N-Arylalkyl-*N*-phenylhydroxylamines as Novel Photo-induced DNA-cleaving Agents

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Single-strand cleavage of DNA was accomplished by photolysis of various *N*-arylalkyl-*N*-phenylhydroxylamines under aerobic conditions for 3 h with 312 nm UV light, which functioned as the trigger to initiate the new and controllable DNA cleavage process.

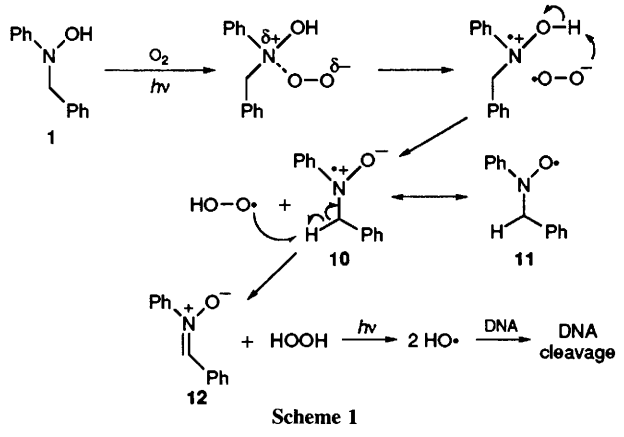
Chemists in the fields of molecular design and synthesis are devoting considerable effort towards the development of DNA-cleaving agents.[1] It is our plan to search for novel organic compounds capable of cleaving DNA under controllable conditions. Herein we report a new and efficient method for the cleavage of DNA by photolysis of *N*-arylalkyl-*N*-phenylhydroxylamines.

By irradiating an acetonitrile solution of **1** (0.015 mmol dm^{-3}) with a 450 W medium-pressure mercury UV lamp through a Pyrex filter (≥300 nm) under aerobic conditions, we obtained the nitrone **12** in quantitative yield at room temperature after 80 min (Scheme 1). In the absence of oxygen, **1** did not lead to **12** in the dark or under photolysis; in the presence of oxygen without light, the conversion of **1** to **12** took 6 days for completion in $CDCl_3$ and 13 days in THF.

Our results support the mechanism shown in Scheme 1 for the photolytic conversion of **1** to **12**,[2-4] in which hydroxylamine **1** first reacted with O_2 through a Type II photosensitized oxidation.[5,6] An EPR signal of the intermediate **11**,[7] a canonical form of **10**, was detected when we irradiated a methanolic solution of **1** with UV light (>300 nm) in the presence of oxygen.

In Scheme 1, the HOO· radical abstracted a benzylic hydrogen in **10** to give nitrone **12** and H_2O_2. Generation of H_2O_2 was confirmed by titration[8] of the resultant solution with KI and $Na_2S_2O_3$. Homolytic fission of H_2O_2 by irradiation *in situ* can lead to HO· radicals,[9] which are often used as an efficient DNA cleaver.[10]

Consequently we considered the cleavage of DNA with HO· radicals generated by photolysis of *N*-substituted-*N*-phenylhydroxylamines in the presence of oxygen.[11] Experiments were performed by use of supercoiled circular φX174 RFI DNA (form I); among various hydroxylamines, **1–9** exhibited single-strand cleaving capability (Table 1). When a phosphate buffer (pH 6.0) containing 10% EtOH, form I DNA (50 μmol dm^{-3}/base pair), and a hydroxylamine was irradiated with 312 nm UV light (16 W) at room temperature, efficient single-strand scission occurred in 3 h to give the relaxed circular DNA (form II) at a concentration of **1** as low as 250 μmol dm^{-3} (form II/form I = 1.1). On the other hand, the strand scission was minimal in the absence of UV light. We also found that the photoinduced cleavage process was pH-dependent over the pH range 5.0–8.0; single-strand cleavage of DNA occurred preferably under acidic conditions. Results from dose measurements of **1** revealed that cleavage of DNA depended upon the concentration of the hydroxylamine; when 125, 250, 500, 1000 or 2000 μmol dm^{-3} of **1** was used, the form II DNA was obtained in 16.8, 26.2, 32.8, 37.5 and 44.4 μmol dm^{-3}/base pair, respectively.



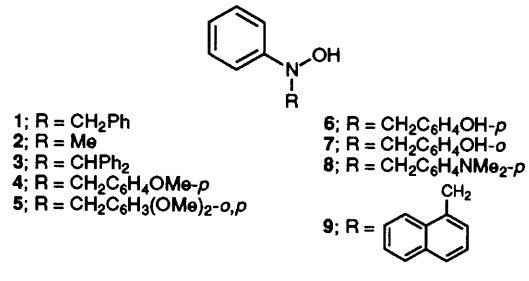
Scheme 1

Water solubility, intercalating capability, and quantum yield associated with a hydroxylamine[12] would influence its DNA-cleaving efficiency. Also the substituent R at the α-carbon of $PhN(OH)C\alpha H_2R$ and substituents on the benzyl ring of $PhN(OH)CH_2Ph$ may facilitate the formation of the crucial intermediate H_2O_2. Based on these concerns, we prepared hydroxylamines **2–5** for comparison. In order to increase the water solubility, we synthesized hydroxylamines **6** and **7** which contained a second hydroxy group and showed useful DNA cleavage reactivity. The second hydroxy group can also be used to link the hydroxylamine to a groove binder (*e.g.*, a triplex-forming oligonucleotide) to allow the possibility of accomplishing the site-specific cleavage of DNA. Incorporation of an amino group in **8** and a naphthyl moiety in

Table 1 Single-strand cleavage of supercoiled circular φX174 RFI DNA (form I) to relaxed circular DNA (form II) by irradiation of hydroxylamines under aerobic conditions at room temperature with 312 nm UV light for 3 h

Hydroxylamine[a]	Relative quantum yield[b]	% form I[c]	% form II[c]	Form II/Form I
1	1	13.1	86.9	6.6
2	0.65	31.4	68.6	2.2
3	0.31	29.4	70.6	2.4
4	1.75	55.1	44.9	0.81
5	2.38	66.6	34.4	0.52
6	1.94	48.0	52.0	1.1
7	0.38	26.7	73.3	2.7
8	2.28	65.4	34.6	0.53
9	2.15	23.6	76.4	3.2
None	—	86.7	13.3	0.15

[a] 0.1 mol dm^{-3} sodium phosphate buffer (Na_2HPO_4 and NaH_2PO_4) at pH 6.0 containing 1000 μmol dm^{-3} of a hydroxylamine, 50 μmol dm^{-3}/base pair of form I DNA (molecular mass 3.50×10^6, 5386 base pairs in length), and 10% EtOH. [b] Quantum yields relative to **1** were determined by following an established method.[15] [c] Analysed by gel electrophoresis with 1% agarose and ethidium bromide staining.



9 provide the possibility of introduction of an amino linker and an intercalating moiety, respectively. We obtained the quantum yields for **2–9** relative to **1**. Our results in Table 1 indicate that the efficiency of DNA cleavage was not quantum-yield dependent.

We obtained the desired hydroxylamines (**1**, **3–9**) in 51–85% overall yields from nitrobenzene in three steps. Hydrogenation of nitrobenzene with hydrazine hydrate and rhodium on carbon gave *N*-phenylhydroxylamine,[13] which was condensed with various aromatic aldehydes to afford the corresponding nitrones. Reduction of those nitrones with $NaBH_4$ in MeOH (for **1**, **4–9**) or alkylation with PhMgBr (for **3**) produced the desired *N*-arylalkyl-*N*-phenylhydroxylamines. *N*-Hydroxylamine **2** was obtained by pyrolysis of $MeEtPhN^+O^-$.[14]

The use of *N*-arylalkyl-*N*-phenylhydroxylamines to react with O_2 for the production of HO· by photolysis provides an efficient way to cleave DNA. This new method allows the use of 312 nm UV light as the trigger to initiate the DNA strand scission, yet it does not require external photosensitizer, H_2O_2, or metal ions. In addition, these hydroxylamines can be readily prepared and easily modified to connect with an intercalator and a groove binder.

This work was supported by the National Science Council of Republic of China (Grant NSC 83-0203-B-001-094) and Academia Sinica. We also thank Professor Lou-sing Kan for discussion.

Received, 24th February 1994; Com. 4/01127I

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