Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 7307–7309

TETRAHEDRON LETTERS

Photochemical DNA cleavage by a Berenil analog

Sally J. Burr, Abdulghani Mselati and Emrys W. Thomas*

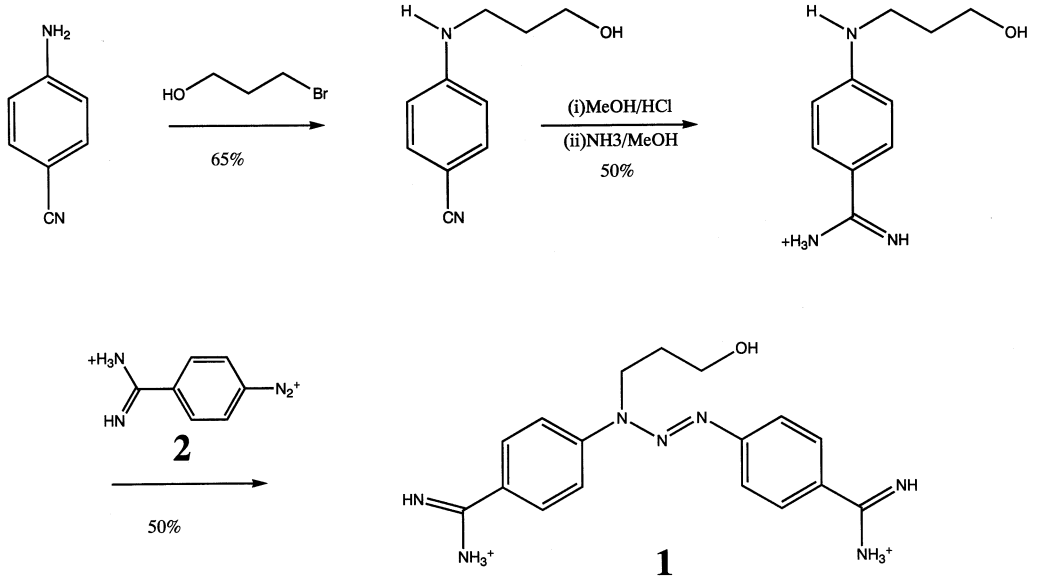
Division of Biological Sciences, Salford University, Salford M5 4WT, UK

Received 6 May 2003; revised 22 July 2003; accepted 1 August 2003

Abstract—Berenil [bis(4-amidinophenyl)1,3-triazene] is a photostable DNA-binding ligand. We describe here the synthesis of *N*-(3-hydroxypropyl)-Berenil, which in contrast to Berenil is photosensitive to 360 nm irradiation, behaving as a caged diazonium salt. The 4-amidinobenzenediazonium fragment produced by photolysis induces DNA modification and cleavage.

A wide variety of compounds[1] are known to act as DNA and RNA photocleavage agents: interest in such compounds lies in their potential as specific nucleases and as phototherapeutic agents. Berenil [bis(4-amidinophenyl)-1,3-triazene] is a paradigmatic minor-groove binder interacting with DNA at AT-rich sites.[2] During an investigation of a series of Berenil analogues incorporating alkyl groups on the triazene nitrogen, it was noticed that several compounds were unstable in daylight; these compounds also showed light-dependent activity in plasmid relaxation assays for DNA cleavage. This report describes the photochemical and DNA-cleaving properties of *N*-(3-hydroxpropyl)-Berenil as a representative active compound in the series.

N-(3-Hydroxypropyl)-Berenil **1** was synthesised[3] as outlined in Scheme 1 (λmax 366 nm, $\varepsilon = 20{,}700$ M^{-1} cm^{-1} (H_2O)).



Scheme 1.

* Corresponding author. E-mail: e.w.thomas@salford.ac.uk

0040-4039/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2003.08.002

Footprinting and binding studies (results not shown) revealed that **1** showed a strong preference for AT-rich binding sites in DNA, with an affinity similar to that of Berenil itself. In contrast to Berenil, however, **1** proved to be photosensitive[†] to 350 nm irradiation, with rapid bleaching of the 366 nm absorption band in aqueous solution. Actinometry showed a modest ($\sim$0.04) quantum yield for photodecomposition: a number of other analogues (including *N*-methyl- and *N*-(2-hydroxyethyl-) had quantum yields in the same range.

Product analyses showed that the initial photoproducts from **1** were the 4-amidinobenzenediazonium dication **2** and 4-amidino-*N*-(3-hydroxypropyl)aniline: **1** can therefore be regarded as a caged diazonium salt. Prolonged 350 nm irradiation led to photolysis of the initial photoproduct **2**, presumably resulting from red edge absorption (**2**, λmax 257 nm, $\varepsilon = 15{,}200$ M^{-1} cm^{-1} (H_2O), with $\varepsilon = 20$ M^{-1} cm^{-1} at 350 nm): 4-hydroxybenzamidine was identifiable as a major product. Photolysis of *N*-alkylated diaryl triazenes in non-polar organic solvents, as reported previously,[5] is dominated by homolytic decomposition pathways. A heterolytic photolysis pathway for diaryl triazenes (presumably facilitated in polar media), as observed here, has not been reported to date.

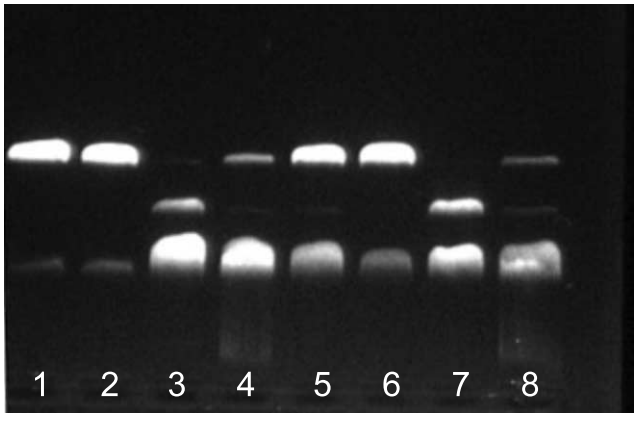


Figure 1. Plasmid relaxation: reaction mixtures contained pBR322 (0.5 μg) in 0.02 M sodium acetate–0.005 M EDTA buffer, and compounds **1** and **2** at the concentrations indicated below. All reaction times were 5 min at 4°C, followed by quenching of excess diazonium by vortexing samples with an equal volume of butanol containing dimethylaniline (0.1% w/v), centrifugation, and rejection of the butanol phase. The quenched aqueous phases were extracted twice with diethyl ether prior to electrophoresis in 1% agarose gels. Lane (1) 0.5 μg DNA+*hv* control; lane (2) 0.5 μg DNA+50 μM **1** dark; lane (3) 0.5 μg DNA+50 μM **1**+*hv*; lane (4) 0.5 μg DNA+5 μM **1**+*hv*; lane (5) 0.5 μg DNA+50 μM **2**, dark; lane (6) 0.5 μg DNA+5 μM **2**,dark; lane (7) 0.5 μg DNA+50 μM **2**+*hv*; lane (8) 0.5 μg DNA+5 μM **2**+*hv*.

[†] Photolyses were conducted with a 350 nm broadband UV source calibrated by actinometry using Aberchrome.[4] Product analyses were by HPLC (reverse phase C18 silica column; 0.1 M sodium dodecyl sulphate, 13% acetonitrile, 0.06 M phosphoric acid for elution; detection at 206 nm).

The light-dependent plasmid relaxing ability of **1** appeared to be due in part to the initial photoproduct **2**: plasmid relaxation was observed in a dark reaction using **2** as prepared by diazotisation of 4-amidinobenzeneamine (Fig. 1, lanes 5 and 6). Similar results were obtained in dark reactions using pre-irradiated **1** (result not shown). Plasmid relaxation did not require an added electron donor (mediating homolytic dediazoniation of the diazonium group) as shown for other diazonium reagents.[6] With broadband 350 nm irradiation, however, an enhanced DNA cleavage by **2** (relative to the dark reaction) was observed (Fig. 1, cf. lanes 5 and 6 with lanes 7 and 8). Photolytic dediazoniation of diazonium salts is well documented[7] and could generate carbocationic species with DNA cleavage potential.

The dark reaction of **2** with double-stranded and single-stranded 5′[^{32}P]-labelled oligonucleotides is shown in Figure 2(A/B).

The appearance of slower-moving bands (see lanes 1, 2, 5, and 6) most likely indicates the formation of covalent adducts between **2** (a relatively electrophilic diazonium salt) and DNA. Some spontaneous chain cleavage takes place (lanes 1 and 5) which is enhanced by heat treatment (lanes 2 and 6). Significantly, DNA modification at pH 7.5 differs from that at pH 5.5 in that specific chain cleavage at purines is apparent (cf. lanes 2 with 6). Detailed studies on the chemistry of DNA modification by **1** and **2** are in progress. In this context, the covalent modification of DNA by electrophilic diazonium reagents, involving both *C*- and *N*-coupling reactions at purine residues, are well documented,[8] as are DNA photocleavage processes involving diazonium salts.[9]

References

1. (a) Armitage, B. *Chem. Rev.* **1998**, *98*, 1171–1200; (b) Fernandez, M.-J.; Grant, K. B.; Herraiz, F.; Yang, X.; Lorente, A. *Tetrahedron Lett.* **2001**, *42*, 5701–5704.
2. (a) Brown, D. G.; Sanderson, M. R.; Skelly, J. V.; Jenkins, T. C.; Brown, T.; Garman, E.; Stuart, D. I.; Neidle, S. *EMBO J.* **1990**, *9*, 1329–1334; (b) Brown, D. G.; Sanderson, M. R.; Garman, E.; Neidle, S. *J. Mol. Biol.* **1992**, *226*, 481–490.
3. Compound **1** was isolated as the dihydrochloride salt, mp 210°C. MS (ES): *m*/*z* 340.35 (M^++1) (calcd for $C_{17}H_{21}N_7O$ 339.40).The synthetic route follows that described for *N*-methyl-Berenil (Ashley, J. N.; Berg, S. S. *J. Chem. Soc.* **1957**, 3089–3093).
4. Heller, H. G.; Langan, J. R. *J. Chem. Soc., Perkin Trans. 2* **1981**, 341–343.
5. Julliard, M.; Vernin, G.; Metzger, J. *Helv. Chim. Acta* **1980**, *63*, 467–472.
6. (a) Griffiths, J.; Murphy, J. A. *J. Chem. Soc., Chem. Commun.* **1992**, 24–26; (b) Arya, D. P.; Warner, P. M.; Jebaratnum, D. *Tetrahedron Lett.* **1993**, *34*, 7823–7826; (c) Arya, D. P.; Jebaratnum, D. *Tetrahedron Lett.* **1995**, *36*, 4369–4372.
7. Gasper, S. M.; Devadoss, C.; Schuster, G. B. *J. Am. Chem. Soc.* **1995**, *117*, 5206–5211.

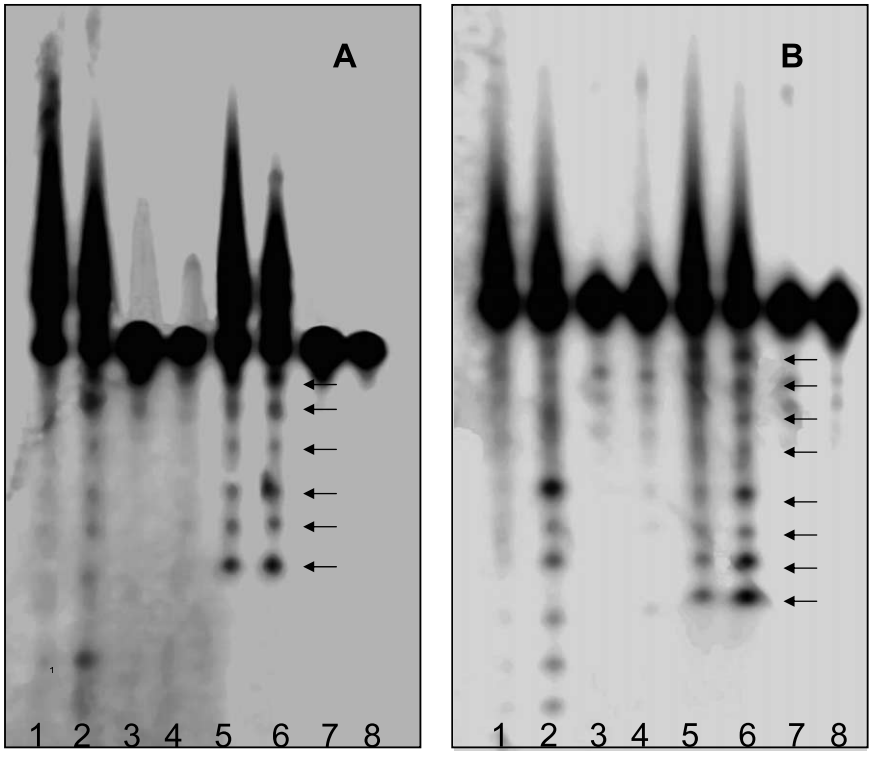


Figure 2. Dark reactions of **2** with: $(dCdGdCdGdAdAdTdTdCdGdCdG)_2$ (dsDNA; panel A) and dAdGdAdCdAdCdAdG-dAdAdTdC (ssDNA; panel B). Oligos were 5′end-labelled with ^{32}P, and treated with **2** (0.0005 M) in either 0.02 M sodium acetate–0.005 M EDTA (pH 5.5), or in 0.05 M sodium cacodylate–0.005 M EDTA (pH 7.5). Reactions were terminated by vortexing the samples with an equal volume of butanol containing 0.1% v/v dimethylaniline, centrifugation, and rejection of the butanol layer. The quenched aqueous phases were extracted twice with diethyl ether prior to electrophoresis in 20% denaturing polyacrylamide gels. **Panel A**: lane (1) ds DNA+0.5 mM compound **2**, pH 5.5, 3 h 20°C; lane (2) as lane 1 but heated at 90°C for 15 min after quenching; lane (3) ds DNA control; lane (4) ds DNA control heated at 90°C 15 min; lane (5) ds DNA+0.5 mM **2**, pH 7.5, 3 h 20°C; lane (6) as lane 5 but heated at 90°C for 15 min after quenching; lane (7) ds DNA control; lane (8) ssDNA control, heated at 90°C for 15 min at pH 7.5. **Panel B**: lane (1) ss DNA+0.5 mM compound **2**, pH 5.5, 3 h 20°C; lane (2) as lane 1 but heated at 90°C for 15 min after quenching; lane (3) ss DNA control; lane (4) ss DNA control heated at 90°C for 15 min; lane (5) ssDNA+0.5 mM **2**, pH 7.5, 3 h 20°C; lane (6) as lane 5 but heated at 90°C for 15 min after quenching; lane (7) ss DNA control; lane (8) ssDNA control, heated at 90°C for 15 min at pH 7.5. Arrows in both panels indicate cleavage products.

8. (a) Chin, A.; Hung, M.-H.; Stock, L. M. *J. Org. Chem.* **1981**, *46*, 2203–2207; (b) Hung, M.-H.; Stock, L. M. *J. Org. Chem.* **1982**, *47*, 448–453; (c) Gannett, P. M.; Powell, J. H.; Rao, R.; Shi, X.; Lawson, T.; Kolar, C.; Toth, B. *Chem. Res. Toxicol.* **1999**, *12*, 297–304.

9. (a) Behr, J.-P. *J. Chem. Soc., Chem. Commun.* **1989**, 101–103; (b) Garcia, A.; Giege, R.; Behr, J.-P. *Nucleic Acids Res.* **1990**, *18*, 89–95; (c) Schmid, N.; Behr, J.-P. *Biochemistry* **1991**, *30*, 4357–4361.