonucleotides, these oligonucleotides exhibit a characteristic absorption in the 320-370 nm region. The photochemical behavior of a number of dinucleoside phosphates containing either thiopyrimidine or thiopurine was shown to give a variety of new photoproducts. The formation of (6-4) bipyrimidines in good overall yield via thietanes, which for the first time were fully characterized by nmr spectroscopy, is relevant to the formation of such lesions in DNA.

Keywords: modified oligonucleotides synthesis; thionucleobases photoadducts; (6-4) bipyrimidines; model DNA photolesions.

INTRODUCTION

Life has developed under continuous sunlight irradiation where the electromagnetic spectrum spreads from infrared to ultraviolet (UV) radiations. Due to the ozone layer, the most energetic solar radiations reaching the earth's surface have wavelengths longer than 290-300 nm, which nevertheless overlap significantly the absorption spectra of the nucleobase components of nucleic acids. Once absorbed these UV photons trigger the formation of lesions within these macromolecules. As nucleic acids play a central role in the transmission and expression of genetic information, these lesions are ultimately responsible for a variety of biological responses such as cell killing, photomutagenesis and photocarcinogenesis. This has prompted the considerable development of the ongoing studies in the field of nucleic acids photochemistry[1].

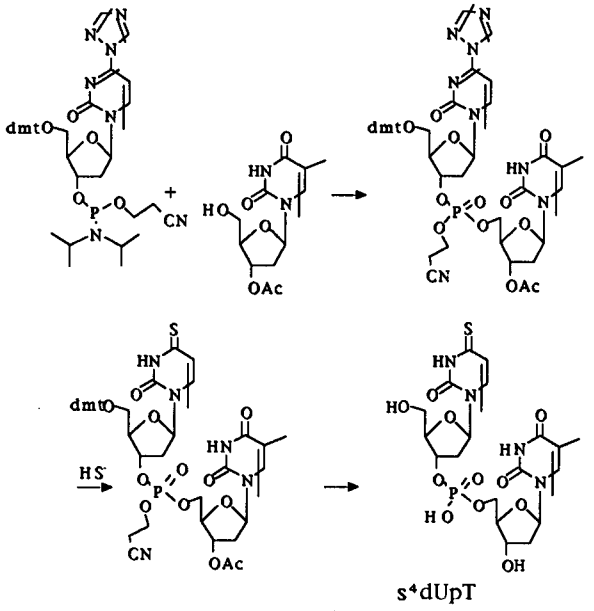
With the advent of the powerful techniques of molecular biology, it became possible to analyze the mutation spectra induced within a mammalian cell context by the introduction of a defined lesion - restricted so far to abasic sites - at a preselected position of a single-stranded or double-stranded shuttle vector[2]. To extend the flexibility of this approach, it is useful to directly trigger the photolesions at a defined site of a DNA fragment, which cannot be easily achieved with standard DNA because all its base components have strongly overlapping absorption spectra centered around 260 nm. This could be potentially accomplished however by using natural or synthetic nucleotides exhibiting red-shifted absorption. Particularly interesting in this respect is the case of 4-thiouridine, which is a minor component of *E.coli* tRNAs. This extremely photoreactive nucleoside strongly absorbs light in the 320-370 nm region. Its selective photoactivation within the *E.coli* cell triggers the formation of the 8-13 link in tRNA that results in a number of specific photobiological effects such as growth delay, photoprotection and cell volume reduction. Another minor thionucleoside present in tRNA, 5-methylaminomethyl-2-thiouridine was also found to be involved in these photobiological effects[3]. Hence thio-substituted nucleic acid bases appear particularly attractive with respect to their absorption and photochemical properties.

As a step towards the goal defined above, we have extended an earlier procedure for the synthesis of oligonucleotides containing thio-substituted residues (4-thiouracil, 4-thiothymine, 6-mercaptopurine and 6-thioguanosine) at selected positions. The investigations concerning the photochemical behaviour of such oligonucleotides have already led to important data in two important domains. The first one is related to the understanding of the mechanisms of the reactions leading to the formation of DNA lesions. The other concerns the deciphering of the tertiary interactions within single strand nucleic acids, which is a problem of considerable interest since only a few methods are available at present for this purpose[4]. The present review article will be focused on the synthesis and the study of the photochemical behaviour of short oligonucleotides containing sulfur-substituted nucleobases.

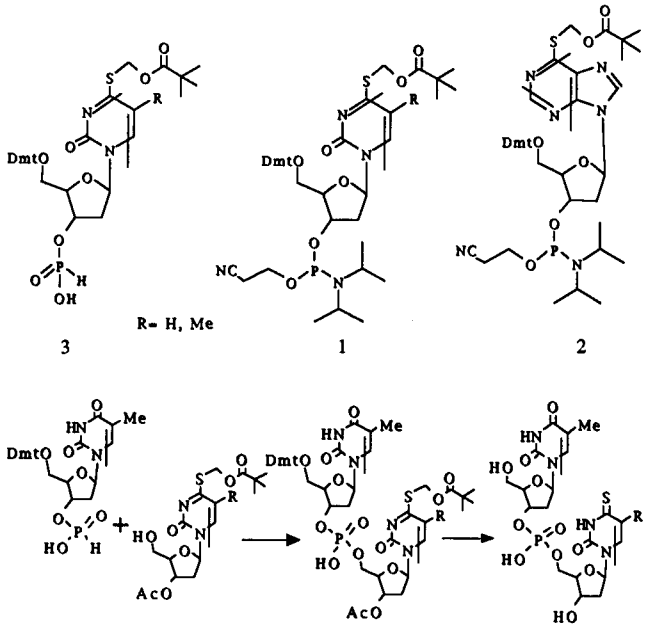
SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING SULFUR-SUBSTITUTED NUCLEOBASES

A few years ago, no method was available to incorporate sulfur-substituted residues into an oligonucleotide. The thioamide group is highly nucleophilic and could interfere with the phosphorylation reactions. It is furthermore highly sensitive to oxidation by iodine, which is a reagent used to convert phosphorus III into phosphorus V derivatives in current synthetic procedures. We first prepared the deoxydinucleoside phosphates ds^{4}UpN and dNps^{4}U, where N is a standard deoxynucleoside and s^{4}U is 4-thiodeoxyuridine. For example, ds^{4}UpT results from the condensation of a phosphoramidite derived from 4-triazolypyrimidinone with 3'-O-acetylthymidine. After oxidation, the triazole group was displaced by the hydrosulfide ion SH^- before the final ammonia deprotection step (Scheme 1)[5].

In order to create a synthetic procedure that is easily applicable in oligonucleotide synthesis, we have searched for a



Scheme 1



Scheme 2

protecting group of the thiocarbonyl function present in 4-thiopyrimidinone and 6-mercaptopurine nucleoside derivatives. To be most appropriate this group should be stable to the acidic conditions used to remove the dimethoxytrityl group attached to the 5'-end of the oligonucleotide, inert to iodine oxidation and should be readily eliminated following the final deprotection step (ammonia treatment). The most satisfactory results were obtained with the pivaloyloxymethyl group used to mask the thiol function. We have thus prepared a series of phosphoramidite synthons (Scheme 2) containing sulfur-substituted pyrimidines or purines (**1a**, **1b**, **2**) that can be directly used for solid phase synthesis with an automatic synthesizer[6]. Hence various oligodeoxynucleotides or mixed deoxyribooligonucleotides were synthesized. After size selection by PAGE, the oligonucleotides were subjected to affinity electrophoresis according to Igloi[7], which gave modified oligonucleotides containing sulfur-substituted nucleic acid bases with yields up to 80%. Recently other synthetic procedures have been proposed for such oligomers[8]. As a general rule they require a longer sequence than ours to obtain the appropriate synthons as well as an additional deprotection step when used in automated synthesis.

In order to prepare in solution large amounts of short oligonucleotides required for our photochemical studies, we have synthesized the corresponding S-protected H-phosphonate intermediates **3** which were applied successfully. This demonstrates the usefulness of the pivaloyloxymethyl group in this phosphorylation procedure[9].

COLLISIONNAL PHOTOREACTIVITY OF 4-THIOURIDINE IN SOLUTION

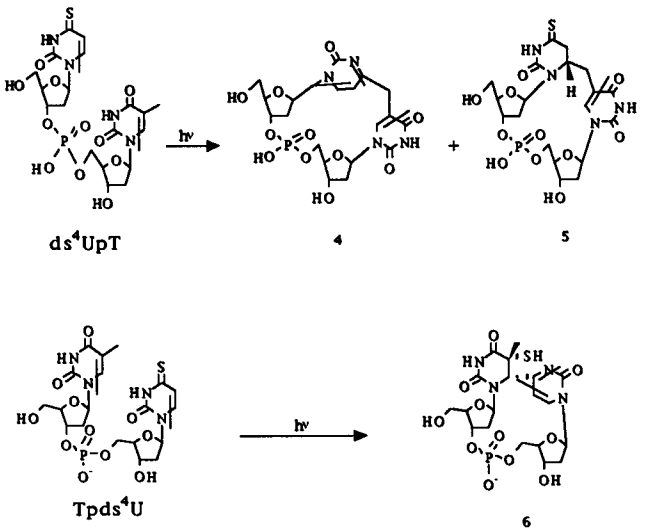
Photoexcited 4-thiouracil derivatives were known to photoreact with the 5-6 double bond of the pyrimidines U, C, s^4U, as well as other olefinic compounds by a (2+2) cycloaddition. In the presence of hydrogen donors, they also yield coupling reactions via hydrogen abstraction and radical recombination[10]. In order to extend the potential use of 4-thiouridine as an intrinsic photoaffinity probe, the interactions of its photoreactive triplet state (τ ~ 200 ns) with current nucleosides at ~300°K in aqueous solution were examined. All current nucleosides at concentrations in the millimolar range are able to quench the s^4U triplet state and stimulate s^4U photolysis by random collisions. Their quenching efficiencies decreased in the following order: T >A >U >C, which closely parallels their ability to stimulate s^4U photolysis (T >A ~U >C).

The formation of stable covalent coupling under the same conditions between the ^{32}P-labelled deoxytrinucleotide ds^4UCC and acceptor nucleosides or homodecanucleotides was monitored by an electrophoresis gel retardation assay. All acceptors were able to yield thermally stable adducts according to a collisional mechanism. The reactivity of free nucleosides, T >U ~A >C >G, was found altered in the presence of decanucleotides due to the increased target size and electrostatic repulsion. The observed efficiency ranging in the order (T_{10}) >$d(U_9A)$ >$d(C_{10})$ >$d(A_{10})$ strongly suggests that in collisional photoadduct formation, which prevails at high acceptor concentrations (>10^{-3}M), base stacking plays a primary role[11].

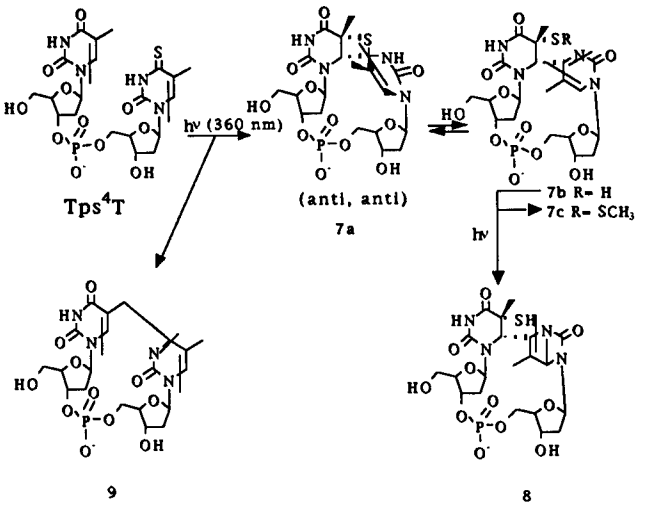
INTRAMOLECULAR PHOTOCROSSLINKING IN DINUCLEOTIDES. MECHANISM OF FORMATION OF (6-4) BIPYRIMIDINE DNA LESIONS

Dinucleotides were used to examine the photochemical behaviour of sulfur-containing bases as they are relatively easy to handle and furthermore represent the simplest model for a polynucleotide chain. As thio-substituted bases are close analogues of the parent natural bases, it was hoped that their photoreactions could, in appropriate cases, allow a better understanding of the mechanism of formation of photoadducts in UV irradiated DNA.

Both isomeric dinucleotides ds^4UpT and $Tpds^4U$ are highly photoreactive since they photoreact in aqueous solution with quantum yields in the range of 1 to $2x10^{-2}$ as compared to less than 10^{-3} for free 4-thiouridine (10^{-4} M) in the same conditions. Surprisingly, their photochemistry is absolutely sequence-dependent. Irradiation of ds^4UpT yields two photoproducts of structure **4** and **5** in agreement with spectroscopic data (Scheme 3)[5]. The detailed three-dimensional reconstruction of the solution state of these compounds was performed using



Scheme 3



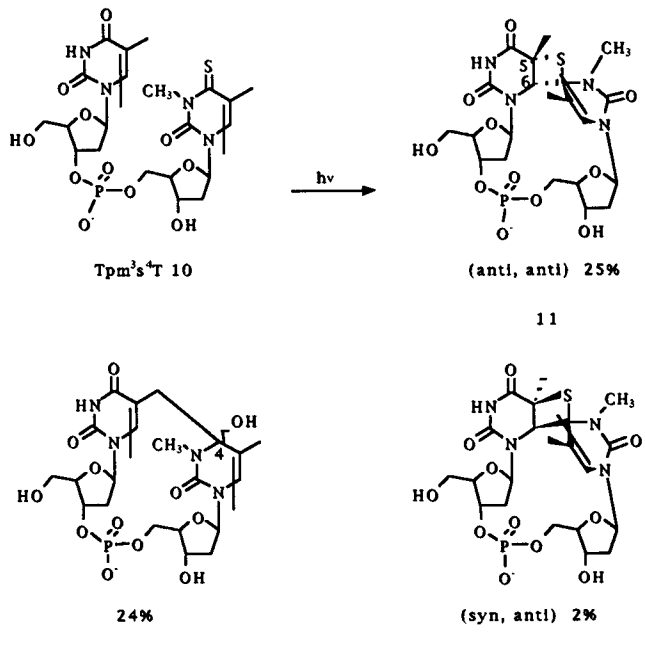
Scheme 4

2D-NMR guided molecular modelling. In particular the new chiral center in **5** displays a 6-(*S*) configuration[12].

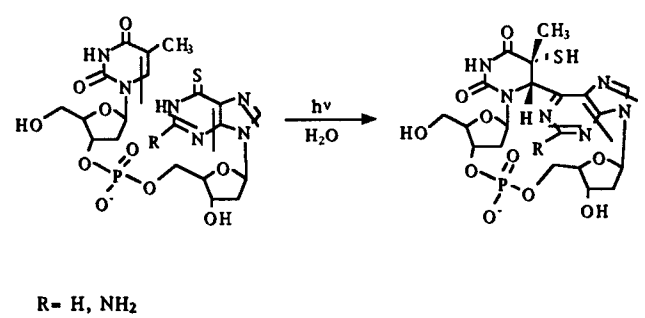
Tpds⁴U on the other hand yields a single (6-4) bipyrimidine photoadduct **6**. This type of adduct corresponds to one of the major photolesions which occur at dipyrimidine sites in DNA when exposed to solar UV, and are known to play a key role in mutagenic and lethal effects. These lesions have also been recognized as causative of tumor development[13]. Accordingly the photoreaction observed in Tpds⁴U could be used to introduce (6-4) bipyrimidines in oligonucleotides. This would allow the study of the structural perturbation induced by this kind of adduct in DNA duplexes and serve to study its repair *in vitro* .

It is worth noting that ds⁴UpT and Tpds⁴U exhibit a radically distinct photochemical behaviour. In the first case, the photoreaction proceeds by a radical mechanism with abstraction of a hydrogen atom from the methyl group of thymine. In the case of Tpds⁴U, on the other hand, it is a (2+2) cycloaddition which, due to the geometric constraints in the dinucleotide, occurs with a regioselectivity distinct of the one observed in tRNA, where s⁴U led to the quantitative formation of a (5-4) bipyrimidine link[1].

Irradiation of Tps⁴T yields four photoproducts (Scheme 4). The main fraction consisted of a mixture of two slowly interconverting compounds **7a**, **7b**, in neutral water (ratio 3/1). **7a** is the primary thietane photoproduct resulting from (2+2) cycloaddition while **7b** is its opened form. Addition of methyl methanethiolsulfonate to this mixture completely displaces the equilibrium towards the methyl disulfide **7c**. The two minor photoproducts are the Dewar product **8** and the addition compound **9** formed by H abstraction and hydrogen sulfide elimination. The characterization of thietane **7a** definitively establishes the stereochemical course of the reaction leading to (6-4) photoproducts. In particular, the cis relationship found between the C-5 hydroxyl amino or thiol groups and the C-6 pyrimidinone rings of the (6-4) photoproducts, respectively, is governed by the stereochemistry of the four-membered ring intermediate (thietane, oxetane, azetidine). Hence during the (2+2) cycloaddition, the two nucleoside partners exhibit an anti conformation, which is retained during the four-membered-ring opening. These data are of interest with respect to formation of (6-4) bipyrimidines in DNA[14]. Furthermore, prevention of the thietane ring opening occurs



Scheme 5



Scheme 6

when the exchangeable H at position N-3 of s^4T is substituted by a stable methyl group as in **10**, which creates two photoadducts after irradiation. The major one **11**, is the thietane formed with both nucleosides in the anti conformation as found in DNA (Scheme 4). The minor one **12** results from a cycloaddition where the 5' nucleoside is syn while the 3' one is anti. It is noteworthy that such a cycloaddition pathway has never been reported or discussed before[15].

Few data are known at present on the photochemical behaviour of thiopurines. The photochemistry of 6-thiodeoxyinosine (s^6dI) and 6-thiodeoxyguanosine (s^6dG) were investigated in the deoxydinucleoside phosphate series. Upon 335 nm irradiation both Tps^6dI and Tps^6dG yielded exclusively the corresponding (6-6) pyrimidine-purine compounds (Scheme 6), again presumably formed through a thietane intermediate. These unprecedented adducts correspond to new "artificial" nucleic acids photoproducts[4].

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