

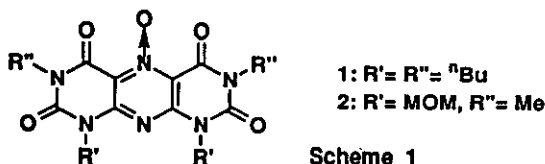
SYNTHESIS AND DNA PHOTO-CLEAVING ACTIVITY OF NOVEL HETEROCYCLIC *N*-OXIDE - ACRIDINE HYBRID MOLECULES †

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Abstract — The novel DNA photo-cleaver consisting of the heterocyclic *N*-oxide, which is an efficient photochemical generator of hydroxyl radicals, an acridine intercalator, and an amide-type methylene linker was designed and synthesized. The preliminary DNA strand-breakage study of the hybrid compounds demonstrated that the DNA photo-cleaving activity of the parent heterocyclic *N*-oxide increased by linking with the acridine intercalator.

Our previous works have documented that pyrimido[5,4-*g*]pteridinetetrone *N*-oxides, (1) and (2), function as an electron acceptor to cause efficiently photochemical oxygenation or dehydrogenation of electron-rich substrates and photochemical generation of hydroxyl (OH) radicals, depending on the nature of the substrates and solvents employed.^{1,2} The most intriguing observation is that water-soluble *N*-oxides (*e.g.*, 2) generate efficiently OH radicals in a bimolecular fashion from a water-solvated excited form upon irradiation with uv-visible light in water.³

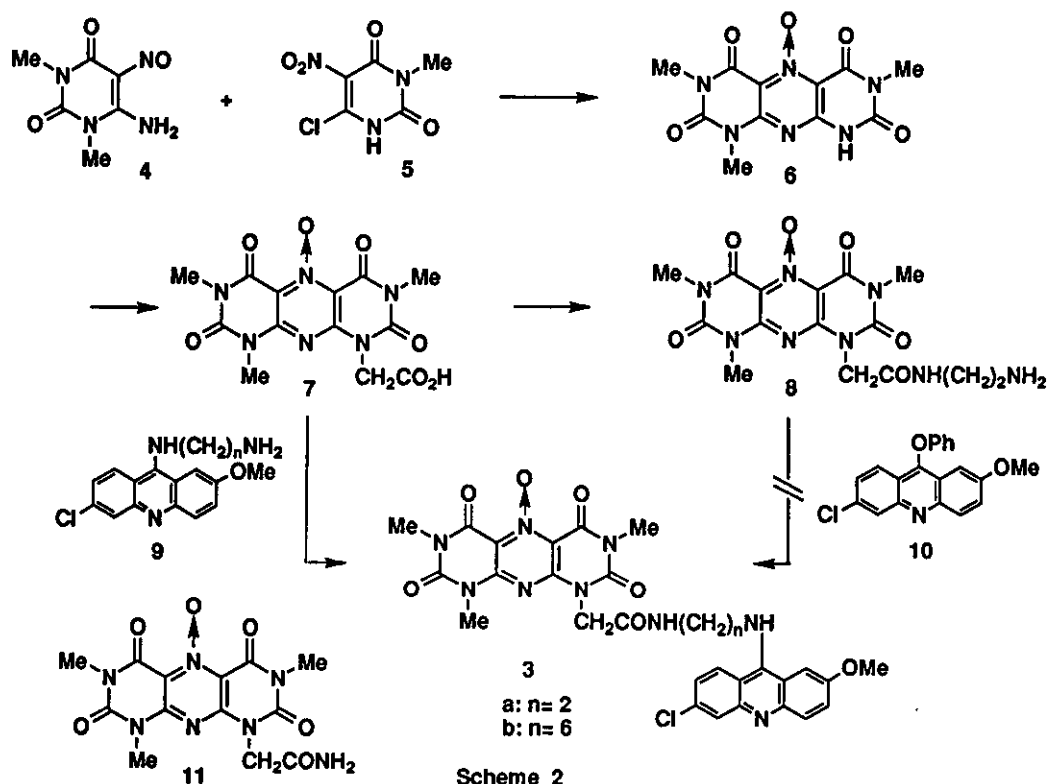


The usefulness of 2 as an OH radical generator was demonstrated by the application to a DNA photo-cleaving agent: the ability of 2 to induce photochemical breakage of DNA strand was estimated by using supercoiled circular Φ X 174 RF-1 (Form I) DNA. Essentially complete conversion of Form I DNA to relaxed circular (Form II) DNA was achieved at a 2.0 μ M concentration of the *N*-oxide (2) after irradiation in a pH 7.5 buffer solution for 10 min.³ No intercalation of 2 with the DNA under the conditions employed was proved by the fluorescence quenching experiments.⁴

In above context, we designed the hybrid molecules (3a,b) in which the heterocyclic *N*-oxide moiety is linked with an acridine intercalator by methylene chains through an amide group, because intercalators have high affinity toward DNA and bring the attached strand-breakage group close to DNA to enhance the cleavage ability.⁵

In this paper, we describe the synthesis and a result in the preliminary DNA strand-breakage study of the novel hybrid compounds (3a,b).

† This paper is dedicated to the memory of the late Dr. Yoshio Ban.



There have been two methods for the construction of the pyrimido[5,4-g]pteridin-2,4,6,8-tetrone ring system involving oxidative dimerization of 1,3-disubstituted 6-amino-5-nitrosouracils by using lead tetraacetate in acetic acid⁶ and thermal condensation of 6-amino-5-nitrosouracils with 6-chloro-5-nitrouracils in dimethylformamide (DMF).⁷ The former method is versatile and efficient for the synthesis of symmetrically *N*-substituted *N*-oxides such as 1 and 2. The latter method is advantageously used for the preparation of unsymmetrically *N*-substituted *N*-oxides. Thus, the preparation of the requisite 1,3,7-trimethylpyrimido[5,4-g]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetrone 5-oxide (6), mp >300°C,⁸ was achieved by refluxing a mixture of 6-amino-1,3-dimethyl-5-nitrosouracil (4) and 6-chloro-3-methyl-5-nitrouracil (5)⁹ in DMF for 3 min (yield: 35%). The reaction of 6 with ethyl bromoacetate in the presence of sodium hydride in dry DMF followed by hydrolysis with 6*N*-hydrochloric acid gave 9-carboxymethyl derivative (7), mp >300°C, in 84% yield. Photolysis of 7 in water (>355 nm, for 1 h) gave a complicated mixture of products resulting from decarboxylation¹⁰ together with deoxygenation. In sharp contrast, the amide derivative of 7, (11), mp >300°C,¹¹ was smoothly converted to give the corresponding deoxygenated product upon irradiation under analogous conditions, indicating efficient generation of OH radicals.³

On the basis of this fact, an amide group was chosen for the linkage between the photo-reactive *N*-oxide moiety and an acridine intercalating moiety.

When 7 was allowed to react with *N*-Boc ethylenediamine in the presence of *N*-hydroxysuccinimide and DCC in dry DMF at room temperature and subsequently was deprotected by acid-treatment, 9-(*N*-aminoethyl)carbamoyl-

methyl derivative (8), mp 268°C, was obtained in 60% yield. The reaction of 8 with excess 9-phenoxyacridine derivative (10) in phenol under reflux, according to the Buchardt's procedure,¹² resulted in the formation of the deoxygenated hybrid compound which was identified by a sample prepared by reduction of 3a with sodium hydrosulfite. Analogous thermal deoxygenation of the *N*-oxide group has already been observed in the reaction of 1 with aromatic hydrocarbons.¹³

Thus, an alternative route was examined and the preparation of the hybrid compounds (3a,b) was achieved as follows: the condensation of 7 with 9-(2-aminoethyl- or 6-aminoethyl)aminoacridine (9a or 9b) obtained by the Buchardt's procedure,¹² was carried out in dry DMF in the presence of *N*-hydroxysuccinimide and DCC. The desired hybrid compounds (3a,b) were obtained in 36% and 69% yields, respectively. The uv spectra of 3a and 3b strongly support the hybrid structures: Uv (H₂O) λ_{max} 422, 368, 265, and 229 nm for 3a; 422, 368, 265, and 230 nm for 3b. Fluorescence quenching experiments showed that the fluorescence of the acridine moiety (490 nm) in 3a,b markedly decreases with concentration dependence by the addition of supercoiled circular DNA as expected, indicating that the acridine moiety of 3a,b strongly intercalates with the DNA. The smooth conversion of 3a to the deoxygenated hybrid compound was observed in the photolysis of 3a in water and the generated OH radicals were detected by ESR spin-trapping method using 5,5-dimethylpyrroline *N*-oxide (DMPO).¹⁴

Table 1. Cleavage of Supercoiled Circular Φ X 174 RF 1 (Form I) DNA into Relaxed Circular (Form II) DNA by Photo-irradiation of Hybrid Compounds(3a,b)and Component Compounds (9a and 8), and its Inhibition with Dimethyl Sulfoxide (DMSO). a)

Compound	Concentration (μ M)	DMSO (%)	Form I (%) b)	Form II (%) b)
9a	5.0	-	86	14
8	1.0	-	65	35
3a	0.1	-	74	26
3a	0.5	-	29	71
3a	1.0	-	N.D.	100
3a	1.0	0.1	4	96
3a	1.0	1.0	27	73
3b	1.0	-	16	84
Blank	-	-	86	14

a) A solution (30 μ l total volume) of Form I DNA (200 ng) in 50 mmol sodium cacodylate buffer (pH 7.5) containing the hybrid molecules (3a,b) or component compounds (9a and 8) at varying concentrations was irradiated in the absence or presence of DMSO at a distance of 5 cm from a 400 W high-pressure mercury arc lamp through a BiCl₃ solution filter (>355 nm) at ambient temperature for 10 min and then analyzed by agarose gel electrophoresis in the presence of ethidium bromide. The employed DNA contains a small amount of Form II DNA (ca. 10%).

b) Yields were estimated by densitometric analyses of a photographic negative of the agarose gel after ethidium bromide staining.

The ability of 3a,b to induce photochemical cleavage of DNA strand was estimated by using Form I DNA (see Table 1). For comparison, the DNA photo-cleaving activity of the 9-aminoacridine derivative (9a) or the 9-carbamoylmethyl *N*-oxide derivative (8) also was measured under the same conditions.

An efficient single-strand breakage of Form I DNA was observed as evidenced by the production of Form II DNA with concentration dependence of **3a**. Essentially complete conversion of Form I DNA to Form II DNA was achieved at a 1.0 μM concentration of **3a** after irradiation for 10 min. The photochemical DNA strand-breakage with **3a** was effectively inhibited by the addition of an OH radical scavenger, dimethyl sulfoxide, with concentration dependence. Practically, no photo-cleaving activity of **9a** was observed. The *N*-oxide (**11**) exhibited the cleaving activity at a 1.0 μM concentration to cause 35% conversion of Form I DNA to Form II DNA. Analogous conversion to Form II DNA in the case of **3a** was attained at *ca.* 0.1 μM concentration. Thus, it is roughly evaluated that the hybridization of the *N*-oxide with the intercalator increases approximately 10 times the photo-cleaving activity of the *N*-oxide itself. The hybrid compound with a long-chain linker (**3b**) showed the cleaving activity at the almost same level comparing with **3a**.

Contrary to our expectation, the activity of the hybrid compounds as a DNA photochemical cleaver is not prominent in comparison with that of the component *N*-oxide. Further studies on the application of the novel heterocyclic *N*-oxide to the construction of more efficient and site-specific DNA photo-cleaver and on the mechanism for the DNA photo-cleavage are now in progress.

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