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Recent achievements in photochemistry of some components and analogues of nucleic acids

Stefan Paszyc

Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

Abstract

The occurrence of a significant reaction between pyrimidine and purine derivatives has recently been shown using dinucleotide monophosphates and related analogues irradiated with conventional monophotonic far-UV light. This reaction gives a new insight into the photochemical behaviour of nucleic acids and may have mutagenic and lethal consequences in vivo.

Photoreactions of amino acids and pyrimidines, as models of nucleic acid–protein cross-linking, play a significant role in the understanding of the deleterious effects of UV irradiation on cells. Unfortunately, only in a few cases have the photo-cross-linked products been identified unequivocally.

Increasing interest in the application of powerful laser sources for the investigation of nucleic acids and their components has been prompted by the possibility of free radical generation. Ultrahigh intensity laser radiation has made it possible to identify the main products resulting from the reactions of nucleic acid components of DNA and DNA itself with radicals such as e_{aq}^-, H and OH.

The luminescence properties of new nucleoside derivatives, photochemically synthesized in our laboratory and emitting in the visible region, are presented and discussed.

Keywords: Nucleic acids; Luminescence; Pyrimidine derivatives; Purine derivatives

1. Introduction

The deleterious effects of far-UV light on living systems can be explained, at least partly, in terms of photoinduced base lesions in nucleic acids [1,2]. The discovery that the action spectrum for killing cells by UV light is at a maximum in the region 260–265 nm implicates DNA and RNA as the principal absorbers. If proteins were involved to any great extent the action spectrum would peak at 280 nm.

The major photoproducts of pyrimidine bases and DNA photochemistry are cyclobutane (5–6) dipyrimidines, hydrates and pyrimidine–pyrimidone (6–4) adducts. Contrary to earlier reports, purines undergo photochemical reactions. The monomerization of cyclobutane dipyrimidines is of great importance, especially Thy〈〉Thy because of its biological significance as a model of photoreactivation processes.

Because DNA is the basis of the storage, transmission and expression of genetic information, any damage caused to it will have important consequences.

The occurrence of a reaction between pyrimidine–purine derivatives has recently been shown using dinucleotide monophosphates and related analogues irradiated with conventional monochromatic far-UV light [3,4].

Photoinduced DNA–protein cross-links represent a major class of far-UV damage in living systems. During the last few years, emphasis has been placed on the isolation and characterization of the far-UV-irradiation-induced photoadducts of the most reactive amino acids to DNA and related model compounds [5].

Growing interest in the non-linear laser photochemistry of nucleic acids, their components and related model compounds is evident [6,7]. The most important feature of a powerful laser is its ability to deliver high energy in a short pulse of nanosecond, picosecond or femtosecond duration. On UV irradiation of purine and pyrimidine DNA with intense picosecond laser radiation, two-quantum excitation to a high-lying S_n or triplet T_n level occurs through the corresponding S_1 and T_1 states [8].

Fluorescent derivatives of nucleic acid bases are of particular interest in view of their possible applications in various studies of nucleic acids, especially dynamics and conformation [9,10].

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2. Photoreactions of pyrimidines, purines and derivatives in solution

2.1. Pyrimidines

It is generally accepted that the photodimerization of pyrimidine residues in nucleic acids is responsible for the majority of the lethal effects induced in living systems by UV light. The formation of four isomeric cyclobutane photodimers of thymine is well established. Four stereoconfigurations of cyclobutadithymidines have also been isolated and identified [11].

Pyrimidine dimer splitting Thy⟨⟩Thy can be induced by several species and methods. These include hydrated electrons, photoexcited electron acceptors, flash photolysis and pulse radiolysis [8]. The electron-donating systems which photosplit dimers by electron donation are indoles, anilines and dihydroflavins. The important role of DNA photolyases in the photoconversion of pyrimidine dimers into pyrimidines in the repair of UVA-damaged DNA should also be mentioned [12,13].

The photosensitized cleavage of cyclobutane rings of pyrimidine dimers has been the subject of intense research primarily because of its biological significance as a model of the photoreactivation process [14,15]. Our interest in the photosensitized monomerization of pyrimidine dimers was stimulated by the observation of stereoselectivity of the reaction. It has been shown that syn-type isomers cleave faster than anti-type isomers under the same reaction conditions [16]. In our work [17], chloranil- and 9,10-dicyanoanthracene-sensitized reactions of 1,3-dimethylthymine dimers were investigated.

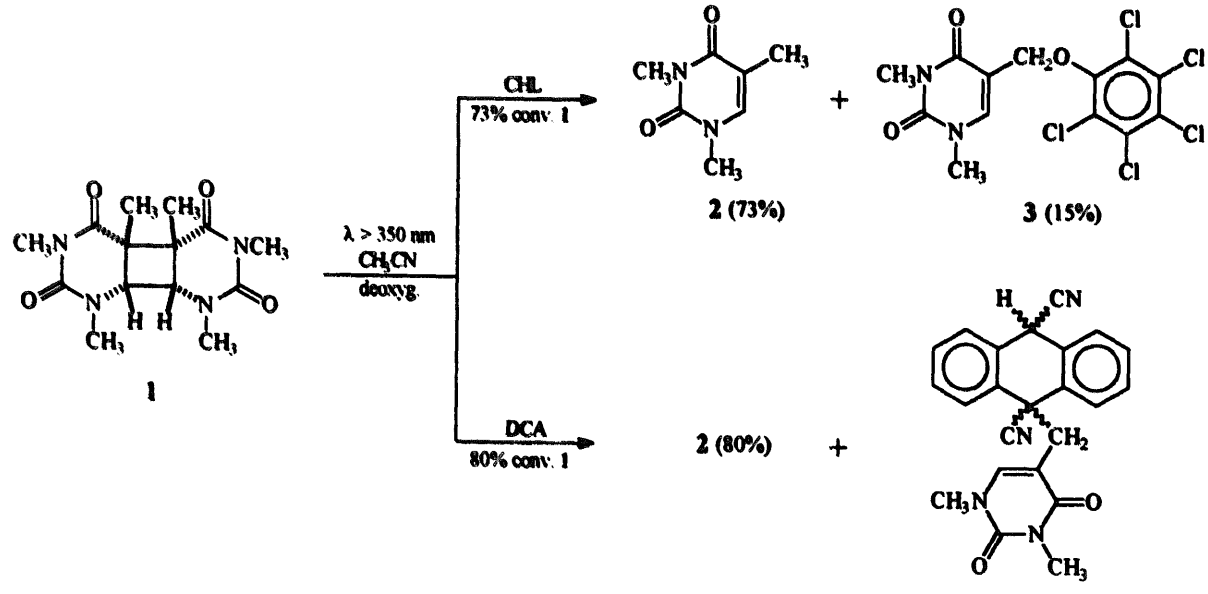
Cis-syn and trans-syn isomers undergo cyclobutane ring opening to yield the parent monomer. Anti-type dimers do not react under these conditions. The effect of solvent polarity on the quantum yield and the quenching of the reaction in the presence of 1,2,4-trimethoxybenzene support the concept that the cycloreversion proceeds via electron transfer from the dimers to the excited sensitizer. The same reaction, although much less efficient, is observed for the cis-syn intramolecular adduct derived from 3,3′-dimethyl-1,1′-trimethylenebisthymine. In the case of all reactive cyclobutanes, the corresponding compound incorporates sensitizer molecules.

As an example, the 9,10-dicyanoanthracene- and chloranil-sensitized reaction of 1,3-dimethylthymine cis-syn dimer is given below in Scheme 1.

Following from the results of our work, the electron-transfer-induced splitting of thymine dimers depends on minute conformational changes in cyclobutanes. This should be taken into account if any conclusions concerning the configuration of cyclobutane-type products formed in nucleic acids are to be drawn on the basis of the data for model compounds.

The second type of photoproduct is pyrimidine photohydrate formed via the singlet excited state. The quantum yields are very low, e.g. 3×10^{-6} at pH 6 for thymine [1]. The nature of the third type of photoproduct, the pyrimidine–pyrimidone (6–4) photoadduct (so-called "spore" photoproduct) is still unknown [8]. Far-UV irradiation of the four main DNA pyrimidine dinucleoside monophosphates in frozen aqueous solution generates products of pyrimidine–pyrimidone-type photoadducts.

Photodimers of cytosines are unstable and undergo deamination in the dark to yield cyclobutadiuracils. Deamination of the photoproduct is also observed in the photo-cross-linked complexes of *E. coli* acetyl valyl tRNA and *Artemia salina* ribosome where a certain degree of conversion of cytosine to uracil is detected [18].



Scheme 1.

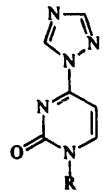
2.2. Pyrimidine–purine photocycloadducts of dinucleotides and analogues

The bichromophoric compounds have been found to be valuable models for the investigation of photochemical reactions occurring in native nucleic acids [3,4].

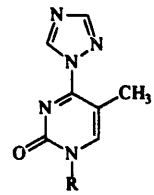
We have been interested for some time in the photochemistry of purines and purine–pyrimidine dinucleotide models. The observation of the photocycloaddition involving C(5)=C(6) pyrimidine and N(7)=C(8) adenine bonds is the most significant result of our study [19] (see below). When adenine is replaced by hypoxanthine, two internal photocycloadducts with azacyclobutane-type structures are obtained [20,21] (see Scheme 2).

We have also shown that an intramolecular cyclobutadipyrimidine-type dimer is formed on irradiation of the dinucleotide bichromophoric model compound cytosine-5-methoxyuracil linked with a trimethylene chain. The photoproduct obtained has been found to undergo deamination in aqueous solution as a dark reaction [22].

In the search for new fluorophores, we have also turned our attention to 4-(1,2,4-triazol-1-yl)-pyrimidones (especially 4-(1,2,4-triazol-1-yl-pyrimidon-2(1H)ones). This chromophore absorbs light in the region of 300–360 nm, i.e. where common nucleic acid bases are transparent, and compounds **9–12** exhibit two bands (230–360 nm) [23].



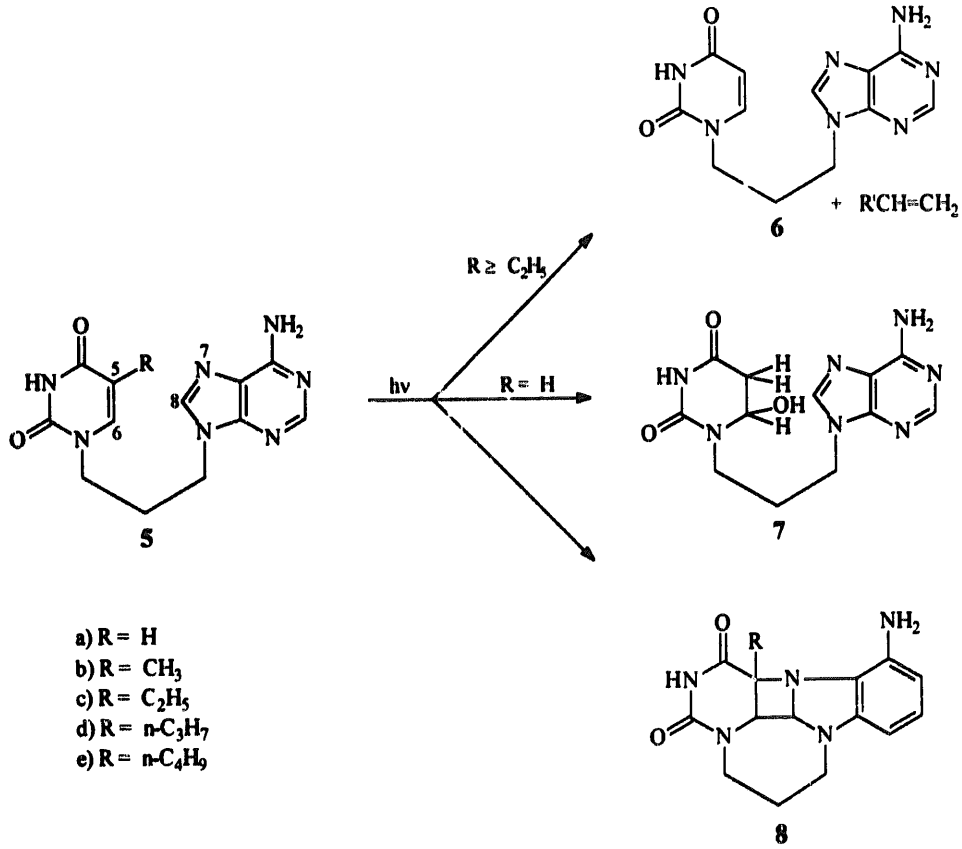
9: R = CH_3
10: R = 2',3',5'-tri-O-acetyl-β-D-ribofuranosyl



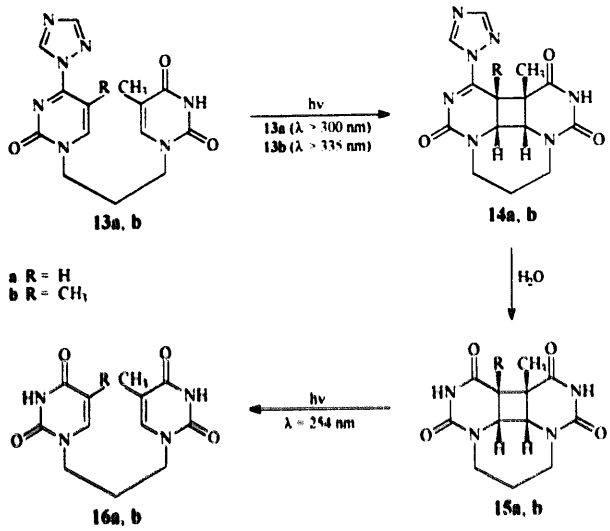
11: R = CH_3
12: R = 3',5'-di-O-acetyl-β-D-deoxyribofuranosyl

The bichromophoric compounds in which triazole-substituted pyrimidines are linked with thymine by a trimethylene bridge were synthesized and their photochemical reaction at 300 nm and $\lambda = 335$ nm was studied. The structures of **13a** and **13b** were established by a comparison of their spectral and other analytical data (TLC, UV, NMR, MS) with those of the authentic samples. The final products, obtained by photochemical ($\lambda = 254$ nm) cyclobutane ring cleavage, were identified in the same way (see Scheme 3).

Evidence for the formation of a photoadduct between adjacent thymine and adenine bases has been obtained from the irradiation of thymidyl(3',5')-2'-deoxy-adenosine (d(TpA)) of larger deoxynucleotides and DNA itself. The main photoproduct of the far-UV irradiation of neutral aqueous solutions of d(TpA) has been isolated and tentatively assigned as an intramolecular adduct [24,25].



Scheme 2.



Scheme 3.

Recently, the mechanism of the photodimerization of adjacent adenine bases on the same strand of DNA has been elucidated by determining the structure of one d(ApA)* of the two major photoproducts formed by UV irradiation of d(ApA) [26]. From a detailed examination of its chemical and spectroscopic properties, it has been deduced that d(ApA)* contains a deoxyadenosine unit covalently linked through its C(8) position to C(4) of an imidazole N(1)-deoxyribonucleoside moiety bearing an *N*-cyanoformamidino substituent at C(5).

Finally, it was inferred that both of the d(ApA) photoproducts were derived from the same azetidine precursor through competing modes of opening of the highly strained four-membered ring.

3. Pyrimidine–amino acid photo-cross-linking

Photoinduced DNA–protein cross-links represent a major class of far-UV damage in living cells. They have been related to aging and carcinogenesis. During the last few years, emphasis has been placed on the isolation and characterization of the far-UV- irradiation-induced photoadducts of the most reactive amino acids to DNA or its components and related model compounds [27].

L-Lysine, alanine, glycine, arginine and tryptophan are among the most reactive amino acids, and the chemical structures of the photoreactions between thymine and L-lysine, 5-bromouridine and tryptophan are known. Irradiation of thymine in solution with tyrosine and the closely related *N*-acetyltyrosine leads to the formation of conjugates between these compounds, together with the well-studied cyclobutane dimeric products of the nucleobase or nucleoside. These photoconjugates have been isolated and characterized [28]. Their characteristic feature is a bond from the aromatic ring of the amino acid to C(5) of the 5,6-saturated nucleobase or nucleoside. The mechanism of formation is proposed to involve the production of a radical cation on the tyrosyl residue and a radical anion on the thymine moiety, followed by proton transfer and radical combination reactions. It seems possible that this type of photoconjugate participates in the cross-linking of proteins to DNA which is observed when DNA–protein complexes are irradiated with either UVC or UVB light.

Recently, lysine was covalently conjugated to calf thymine DNA by irradiation with UV light ($\lambda = 253.7$ nm). It seems that lysine is photoconjugated across the 5,6 double bonds of thymine and cytosine bases in DNA, giving thymine–lysine and cytosine–lysine photoconjugates [29].

4. Laser-pulse-induced reactions

Pulse laser sources of high power (UV) radiation have readily been applied for biological investigations [30–32]. Exposure of DNA to high energy irradiation (short wavelength) or high photon density (high power and short pulses) usually leads to photoprocesses other than the conventional S_1 state photochemistry. Laser pulses of high intensity (nanosecond, picosecond) in protein-free DNA cause single-strand breaks. Free radicals formed by γ irradiation or laser UV irradiation of water [33] play a significant role in the production of pyrimidine, purine and DNA radicals. Most of the studies concerning laser-pulse-induced photolysis and generating such species involve sequential two-photon excitation in two steps at 266 nm and 248 nm and monophotonic excitation in one step at 532 nm [34,35].

The water radical $OH^{\cdot}$ can enter into three processes with thymine: (1) addition at C(5); (2) addition at C(6); (3) hydrogen abstraction from the methyl group.

Pyrimidines react with the solvated electron with a diffusion-controlled rate constant ($k \sim 10^{10}$ dm^3 mol^{-1} s^{-1}). The corresponding pyrimidine radical anions have ketyl character and are subsequently protonated by H^+ or water.

The main reactions of $H^{\cdot}$ occur at the 5 and 6 double bond positions strongly depending on the kind of substituent.

Purines (for example deoxyguanine derivatives) are readily attacked by $OH^{\cdot}$ at different sites of the molecule, forming various radicals which have oxidizing and reducing properties. Guanines [36] and other purines react very rapidly with e_{aq}^- [37]. The negative charge in the radical anion formed is mainly localized on the nitrogens and such anions are easily protonated to give the N-protonated radicals. Purines are less reactive with $H^{\cdot}$ than pyrimidines. The photochemistry of DNA and the consequences of its photoionization using powerful laser sources have been the subject of several review articles [6,31,32,34,35]. Generally, from the results recently obtained, it can be concluded that the damage produced in DNA migrates to specific sites, the positive charge being localized on guanine and the negative charge on thymine [8]. It has also been shown that nucleic acids irradiated with laser UV light, usually at 248 and 266 nm, produce the key species radical cations of nucleobases. Their reaction pathways play

an important role in explaining some chemical and biological processes in DNA. The mechanism of photosensitized DNA cleavage according to the nature of the sensitizer has been reviewed recently [38]. The important role of attack by hydroxyl radicals, the electron transfer process and oxidation by singlet oxygen have been outlined.

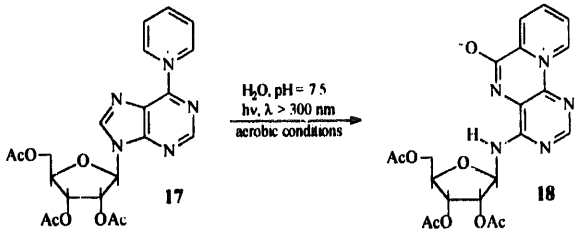
Radical cations produced by irradiation below approximately 210 nm are important intermediates in photodamage. However, a significant role of the sugar phosphate moiety ($\lambda_{irr} \approx 193$ nm) and/or water radicals ($\lambda_{irr} < 180$ nm) should not be neglected [35]. It has been suggested by some workers that picosecond UV irradiation of nucleic acid components yields the singlet channel of two-step excitation, while nanosecond irradiation yields the triplet channel [30].

5. New highly fluorescent derivatives of nucleic acid bases

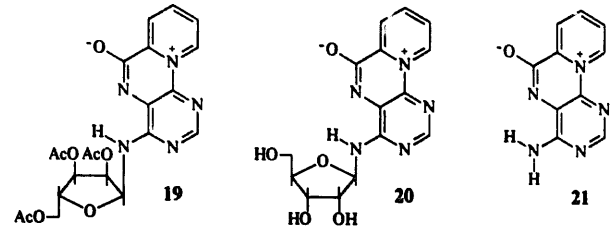
A number of fluorescent molecules [40], among them nucleoside derivatives [41,42], are readily applied as non-isotopic probes in studies investigating the structure, conformation and stereodynamics of nucleic acids. Requirements for a good probe include the following: (1) a high quantum yield of fluorescence, ideally $\Phi_F = 1$; (2) conjugation of the probe to the substrate should require mild conditions so as not to disturb the conformation of the substrate; (3) the probe should be photostable; (4) the luminescence properties of the probe should not change significantly after coupling; (5) longest possible wavelength emission (500–700 nm) mainly in the area of immunoassays. However, few probes emitting in the visible region of the spectrum at room temperature are available.

In the search for suitable fluorescent probes meeting such requirements, we have reported [9] the photochemical transformation of the blue emitting compound **17** in aqueous solution into another nucleoside which emits an intense green fluorescence, termed 2′,3′,5′-tri-O-acetyl-β-luminarosine (**18**) (see Scheme 4).

The α-anomer (**19**), the parent ribonucleoside (**20**) (luminarosine) and the aglycone (**21**) have also been obtained [43].

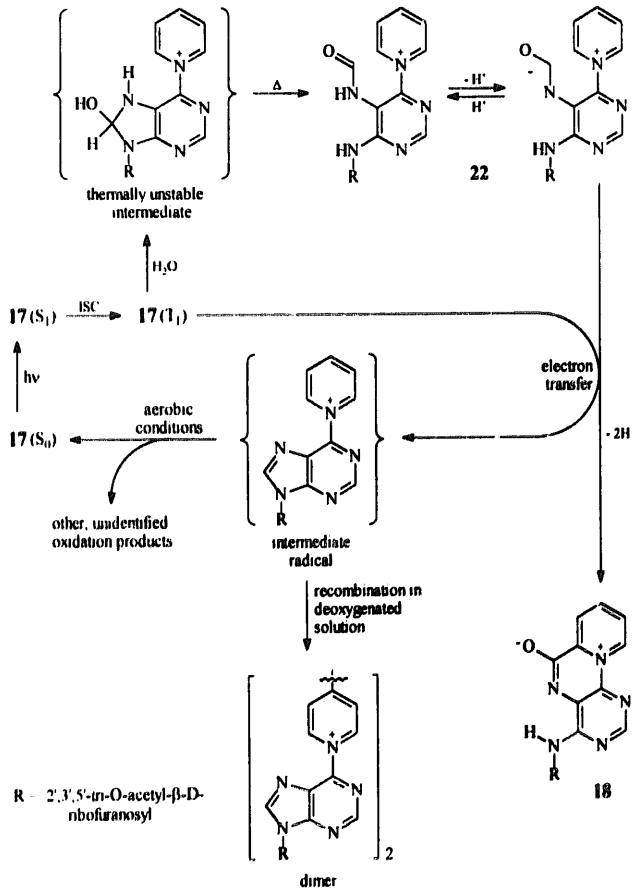


Scheme 4.

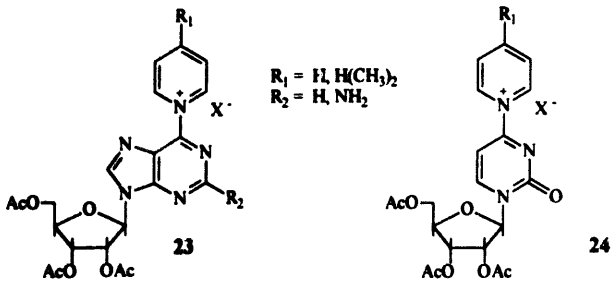


The high photochemical reactivity of salt **17** and the formation of **18** are associated with efficient intersystem crossing to the excited triplet state in the compound. The fluorescence maximum of **18** is at $\lambda = 528$ nm, $\Phi_F = 0.62$ and $\tau = 8.3$ ns in aqueous solution. The mechanism of phototransformation of **17** into **18** in aqueous solution in the pH range 6–8 under aerobic and anaerobic conditions has been explained in the elegant work by Skalski et al. [44] (see Scheme 5).

In the first step of the mechanism, **17** undergoes light-induced hydrolytic ring opening in the imidazole part of the purine form. At pH > 7, **22** exists partly in the electrically neutral zwitterionic form which, via electron- transfer-induced ring closure, gives luminarosine **18**. This particular process is sensitized by the excited triplet state of **17** which is an efficient electron acceptor. In the absence of air, the resulting pyridynyl radicals undergo dimerization. The photophysical properties of luminarosine and luminarine have



Scheme 5.



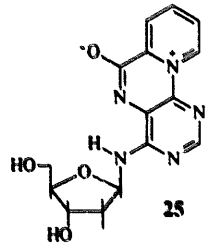
Scheme 6.

also been investigated [43,45]. The absorption and fluorescence spectra of these compounds, as well as the dependence of λ_{em} on the solvent polarity, suggest that they may be useful as fluorescent probes of nucleic acids and other biologically important molecules.

It has been shown recently that among the *N,N*-dimethylaminopyridinium derivatives [46], that derived from guanosine ($R_1 \equiv N(CH_3)_2$, $R_2 \equiv NH_2$), abbreviated as GDMAP, exhibits strong fluorescence at room temperature [47] (see Scheme 6).

This derivative can be inserted into synthetic fragments of DNA. We have performed preliminary photophysical studies of this compound in view of its application as a fluorescent probe in DNA [10], and have found that its fluorescence intensity increases and exhibits dual-exponential decay in the presence of common nucleosides. The formation of ground state complexes with nucleosides has been deduced from absorption and emission measurements.

Finally, the photochemical synthesis of the deoxy analogue, deoxyluminarosine [48], and two routes of sequence-specific and chemical introduction into oligonucleotide chains have been elaborated. Oligomers containing **25** exhibit strong fluorescence ($\Phi_F = 0.62$, pH 7.5) comparable with that of monomeric luminarosine [42] using fluorescein in 0.1 M NaOH as standard.



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