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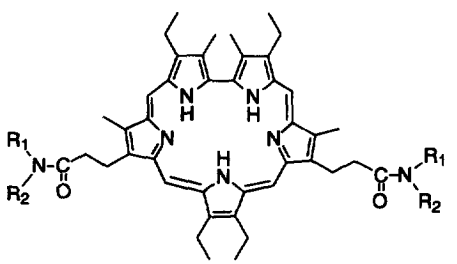
ENHANCED DNA PHOTOCLEAVAGE AND BINDING PROPERTIES OF SAPPHYRIN-POLYAMINE CONJUGATES

Jonathan L. Sessler,* Andrei Andrievsky, Petra I. Sansom, Vladimír Král, and Brent L. Iverson*

Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas 78712

Abstract: The synthesis of several water-soluble sapphyrin derivatives is described. Of these, the sapphyrins **2-4**, bearing appended polyamines, exhibit enhanced DNA photocleavage and binding over sapphyrin **1** as determined from pBR322 plasmid cleavage experiments and an ethidium bromide displacement fluorescence assay. © 1997 Elsevier Science Ltd.

The design of photosensitizers that cleave DNA upon photochemical activation is of great interest in the development of DNA-targeting therapeutics and probes for DNA structure and sequence.[1] We have focused on the photocleavage capabilities of chromophores that modify DNA when irradiated at red-shifted wavelengths (>600 nm).[2] This region is preferable, as it is here that the bodily tissues are most transparent.[3] In the context of this program we have recently reported that *sapphyrin* **1** (a pentapyrrolic "expanded" porphyrin) is capable of photocleaving DNA when irradiated at wavelengths above 620 nm.[2,4]



1 $R_1 = R_2 =$ –CH$_2$CH$_2$OH

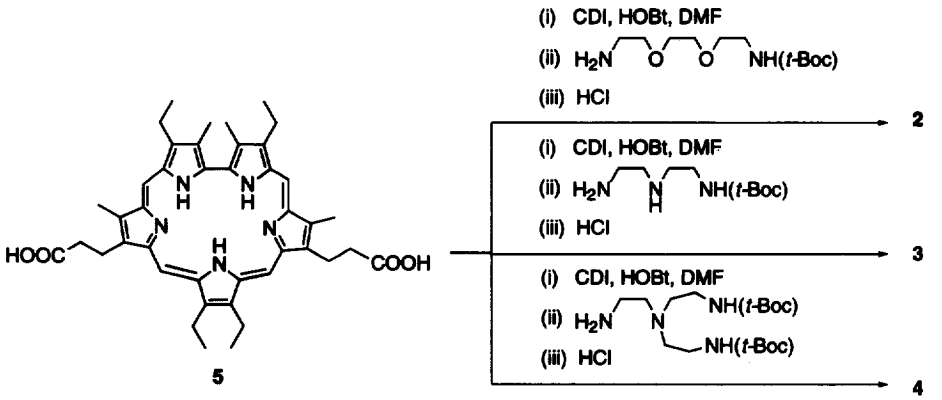
2 $R_1 =$ –CH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$NH$_2$; $R_2 = H$

3 $R_1 =$ –CH$_2$CH$_2$NHCH$_2$CH$_2$NH$_2$; $R_2 = H$

4 $R_1 =$ –CH$_2$CH$_2$N(CH$_2$CH$_2$NH$_2$)$_2$; $R_2 = H$

The efficiency of photocleavage is expected to be directly proportional to the strength of photosensitizer binding to the DNA double helix. Sapphyrin **1**, which has a mono-protonated pentapyrrolic core at neutral pH, binds nucleic acids through a combination of several modes of interaction, including electrostatic phosphate chelation of the phosphodiester backbone.[5] Polyamines, when protonated, have been shown to bind to the negatively charged major groove of the DNA double helix via Coulombic interactions.[6] Sapphyrins **2-4** were derivatized on the periphery with polyamine moieties to provide for more water-solubility and enhanced DNA binding, ultimately leading to increased photocleavage compared to **1** (vide infra).

Sapphyrins **2-4** were prepared by coupling the known sapphyrin bisacid **5**[7] with the appropriate *t*-Boc-protected amine, followed by acid-mediated cleavage of the *t*-Boc protective groups (Scheme 1).[8] The relative photocleavage capabilities of the polyprotonated sapphyrin derivatives **1-4** were then screened using a supercoiled pBR322 plasmid DNA assay.[9] Specifically, DNA cleavage was followed by monitoring the conversion of supercoiled (form I) plasmid DNA to nicked circular and linear (forms II and III) DNA. At wavelengths above 300 nm, amino-functionalized sapphyrin derivatives **2-4** showed significantly more effective cleavage than compound **1** (Figure 1).[10]



Scheme 1.

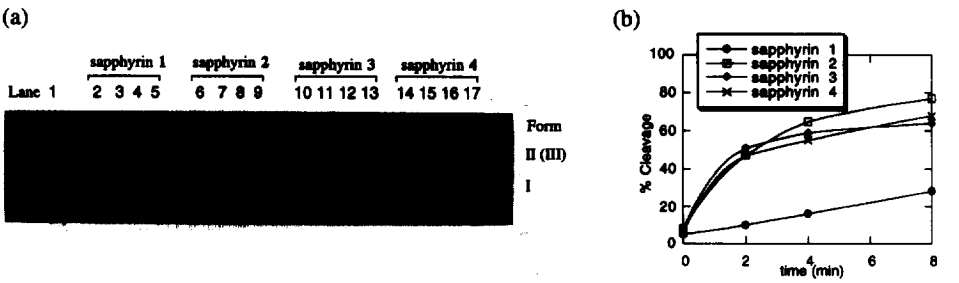


Figure 1. (a) Photograph of a 0.8% agarose gel containing ethidium bromide showing the results of a pBR322 plasmid DNA cleavage study. In accord with the general procedure of ref 9, 0.04 μg/μL DNA in 10 mM bis-tris, 10 mM NaCl buffer (pH 7) was irradiated in clear polypropylene microcentrifuge tubes (Intermountain Scientific) in the presence of sapphyrins **1-4**. Irradiation was effected at room temperature using light from a high-pressure xenon lamp (Oriel) passed through a 300 nm filter. Each sample received approximately 790 mW/cm^2. The following conditions pertained: Lane 1: 0 μM sapphyrin, $t = 8$ min. Lanes 2-17: 1 μM sapphyrin. Lanes 2, 6, 10, 14: $t = 0$ min. Lanes 3, 7, 11, 15: $t = 2$ min. Lanes 4, 8, 12, 16: $t = 4$ min. Lanes 5, 9, 13, 17: $t = 8$ min. (b) Time course of cleavage effected by sapphyrins **1-4** as obtained in the study shown in part (a).

In order to ascertain the role sapphyrin-DNA binding could play in modulating the photocleavage process, we carried out pBR322 plasmid cleavage experiments under conditions where these binding interactions are minimized. This was accomplished by increasing the buffer ionic strength via the addition of sodium chloride or by sequestering the molecules using sodium dodecyl sulfate (SDS). Indeed, under both sets of conditions, the photodynamic activity of the sapphyrins was reduced (Table 1).[9,11] This effect is consistent with the binding between the agent and the DNA playing an important role in regulating the observed photocleavage phenomenon.

Table 1.

Compound	Cleavage Inhibition 2 M NaCl	Cleavage Inhibition 0.2 M SDS
Sapphyrin 1	92%	100%
Sapphyrin 2	66%	31%
Sapphyrin 3	87%	47%
Sapphyrin 4	93%	41%

Further insight into the interactions between sapphyrins **1-4** and DNA was obtained using UV-visible spectroscopy. Titration of each of the four derivatives with ds-calf thymus DNA showed similar spectral characteristics (e.g., Figure 2). These results are consistent with each of these derivatized sapphyrins exhibiting similar interactions with dsDNA.

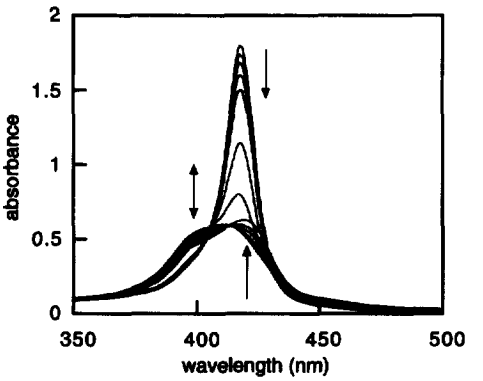


Figure 2. Overlay UV-visible absorption spectra for a 5 μM solution of sapphyrin **2** recorded in the presence of increasing concentrations of dsDNA-phosphate (from [P]/[S] = 0 to 320) in 10 mM bis-tris, 10 mM NaCl buffer, pH 7.0. The initial absorption at 418 nm ([P]/[S] = 0) gives way to a new blue-shifted absorption band at 400 nm at a [P]/[S] ratio of 25. Upon addition of additional dsDNA-phosphate, the apparent λ_{max} red-shifts to yield an absorption centered at ca. 422 nm at a [P]/[S] ratio of 170. The arrows indicate the growth or decay of the indicated absorption band throughout the titration. The double arrow shows the growth and subsequent decay at 400 nm.

Relative affinities of sapphyrins **1-4** to ds-calf thymus DNA were estimated using a fluorimetric assay with ethidium bromide as previously described (Table 2).[12] The C_{50} value refers to the concentration of test compound required to displace 50% of the ethidium bromide from the DNA as measured by fluorescence spectroscopy. The measured C_{50} value of the standard, Tren (tris(2-aminoethyl)amine), was the same order as its literature value of 22.[6b] Sapphyrins **1-4** all showed significantly lower C_{50} values than this standard. Furthermore, sapphyrins **2-4** showed approximately 10-fold lower C_{50} values than sapphyrin **1**, which is consistent with higher binding affinities of these polyamino derivatives to the dsDNA. Interestingly, the higher number of protonated nitrogens on sapphyrins **3** and **4** than sapphyrin **2** did not significantly affect their binding affinities.

Table 2.

Compound	Number of basic nitrogens	C_{50} Value CT-DNA (μM)
Sapphyrin **1**	1	1.2
Sapphyrin **2**	3	0.062
Sapphyrin **3**	5	0.127
Sapphyrin **4**	7	0.114
Tren	3	28

In conclusion, polyamines appended to the sapphyrin macrocycle were found to augment both DNA binding interactions and photocleavage. More detailed investigations into the nature of these interactions are in progress.

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7. The synthesis of sapphyrin **5** has been described previously: Král, V.; Sessler, J. L.; Furuta, H. *J. Am. Chem. Soc.* **1992**, *114*, 8704.

8. The general synthetic procedure is as follows: Sapphyrin bisacid **5** (1 equiv) was dissolved under argon in anhydrous DMF. 1,1'-Carbonyldiimidazole (CDI) (3 equiv) was added, followed by a catalytic amount of 1-hydroxybenzotriazole hydrate (HOBt). The resulting mixture was stirred at room temperature under argon for 2 h. A solution of *t*-Boc-protected amine (3 equiv) in DMF was added, and the resulting mixture was stirred at room temperature under argon for 24 h. The solvent was evaporated off, and the protected amine was purified via column chromatography on silica gel using ammonia-saturated methanol/dichloromethane (2-20% v/v gradient) as the eluent. The solvent was evaporated and the compound was deprotected using HCl-saturated chloroform/methanol (3:1) for ca. 2-7 h (TLC control). The solvents were evaporated, and the product was dried in vacuo. Satisfactory spectroscopic and analytical data were obtained for all new compounds.

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10. Photocleavage was monitored using wavelengths above 300 nm since the cleavage efficiency is maximal in this range.

11. The high level of inhibition for sapphyrin **1** may be rationalized by its relatively low DNA binding affinity and photocleavage as compared with those of sapphyrins **2-4**.

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