Sapphyrin−Oligonucleotide Conjugates. Novel Sequence-Specific DNA Photomodifying Agents with Increased Binding Affinity

Jonathan L. Sessler,*,† Petra I. Sansom,† Vladimír Král,† Donald O'Connor,‡ and Brent L. Iverson*,†

Contribution from the Department of Chemistry and Biochemistry and The Center for Fast Kinetics Research, The University of Texas at Austin, Austin, Texas 78712

Received May 17, 1996[⊗]

Abstract: The synthesis of a novel sapphyrin−oligonucleotide conjugate is described along with its DNA photocleavage and nucleic acid binding properties. The sapphyrin−oligonucleotide conjugate produces photodamage on a complementary oligonucleotide target when irradiated at wavelengths above 620 nm. Upon piperidine treatment, guanine residues on the target strand in the vicinity of the sapphyrin macrocycle are found to be cleaved more effectively than guanines remote from the sapphyrin subunit. No sequence-specific photomodification was observed when noncomplementary oligonucleotides were used as a target. The duplexes formed between the sapphyrin conjugate and complementary nucleic acids were found to have higher melting temperatures than analogous control systems consisting of unmodified oligonucleotides of the same sequence. Melting temperature studies using variable salt concentrations and oligonucleotide targets indicate that the binding enhancement is due to hydrophobic interactions. The sapphyrin unit attached to an oligonucleotide can thus serve a dual role; it can act as a sequence-specific photomodification agent at irradiation wavelengths >620 nm, and it can increase the affinity of a sapphyrin-bearing oligonucleotide to a complementary sequence.

Introduction

The study of chemically modified oligonucleotides is currently a topic of widespread interest in part because these compounds have therapeutic potential for regulating gene expression.[1] One approach being pursued involves the use of modified antisense agents containing photoactive groups.[2−8] Such groups provide the advantage of being inert in the dark while being easily "switched on" upon irradiation with light of an appropriate wavelength. To date, photoactivatable oligonucleotide conjugates containing porphyrin moieties,[2] azido derivatives,[3] ellipticines,[4] psoralens,[5] proflavins,[6] and fullerenes[7] have been described. All of these derivatives modify DNA when irradiated at wavelengths below 700 nm. For use *in vivo*, however, photoactivation using red-shifted wavelengths is preferable since bodily tissues are most transparent in these spectral regions.[9] Recently, it was found that oligonucleotide conjugates bearing lutetium(III) texaphyrin photosensitizers (e.g., unmodified texaphyrins **1** and **2**) are capable of photomodifying DNA when irradiated at wavelengths over 700 nm.[8] It was also found that the water-soluble sapphyrin derivative **3** is able to efficiently cleave plasmid pBR322 DNA when irradiated at wavelengths of 300 nm or higher and, to a lesser degree, when irradiated at wavelengths over 700 nm.[8] As the result of these previous findings, we became interested in synthesizing sapphyrin−oligonucleotide conjugates, such as **9**, and testing whether such systems could serve as oligonucleotide-based photocleaving agents that are activated when irradiated in these low-energy spectral regions.

In addition to being functionalized with photoactivating groups, oligonucleotides have also been modified to augment their binding affinity.[10−14] Efforts to increase the stability of oligonucleotide assemblies include the use of tethered interca-

† Department of Chemistry and Biochemistry.

‡ The Center for Fast Kinetics Research.

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1996.

(1) (a) De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366−374. (b) *Antisense Research and Applications*; Crooke, S. T., Lebleu, B., Eds.; CRC Press: Boca Raton, FL, 1993. (c) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543−584. (d) Hélène, C.; Toulmé, J.-J. *Biochim. Biophys. Acta* **1990**, *1049*, 99−125. (e) *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*; Wickstrom, E., Ed.; Wiley-Liss, Inc.: New York, 1991.

(2) (a) Mastruzzo, L.; Woisard, A.; Ma, D. D. F.; Rizzarelli, E.; Favre, A.; Le Doan, T. *Photochem. Photobiol.* **1994**, *60*, 316−322. (b) Fedorova, O. S.; Savitskii, A. P.; Shoikhet, K. G.; Ponomarev, G. V. *FEBS Lett.* **1990**, *259*, 335−337. (c) Vlassov, V. V.; Deeva, E. A.; Ivanova, E. M.; Knorre, D. G.; Maltseva, T. V.; Frolova, E. I. *Nucleosides Nucleotides* **1991**, *10*, 641−643. (d) Le Doan, T.; Praseuth, D.; Perrouault, L.; Chassignol, M.; Thuong, N. T.; Hélène, C. *Bioconjugate Chem.* **1990**, *1*, 108−113.

(3) (a) Le Doan, T.; Perrouault, L.; Praseuth, D.; Habhoub, N.; Decout, J.-L.; Thuong, N. T.; Lhomme, J.; Hélène, C. *Nucleic Acids Res.* **1987**, *15*, 7749−7760. (b) Levina, A. S.; Berezovskii, M. V.; Venjaminova, A. G.; Dobrikov, M. I.; Repkova, M. N.; Zarytova, V. F. *Biochimie* **1993**, *75*, 25−27. (c) Praseuth, D.; Perrouault, L.; Le Doan, T.; Chassignol, M.; Thuong, N.; Hélène, C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1349−1353.

(4) Perrouault, L.; Asseline, U.; Rivalle, C.; Thuong, N. T.; Bisagni, E.; Giovannangeli, C.; Le Doan, T.; Hélène, C. *Nature* **1990**, *344*, 358−360.

(5) (a) Bhan, P.; Miller, P. S. *Bioconjugate Chem.* **1990**, *1*, 82−88. (b) Teare, J.; Wollenzien, P. *Nucleic Acids Res.* **1989**, *17*, 3359−3372. (c) Pieles, U.; Englisch, U. *Nucleic Acids Res.* **1989**, *17*, 285−299. (d) Takasugi, M.; Guendouz, A.; Chassignol, M.; Decout, J. L.; Lhomme, J.; Thuong, N. T.; Hélène, C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5602−5606.

(6) Praseuth, D.; Le Doan, T.; Chassignol, M.; Decout, J.-L.; Habhoub, N.; Lhomme, J.; Thuong, N. T.; Hélène, C. *Biochemistry* **1988**, *27*, 3031−3038.

(7) Boutorine, A. S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Hélène, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2462−2465.

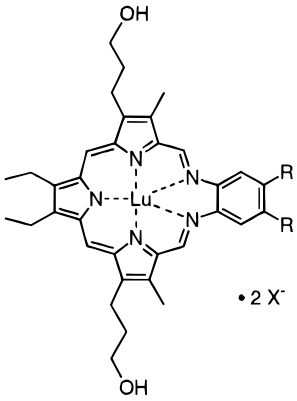
(8) Magda, D.; Wright, M.; Miller, R. A.; Sessler, J. L.; Sansom, P. I. *J. Am. Chem. Soc.* **1995**, *117*, 3629−3630.

(9) Wan, S.; Parrish, J. A.; Anderson, R. R.; Madden, M. *Photochem. Photobiol.* **1981**, *34*, 679−681.

(10) (a) Letsinger, R. L.; Schott, M. E. *J. Am. Chem. Soc.* **1981**, *103*, 7394−7396. (b) Asseline, U.; Delarue, M.; Lancelot, G.; Toulmé, F.; Thuong, N. T.; Montenay-Garestier, T.; Hélène, C. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3297−3301. (c) Giovannangeli, C.; Montenay-Garestier, T.; Rougée, M.; Chassignol, M.; Thuong, N. T.; Hélène, C. *J. Am. Chem. Soc.* **1991**, *113*, 7775−7777.

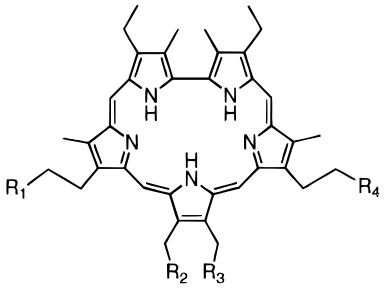
(11) (a) Letsinger, R. L.; Chaturvedi, S. K.; Farooqui, F.; Salunkhe, M. *J. Am. Chem. Soc.* **1993**, *115*, 7535−7536. (b) Gryaznov, S. M.; Lloyd, D. H. *Nucleic Acids Res.* **1993**, *21*, 5909−5915.

S0002-7863(96)01672-1 CCC: $12.00



1 R = $OCH_2CH_2CH_2OH$; X = Cl

2 R = $(OCH_2CH_2)_3OCH_3$; X = OAc



3 $R_1 = R_4 = CON(CH_2CH_2OH)_2$; $R_2 = R_3 = CH_3$

4 $R_1 = R_4 = CH_2OH$; $R_2 = R_3 = CH_3$

5 $R_1 = CH_2OH$; $R_2 = R_3 = CH_3$; $R_4 = CH_2ODMT$

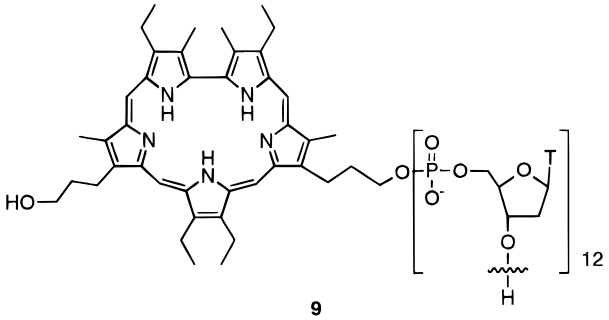
6 $R_1 = CH_2OPH(O)O^- HNEt_3^+$; $R_2 = R_3 = CH_3$; $R_4 = CH_2ODMT$

7 $R_1 = R_4 = H$; $R_2 = CH_2COOH$; $R_3 = H$

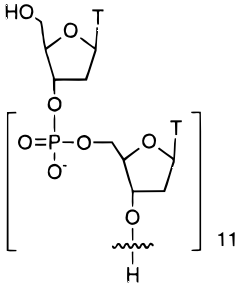
8 $R_1 = R_4 = H$; $R_2 = R_3 = CH_3$

lators,[10] hydrophobic groups,[11] cationic groups,[12] aliphatic and aromatic bridges,[13] and circular oligonucleotides.[14] Recently, we reported that sapphyrin **3** interacts with nucleic acids by several modes of interaction, including phosphate chelation of the phosphodiester backbone and hydrophobic interactions with the nucleobases.[15] The sapphyrins (e.g., **3**−**8**) therefore represent potential candidates for use in oligonucleotide affinity enhancement studies.

In this paper, we describe the synthesis of the novel sapphyrin−oligonucleotide conjugate **9**. This compound not only shows sequence-specific photomodification of DNA upon irradiation with wavelengths above 620 nm but also exhibits increased binding affinities to complementary nucleic acids over the control oligonucleotide $(dT)_{12}$ (**10**).



9



10

Experimental Section

General Methods. Proton and carbon-13 NMR spectra were recorded on either a General Electric QE-300 (300 MHz) or a GE GN500 instrument. Phosphorus-31 NMR spectra were recorded on a Varian Unity Plus (300 MHz) instrument with phosphoric acid used as an external reference. UV−vis spectra were recorded on a Beckman DU 640 instrument using cuvettes of 1 cm path length. Doubly distilled water (ddH_2O) was used in the preparation of all aqueous solutions. MALDI-MS studies were done on a LaserTec laser desorption mass spectrometer, linear mode (PerSeptive Biosystems).

Syntheses. The syntheses of texaphyrin derivatives **1** and **2** and sapphyrin derivatives **3**, **4**, **7**, and **8** were described previously.[16−20]

8-[[(4,4′-Dimethoxytrityl)oxy]propyl]-17-(hydroxypropyl)-3,12,13,22-tetraethyl-2,7,18,23-tetramethylsapphyrin (5). 8,17-Bis-(hydroxypropyl)-3,12,13,22-tetraethyl-2,7,18,23-tetramethylsapphyrin bistosylate salt **4** (492 mg, 0.49 mmol), pyridine (948 mg, 12 mmol), and one drop of 4-(dimethylamino)pyridine as a catalyst were dissolved in 100 mL of dichloromethane under nitrogen. To this green solution was added 4,4′-dimethoxytrityl chloride (203 mg, 0.6 mmol) dissolved in 50 mL of dichloromethane over 1 h at room temperature with stirring. The resulting green solution was stirred for an additional 4 h and was then washed two times with brine. The combined organic fractions were dried over sodium sulfate, and dichloromethane was removed using a rotary evaporator. The product was isolated by column chromatography on silica gel using dichloromethane/methanol (1−10% v/v gradient) containing 0.1% triethylamine as the eluent to give a 59% yield (279 mg) of the product **5**. ^1H NMR (300 MHz, $CDCl_3$): δ 2.14−2.23 (m, 12H, CH_2CH_3), 2.94−2.99 (m, 4H, $CH_2CH_2CH_2O$), 3.79 (s, 6H, OCH_3), 3.85 (t, 2H, $CH_2CH_2CH_2O$), 4.16 (s, 6H, CH_3), 4.17 (s,

(12) (a) Letsinger, R. L.; Singman, C. N.; Histand, G.; Salunkhe, M. *J. Am. Chem. Soc.* **1988**, *110*, 4470−4471. (b) Nara, H.; Ono, A.; Matsuda, A. *Bioconjugate Chem.* **1995**, *6*, 54−61. (c) Hashimoto, H.; Nelson, M. G.; Switzer, C. *J. Am. Chem. Soc.* **1993**, *115*, 7128−7134. (d) Fathi, R.; Huang, Q.; Syi, J.-L.; Delaney, W.; Cook, A. F. *Bioconjugate Chem.* **1994**, *5*, 47−57. (e) Tung, C.-H.; Breslauer, K. J.; Stein, S. *Nucleic Acids Res.* **1993**, *21*, 5489−5494. (f) Takeda, T.; Ikeda, K.; Mizuno, Y.; Ueda, T. *Chem. Pharm. Bull.* **1987**, *35*, 3558−3567. (g) Vinogradov, S. V.; Suzdaltseva, Y. G.; Kabanov, A. V. *Bioconjugate Chem.* **1996**, *7*, 3−6.

(13) (a) Durand, M.; Chevrie, K.; Chassignol, M.; Thuong, N. T.; Maurizot, J. C. *Nucleic Acids Res.* **1990**, *18*, 6353−6359. (b) Richardson, P. L.; Schepartz, A. *J. Am. Chem. Soc.* **1991**, *113*, 5109−5111. (c) Salunkhe, M.; Wu, T.; Letsinger, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8768−8772. (d) Durand, M.; Peloille, S.; Thuong, N. T.; Maurizot, J. C. *Biochemistry* **1992**, *31*, 9197−9204. (e) Ma, M. Y.-X.; Reid, L. S.; Climie, S. C.; Lin, W. C.; Kuperman, R.; Sumner-Smith, M.; Barnett, R. W. *Biochemistry* **1993,** *32*, 1751−1758.

(14) Prakash, G.; Kool, E. T. *J. Am. Chem. Soc.* **1992**, *114*, 3523−3527.

(15) (a) Iverson, B. L.; Shreder, K.; Král, V.; Sansom, P.; Lynch, V.; Sessler, J. L. *J. Am. Chem. Soc.* **1996**, *118*, 1608−1616. (b) Iverson, B. L.; Shreder, K.; Král, V.; Sessler, J. L. *J. Am. Chem. Soc.* **1993**, *115*, 11022−11023.

(16) Sessler, J. L.; Mody, T. D.; Hemmi, G. W.; Lynch, V. *Inorg. Chem.* **1993**, *32*, 3175−3187.

(17) Young, S. W.; Woodburn, K. W.; Wright, M.; Mody, T. D.; Fan, Q.; Sessler, J. L.; Dow, W. C.; Miller, R. A. *Photochem. Photobiol.*, in press.

(18) Král, V.; Furuta, H.; Shreder, K.; Lynch, V.; Sessler, J. L. *J. Am. Chem. Soc.* **1996**, *118*, 1595−1607.

(19) Iverson, B. L.; Shreder, K.; Morishima, T.; Rosingana, M.; Sessler, J. L. *J. Org. Chem.* **1995**, *60*, 6616−6620.

(20) Bauer, V. J.; Clive, D. L.; Dolphin, D.; Paine, J. B., III; Harris, F. L.; King, M. M.; Loder, J.; Wang, S.-W. C.; Woodward, R. B. *J. Am. Chem. Soc.* **1983**, *105*, 6429−6436.

3H, CH_3), 4.18 (t, 2H, $CH_2CH_2CH_2O$), 4.21 (s, 3H, CH_3), 4.60 (q, 4H, CH_2CH_3), 4.73 (q, 4H, CH_2CH_3), 4.89 (t, 4H, $CH_2CH_2CH_2O$), 6.92 (d, 4H, *m*-anisole-*H*), 7.30 (t, 1H, *p*-phenyl-*H*), 7.41 (t, 2H, *m*-phenyl-*H*), 7.51 (d, 4H, *o*-anisole-*H*), 7.60 (d, 2H, *o*-phenyl-*H*), 11.54 (s, 2H, meso-*H*), 11.55 (s, 2H, meso-*H*). ^{13}C NMR (500 MHz, $CDCl_3$): δ 11.85, 14.85, 16.88, 17.32, 17.55, 17.63, 19.68, 19.83, 19.92, 22.57, 23.99, 33.30, 34.36, 34.40, 54.25, 57.27, 60.69, 62.68, 75.26, 75.29, 75.36, 75.41, 85.42, 90.99, 97.16, 112.22, 119.92, 124.37, 125.88, 126.13, 126.94, 127.41, 128.22, 128.37, 129.02, 129.28, 131.62, 131.64, 134.03, 134.51, 134.81, 135.65, 136.31, 138.08, 138.57, 140.65, 140.80, 141.65, 141.95, 144.48, 157.58. HRMS FAB, M^+: *m/z* 962.5599 (calcd for $C_{63}H_{72}N_5O_4$, *m/z* 962.5584).

8-[[(4,4′-Dimethoxytrityl)oxy]propyl]-17-(hydrogen phosphonate propyl)-3,12,13,22-tetraethyl-2,7,18,23-tetramethylsapphyrin (6). Triethylamine (871 mg, 8.6 mmol) and **5** (46 mg, 0.048 mmol) were dissolved in 50 mL of dichloromethane at room temperature under nitrogen. While this solution was stirred at room temperature, tris-(1,1,1,3,3,3-hexafluoro-2-propyl) phosphite[21] (200 mg, 0.38 mmol) was added in two equal portions with a 15 min interval between additions. The resulting green solution was stirred for an additional 30 min at room temperature and then was washed two times with an aqueous 1.0 M solution of triethylammonium bicarbonate (pH 8.5). The combined organic fractions were dried over sodium sulfate, and the dichloromethane was removed using a rotary evaporator. The product **6** was isolated in 70% yield (38 mg) using column chromatography on silica gel using dichloromethane/methanol (1−15% v/v gradient) containing 0.1% triethylamine as the eluent. ^{1}H NMR (300 MHz, 2% $CD_3OD/CDCl_3$): δ 2.14−2.24 (m, 12H, CH_2CH_3), 2.99 (m, 4H, $CH_2CH_2CH_2O$), 3.74 (s, 6H, OCH_3), 3.77 (t, 2H, $CH_2CH_2CH_2O$), 3.99 (s, 3H, CH_3), 4.16 (t, 2H, $CH_2CH_2CH_2O$), 4.20 (s, 3H, CH_3), 4.23 (s, 6H, CH_3), 4.62−4.75 (m, 8H, CH_2CH_3), 4.89 (t, 4H, $CH_2CH_2CH_2O$), 6.86 (d, 4H, *m*-anisole-*H*), 7.28 (t, 1H, *p*-phenyl-*H*), 7.36 (t, 2H, *m*-phenyl-*H*), 7.54 (d, 4H, *o*-anisole-*H*), 7.69 (d, 2H, *o*-phenyl-*H*), 10.92 (s, 1H, P*H*), 11.56 (s, 4H, meso-*H*). ^{13}C NMR (500 MHz, 2% $CD_3OD/CDCl_3$): δ 12.30, 12.79, 16.26, 16.31, 17.64, 17.89, 18.00, 18.57, 20.15, 20.77, 20.88, 22.29, 24.99, 29.03, 31.49, 31.55, 32.69, 32.74, 34.08, 55.17, 59.55, 59.63, 63.73, 86.32, 89.72, 90.29, 90.30, 97.48, 98.91, 113.10, 126.81, 127.85, 128.33, 129.60, 130.17, 131.5, 132.45, 132.75, 132.95, 133.65, 134.29, 135.36, 136.62, 138.50, 138.79, 139.07, 141.37, 142.22, 143.39, 144.89, 145.39, 158.42. ^{31}P NMR (500 MHz, 2% $CD_3OD/CDCl_3$): δ 0.047 (J_{P-H} = 623 Hz). HRMS FAB, M^+: *m/z* 1026.5310 (calcd for $C_{63}H_{73}N_5O_6P$, *m/z* 1026.5298).

17-[Dodeca(deoxythymidine)]-8-(hydroxypropyl)-3,12,13,22-tetraethyl-2,7,18,23-tetramethylsapphyrin (9). Syntheses were performed on a Cruachem PS 150 DNA synthesizer. Trimethylacetyl chloride (pivaloyl chloride) was distilled at atmospheric pressure and stored under nitrogen. Pyridine was predistilled from calcium hydride before being redistilled over ninhydrin just prior to use. Acetonitrile and dichloromethane were distilled from calcium hydride. Tetrahydrofuran was distilled over benzophenone and sodium. The synthesis column was packed with $(dT)_{12}$, synthesized on a 0.2 μM scale, still being attached to the control pore glass (CPG) beads (Ransom Hill Bioscience). During coupling, the system was maintained under an argon atmosphere of 6 psi. The coupling was effected using the following modified hydrogen phosphonate oligonucleotide protocol:[22] (1) wash—acetonitrile (2 min); (2) deprotection—3% dichloroacetic acid in dichloromethane (4 min); (3) wash—acetonitrile (2 min); (4) wash—acetonitrile/pyridine (1/1) (2 min); (5) coupling—10 mM **6** in dichloromethane, 50 mM pivaloyl chloride in acetonitrile/pyridine (1/1) (30 μL alternating), wait 2 min, reinject collected sapphyrin solution with 50 mM pivaloyl chloride in acetonitrile/pyridine (1/1) (60 μL of sapphyrin solution and 30 μL of pivaloyl chloride solution alternating), reinject collected sapphyrin solution three times; (6) washes—acetonitrile (2 min), acetonitrile/pyridine (1/1) (2 min), acetonitrile (1 min), dichloromethane (2 min), acetonitrile (1 min); (7) deprotection—3% dichloroacetic acid in dichloromethane (4 min); (8) washes—acetonitrile (2 min), acetonitrile/pyridine (1/1) (2 min); (9) oxidation—0.1 M I_2 in pyridine/*N*-methylimidazole/water/tetrahydrofuran (4/1/5/90) (1 mL over 1 min), 0.1 M I_2 in triethylamine/water/tetrahydrofuran (5/5/90) (1 mL over 1 min); (10) washes—acetonitrile/pyridine (1/1) (2 min), acetonitrile (2 min), methanol (2 min), acetonitrile (2 min).

The dark green CPG was added to 2.5 mL of concentrated ammonium hydroxide, and the heterogeneous mixture was stirred at room temperature for 1 h. The light green solution was then removed from the white CPG and was concentrated to form a light green solid. The coupling yield was 56% based on dimethoxytrityl cation concentrations measured by visible spectroscopy during deprotection. The green solid was dissolved in water and was purified by a Pharmacia FPLC system using a PepRPC column (porous silica, C_2/C_{18}, Pharmacia) and 0−50% gradient 100 mM triethylammonium acetate buffer (pH 7.0)/acetonitrile as the eluent. UV−vis (10 mM bis-tris, 10 mM NaCl buffer, pH 7.0) (λ (nm) (ϵ)): 266 (96 000), 428 (52 000), 452 (550 000), 621 (14 000), 677 (10 400). Reversed phase HPLC (Waters) analysis with a C_{18} column, using 70% 100 mM triethylammonium acetate (pH 7.0)/acetonitrile as the eluent, gave a retention time of 6.7 min for **9** and 1.3 min for $(dT)_{12}$ (**10**) (Midland Certified Reagent Co.). Polyacrylamide gel electrophoresis with a 19%, 7 M urea denaturing gel run at 250 V for 4 h with TBE (89 mM tris-borate, 2 mM EDTA) running buffer gave a run distance of 3.9 cm from the origin of the gel for conjugate **9** and 6.0 cm for **10**. Matrix-assisted laser desorption-ionization mass spectrometry (MALDI-MS), *m/z* 4312.1 (calcd for **9**·-$2H^+$ ($C_{162}H_{211}O_{86}N_{29}P_{12}$), *m/z* 4312.3), parent molecular ion, and *m/z* 8629.9 (calcd for $[\mathbf{9}\cdot 2H^+]_2$ ($C_{324}H_{422}O_{172}N_{58}P_{24}$), *m/z* 8624.6), dimer molecular ion.

Site-Directed Photocleavage Studies. Synthetic DNA sequences **11**, **12**, and **13** (Keystone Laboratories) were selected as substrates and were 5′-end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (NEN Research Products, Dupont). Duplex **13** was labeled on the oligonucleotide strand containing the homothymidylate sequence. Samples, consisting of substrate and conjugate **9** or $(dT)_{12}$ (**10**), were made up to a total volume of 100 μl in clear, 0.65 mL Eppendorf tubes. These samples also contained 10 mM bis-tris (1,3-bis[[tris(hydroxymethyl)methyl]amino]propane) and 100 mM or 250 mM NaCl and were buffered to pH 7.0. Samples were cooled for 30 min in an ice−water bath and were irradiated at 5 °C with the light of a high-pressure xenon lamp (Oriel) passed through either a >300 or >620 nm filter. The sample, surrounded by an ice bath, received approximately 400 mW/cm^2 (λ > 300 nm) or 320 mW/cm^2 (λ > 620 nm). After irradiation, the sample solution was frozen and lyophilized. Alkali-labile sites were characterized by piperidine treatment.[23] The samples were then redissolved in loading buffer (98% formamide, 10 mM EDTA containing xylene cyanole FF and bromophenol blue), and approximately 10 μL (1×10^5 cpm) of each sample was run on a 19% denaturing polyacrylamide gel containing 7 M urea. The gels were run at 1600 V for 4 h using TBE running buffer. Autoradiograms were obtained by exposing the gels to Amersham autoradiography film at 0 °C for 24 h. Gel exposures were analyzed by quantitative densitometry.[24]

pBR322 Photocleavage Studies. DNA cleavage was quantified by monitoring the conversion of supercoiled (form I) plasmid DNA to nicked circular (form II) DNA. Plasmid pBR322 DNA was bought from Gibco BRL. In accord with the general procedure of ref 25, 20 ng/μL DNA was irradiated in quartz cuvettes in the presence of 4 μM sapphyrin **3** and 10 mM bis-tris, 10 mM NaCl buffer (pH 7.0) under specified conditions. Irradiation was effected at room temperature using light from a high-pressure xenon lamp (Oriel) passed through either a >300 or a >620 nm filter. The sample received approximately 400 mW/cm^2 (λ > 300 nm) or 320 mW/cm^2 (λ > 620 nm). A 12 μL portion of each sample was added to 5 μL of loading buffer (30% glycerol/ddH_2O containing bromophenol blue) and was run on a 0.8% agarose gel prepared with a 2 μM solution of ethidium bromide in TEA buffer (40 mM tris-acetate, 1 mM EDTA). After electrophoresis using TEA

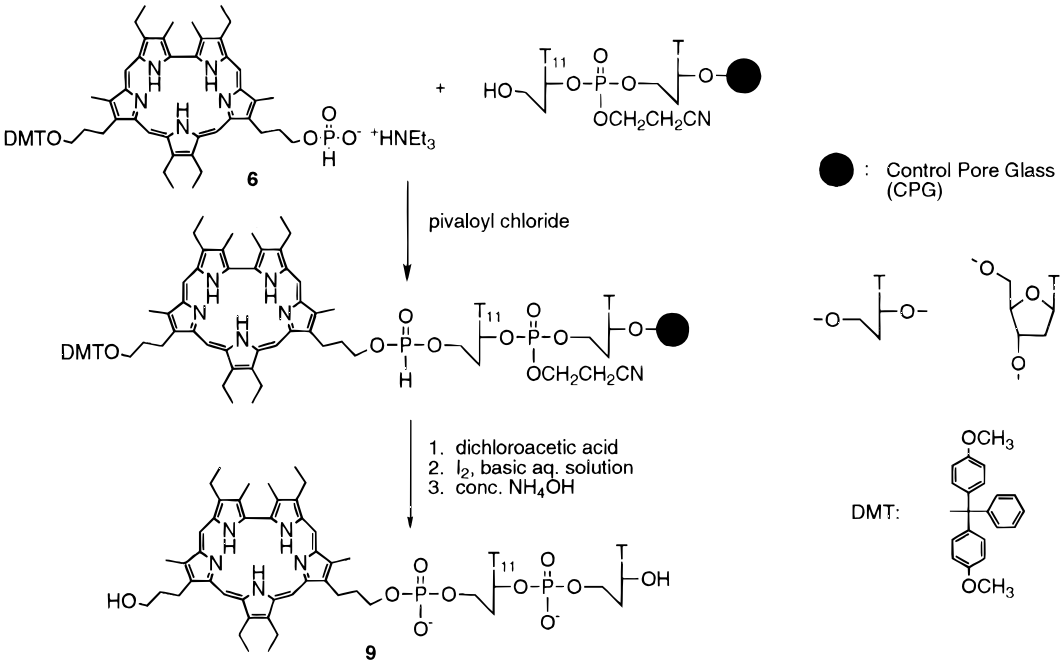
(21) (a) Sakatsume, O.; Yamane, H.; Takaku, H.; Yamamoto, N. *Tetrahedron Lett.* **1989**, *30*, 6375−6378. (b) Denney, D. B.; Denney, D. Z.; Hammond, P. J.; Liu, L.-T.; Wang, Y.-P. *J. Org. Chem.* **1983**, *48*, 2159−2164.

(22) (a) Froehler, B. C.; Ng, P. G.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 5399−5407. (b) Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1988**, *29*, 2619−2622.

(23) Sambrook, J.; Frisch, E. F.; Maniatis, T. *Molecular Cloning, A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: New York, 1989.

(24) Poulsen, L. L.; Ziegler, D. M. U.S. Patent No. 5,194,949, 1993.

Scheme 1. Synthesis of Sapphyrin−Oligonucleotide Conjugate **9**



as the running buffer, the DNA was visualized using a 312 nm UV light box. Gel pictures were analyzed by quantitative densitometry.[24,25]

Melting Temperature Studies. Optical melting curves were obtained using a Hewlett-Packard 8452A diode array spectrophotometer equipped with a stirred cell holder connected to a 386 personal computer. The jacketed cell holder was linked to a Fisher Scientific isotemp refrigerated circulator. Solutions (1.5 mL) were placed in a quartz cuvette with a path length of 1 cm. Absorbance values were measured every 30 s and were collected by the personal computer. The temperature increased approximately 1 °C/min, and the values were manually collected in conjunction with the absorbances using a digital thermometer with a Cu−CuNi thermocouple (Omega) placed directly in the cuvette. Melting temperature curves (the change of relative absorbance at 260 nm over a temperature range), their first-derivative curves, and the inflection point of the first-derivative curve (the melting temperature) were determined using KaleidaGraph 3.0. Melting temperatures were an average of two to three separate studies. Poly(A) and $d(T)_{12}$ (**10**) oligonucleotides used in this study were bought from Midland Certified Reagent Co., and oligodeoxyribonucleotide substrates **11**−**20** were purchased from Keystone.

Results and Discussion

Sapphyrin−Oligonucleotide Conjugate Characteristics. The sapphyrin−oligonucleotide conjugate **9** was synthesized from the sapphyrin derivative **6** (Scheme 1). Monoprotected sapphyrin hydrogen phosphonate **6** was obtained in two steps *via* monoprotection and subsequent phosphorylation of **4**. The stability of sapphyrin to strongly acidic and basic conditions allowed for its use as a "nucleotide building block" in the standard hydrogen phosphonate oligonucleotide synthesis protocol.[22] Specifically, sapphyrin **6** was activated using pivaloyl chloride and was coupled to the 5′ end of a $(dT)_{12}$ oligonucleotide (synthesized by the phosphoramidite method) that was still attached to control pore glass (CPG) beads. The resulting intermediate was deprotected at the 5′ end by treatment with dichloroacetic acid, and the hydrogen phosphonate linkage was oxidized with basic aqueous iodine solutions. Finally, the phosphodiester linkages on the $(dT)_{12}$ portion of the molecule were deprotected, and the compound was cleaved from the CPG with concentrated aqueous ammonium hydroxide.

The conjugate **9** was purified by preparative reversed phase chromatography and characterized by reversed phase HPLC, polyacrylamide gel electrophoresis, UV−vis spectroscopy, and MALDI-MS. The sapphyrin moiety effects a retardation in the mobility of conjugate **9** over $(dT)_{12}$ (**10**) in reversed phase HPLC and during polyacrylamide gel electrophoresis. Such a retardation was expected in view of the size and hydrophobicity of the sapphyrin subunit. The UV−vis spectrum of purified **9** includes characteristic sapphyrin and DNA absorbances (Figure 1). On the basis of earlier studies, the absorbance band with a λ_{max} of 452 nm is assigned to the sapphyrin moiety of **9** in its monomeric form, whereas the shoulder at 428 nm is assigned to the sapphyrin subunit in a noncovalent dimeric form.[15a,26] Conjugate **9** is thus the first water-soluble derivative of sapphyrin in which the macrocycle is nearly completely monomeric. Importantly, the ratio of the extinction coefficients for the absorbances at 452 and 260 nm ($\epsilon_{452}/\epsilon_{260}$) is 5.8 for **9**. This value agrees well with 5.5, the ratio ($\epsilon_{452}/\epsilon_{260}$) of the

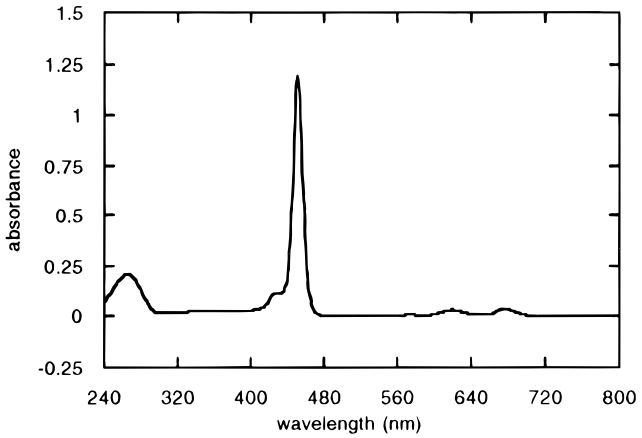


Figure 1. UV−vis spectrum of sapphyrin−oligonucleotide **9** recorded as a *ca.* 2.2 μM aqueous solution (10 mM bis-tris, 10 mM NaCl buffer, pH 7.0).

(25) (a) Praseuth, D.; Gaudemer, A.; Verlhac, J. -B.; Kraljic, I.; Sissoëff, I.; Guillé, E. *Photochem. Photobiol.* **1986**, *44*, 717−724. (b) Croke, D. T.; Perrouault, L.; Sari, M. A.; Battioni, J. -P.; Mansuy, D.; Hélène, C.; Le Doan, T. *J. Photochem. Photobiol., B.* **1993**, *18*, 41−50.

(26) Král, V.; Andrievsky, A.; Sessler, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 2953−2954.

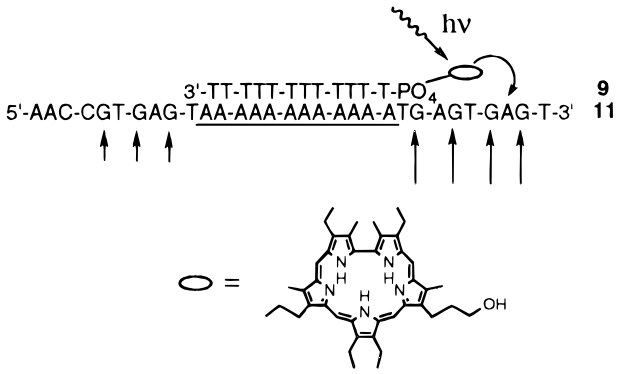


Figure 2. Schematic representation of the site-directed photocleavage of substrate **11** effected by conjugate **9**.

extinction coefficients for monomeric $[2H\cdot\mathbf{4}]^{2+}\cdot 2OTs^-$ in CH_2Cl_2 (at 452 nm) and $(dT)_{12}$ (**10**) in water (at 260 nm).[27]

Wavelength Dependent pBR322 Photocleavage Studies. Sapphyrin produced substantial photocleavage of supercoiled plasmid pBR322 upon irradiation using wavelengths >620 nm. For example, in experiments involving sapphyrin **3** and pBR322 DNA, a 3 h illumination at > 620 nm (320 mW/cm^2) produced conversion of 47% supercoiled pBR322 to the open-circular form (Supporting Information). Some cleavage could be effected upon irradiation with wavelengths >700 nm,[8] but with less efficiency than that observed using >620 nm irradiation.

Site-Directed Photocleavage Studies. Sequence-specific photocleavage upon irradiation at >620 and >300 nm was investigated using conjugate **9** and the complementary oligonucleotide **11** (Figure 2). Sequence **11** was chosen on the basis of earlier work involving similar porphyrin−, as opposed to sapphyrin−, oligonucleotide conjugates.[2d] The autoradiogram (Figure 3) shows the photoproduct bands generated upon irradiation at 5 °C of the complex formed between the sapphyrin−oligonucleotide conjugate **9** and its target substrate **11**, the sequence of which is shown on the left side of the figure.[30] Irradiation at wavelengths of both >300 and >620 nm led to the formation of two new species (labeled as I and II, with a possible third species which migrates as a faint band slower than I and II). These new bands migrate slower than the starting material (lanes 8 and 12 for >300 nm and lane 13 for >620 nm) and are thus, in analogy to what was previously observed with porphyrins,[2d] azido derivatives,[3] and proflavins,[6] ascribed to cross-linked products formed between **9** and **11**. When the irradiated samples were treated with 1 M piperidine at 90 °C for 20 min (lanes 10 and 14 for >300 nm and lanes 11 and 15 for >620 nm), shorter fragments were formed that comigrated with a Maxam−Gilbert (G) sequencing lane (lane 2) and the putative cross-linking bands diminished in intensity.

5'-AAC-CGT-GAG-TAA-AAA-AAA-AAA-ATG-AGT-GAG-T-3' **11**

5'-AAC-CGT-GAG-TTT-TTT-TTT-TTT-TTG-AGT-GAG-T-3' **12**

5'-AAC -CGT-GAG-TTT-TTT-TTT-TTT-TTG-AGT-GAG-T-3'
3'-TTG-GCA-CTC-AAA-AAA-AAA-AAA-AAC-TCA-CTC-A-5' **13**

There was greater cleavage at the three guanine residues near the 3′ end of the substrate compared to the three guanine residues near the 5′ end of the substrate.[31] In particular, the cleavage at the 3′-guanine residues of the substrate was 70−80% of the total cleavage observed (e.g., see lanes 10, 11, 14, and 15 in Figure 3). This is particularly important as the 3′ region of substrate is the expected vicinity of the sapphyrin subunit in an antiparallel duplex.

Controls demonstrated that light, the sapphyrin moiety, and hybridization were all required to effect observed site-directed cleavage. There was no cross-linking or cleavage observed in the absence of illumination. This was true whether or not the reactions were subject to piperidine treatment (lanes 4 and 6).[32] Likewise, neither cleavage nor cross-linking was observed when sapphyrin-free $(dT)_{12}$ (**10**) was irradiated (lanes 5 and 7) for 40 min (>300 nm) in the presence of substrate **11** with or without piperidine treatment. Furthermore, when conjugate **9** was irradiated in the presence of the noncomplementary substrate **12** (the sequence of which is shown on the right side of Figure 3), no cleavage was observed, even under maximum irradiation conditions (20 min, λ > 300 nm, or 40 min, λ > 620 nm) and when subject to followup piperidine treatment (lanes 19 and 20).[32] Finally, no other residues (C, A, T) were found to be significantly cleaved when **9** and **11** were irradiated together either with or without piperidine treatment. This latter result is considered particularly important, since it means that self-destruction of a sapphyrin conjugate not containing guanine residues is likely improbable.

Photocleavage was investigated using different salt concentrations. By increasing the salt concentration from 100 to 250 mM, greater cleavage efficiencies of the three guanines at the 5′ and 3′ ends of the substrate were observed. This result can be rationalized in terms of the known finding that DNA duplexes become more stable as the overall ionic strength of the solution is raised.[33] Triple helix formation is also common at high ionic concentrations,[34] and such complexation cannot be ruled out. It is, however, not favored as playing a role in these photocleavage studies. In binding studies using conjugate **9** and substrate **11**, triple helix formation was not observed (melting temperature studies, *vide infra*). Furthermore, photocleavage was observed at both the 5′ and 3′ ends of the substrate during studies conducted at room temperature, a temperature at which triple helix formation is not favored.[35] Finally, photocleavage experiments based on triple helix formation were attempted using the duplex substrate **13**; however, no cleavage was observed.

Mode of Photomodification. Two limiting mechanistic possibilities can be considered for the sapphyrin-mediated photomodification reaction. The first possibility involves direct electron transfer (ET) between certain DNA bases (e.g., G residues) and the sapphyrin subunit in either a triplet or singlet excited state. The second scenario involves a photoexcited sapphyrin in its triplet state reacting with molecular oxygen to form singlet oxygen, a species that reacts readily with guanine.[36]

The cross-linked adducts formed upon photoexcitation (Figure 3, bands I and II) are most likely due to the electron transfer

(27) The solvent CH_2Cl_2 is necessary to keep the macrocycle monomeric.
(28) Iverson, B. L.; Dervan, P. B. *Nucleic Acids Res.* **1987**, *15*, 7823−7830.
(29) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499−560.
(30) The low reaction temperature (5 °C) was chosen to maximize duplex formation.
(31) Cleavage at six guanine residues, namely, G5, G7, G9, G24, G26, and G28, was analyzed by gel densitometry. Densitometry measurements involving G30 could not be made accurately.
(32) Background cleavage was minor, being observed to be less than 2% of the intact substrate strand.
(33) The increased stability is thought to be a consequence of the decline in the repulsive interactions between the negatively charged phosphodiester backbones: Saenger, W. *Principles of Nucleic Acids Structure*; Springer-Verlag: New York, 1984; p 145.
(34) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645−650.
(35) Shea, R. G.; Ng., P.; Bischofberger, N. *Nucleic Acids Res.* **1990**, *18*, 4859−4866.
(36) (a) Foote, C. S. *Science* **1968**, *162*, 963−970. (b) Rosenthal, I.; Pitts, J. N., Jr. *Biophys. J.* **1971**, *11*, 963−996.

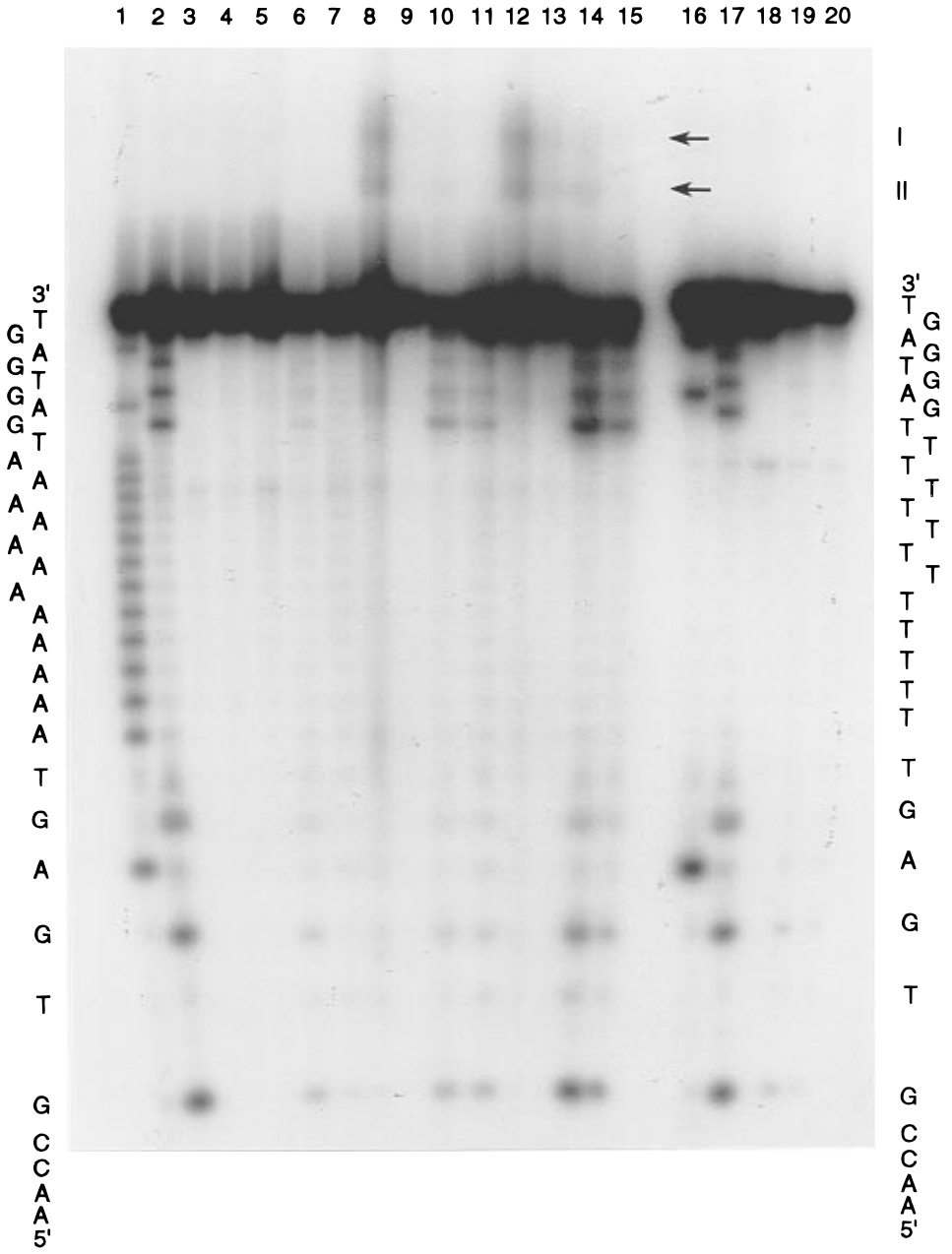


Figure 3. Photocleavage of synthetic DNA 31-mers **11** and **12** effected using the sapphyrin−oligonucleotide conjugate **9**. The following conditions pertained: lanes 1 and 16, Iverson−Dervan (A) sequencing reaction;[28] lanes 2 and 17, Maxam−Gilbert (G) sequencing reaction;[29] lanes 1−15, 32 nM substrate **11**; lanes 16−20, 32 nM substrate **12**; lanes 4, 6, 8−15, 19, and 20, 500 nM conjugate **9**; lanes 5 and 7, 500 nM $(dT)_{12}$ (**10**); lanes 3, 4, 6, and 18, dark controls; lanes 5 and 7, 40 min, >300 nm; lanes 8 and 10, 10 min, >300 nm; lanes 9 and 11, 20 min, >620 nm; lanes 12, 14, and 19, 20 min, >300 nm; lanes 13, 15, and 20, 40 min, >620 nm; lanes 6, 7, 10, 11, 14, 15, 19, and 20, piperidine treatment.

pathway. This is in analogy to the proposed electron transfer mediated cross-linking observed with porphyrins,[2d] azido derivatives,[3] and proflavins.[6] Sapphyrin could react directly with guanine bases to form radical species which may cross-link in a localized fashion. The formation of duplex structures could orient the sapphyrin macrocycles such that the requisite electron transfer processes are efficient and site-directed. These cross-linked adducts are cleaved after piperidine treatment to expose the photomodification to be at adjacent guanine residues.

The total observed photomodification, however, cannot be ascribed exclusively to cross-linking. Densitometry showed that the cross-linked products accounted for 4−7% of the total substrate radioactivity for photolysis reactions carried out in 100 mM sodium chloride. Yet, upon piperidine treatment, cleavage products accounted for 15−23% of the total substrate radioactivity in these very same reactions. Thus, the observed photomodification must reflect alkali-labile products that migrate as intact substrate on the gel.[37] These alkali-labile sites presumably involve the guanine bases and could be peroxyl derivatives of guanine radical cations formed *via* electron transfer reactions. They could also be the result of singlet oxygen reactions. Sapphyrin is known to be a moderately effective singlet oxygen sensitizer in organic solvents.[38]

A number of supercoiled pBR322 DNA cleavage studies were carried out to investigate the possible role of oxygen in the sapphyrin photomodification reaction. Running the photocleav-

(37) This is observed in photomodification reactions involving porphyrin macrocycles in which substrates with photooxidized guanines exhibit such behavior.[2d]

(38) (a) Maiya, B. G.; Cyr, M.; Harriman, A.; Sessler, J. L. *J. Phys. Chem.* **1990**, *94*, 3597−3601. (b) Judy, M. M.; Matthews, J. L.; Newman, J. T.; Skiles, H. L.; Boriack, R. L.; Sessler, J. L.; Cyr, M.; Maiya, B. G.; Nichol, S. T. *Photochem. Photobiol.* **1991**, *53*, 101−107.

Table 1. Melting Temperatures (°C) of the Complex of Conjugate **9** or $(dT)_{12}$ (**10**) with Target **11**[a]

	0 mM NaF, 0 mM $Na(H_2PO_4)$	5 mM NaF, 0 mM $Na(H_2PO_4)$	0 mM NaF, 250 mM $Na(H_2PO_4)$
conjugate **9**/substrate **11**	26.0 ± 0.1	28.7 ± 0.3	39.9 ± 0.1
$(dT)_{12}$ (**10**)/substrate **11**	23.3 ± 0.3	24.1 ± 0.1	35.5 ± 0.2

[a] All studies were done in 10 mM bis-tris, 100 mM NaCl buffer, pH 7.0. The concentration of each oligonucleotide component was 4 μM.

age experiments under air, argon, and oxygen atmospheres produced cleavage yields of 79%, 39%, and 80%, respectively. Since singlet oxygen travels farther in D_2O than H_2O,[39] studies were also carried out in a buffer containing aerated D_2O. Under these conditions, a cleavage yield of 64% was observed vs the 70% recorded in H_2O. Finally, experiments were carried out in the presence of sodium azide (150 mM), a known singlet oxygen quencher.[40] Under these conditions, lower cleavage yields were obtained than when sodium chloride (also 150 mM) was used (23% vs 50%, respectively). Although neither increasing the dissolved O_2 concentration nor using D_2O affected the cleavage yields, the fact that adding sodium azide and purging with argon diminished the effective per-photon cleavage yields is consistent with oxygen (especially singlet oxygen) playing some role in the photomodification process.

Further consistent with the above conclusions is the finding that the photocleavage is not completely site-selective. Some modification of the 5′ end of the substrate (i.e., remote from the site of the sapphyrin) is observed in experiments involving **9** and **11**. Certainly, during these site-directed studies, singlet oxygen is expected to be concentrated at the 3′ end of the substrate (i.e., in the vicinity of the bound sapphyrin). It is likely, however, that some fraction of the singlet oxygen migrates to the 5′ end of the duplex where it could effect oxidative modification of guanine residues in the usual way. Indeed, in photocleavage studies carried out in the presence of sodium azide, a known singlet oxygen quencher, no cleavage was observed at the 5′ end of the substrate.[41] Interestingly, the cleavage observed at the 3′ end of the substrate was analogous to that observed in the absence of sodium azide, although only half as effective.

Singlet oxygen is presumably derived from the triplet excited state of sapphyrin, so it was of interest to determine the lifetime of this state. Sapphyrin **7** was found to have a relatively long triplet lifetime of 151 ± 8 μs in N_2-saturated methanol.[42] Since lutetium(III) texaphyrin **1** had shown distinct dependence on O_2 concentration with respect to DNA photocleavage,[8] the triplet lifetimes of lutetium(III) texaphyrin and sapphyrin were compared in the same solvent system. In N_2-saturated methanol, lutetium(III) texaphyrin **2** showed a considerably shorter lifetime of 19.4 ± 1 μs.[42] It became clear that, in the case of lutetium(III) texaphyrin with its short triplet state lifetime, the available concentration of O_2 played a critical role in mediating the bimolecular photosensitized formation of singlet oxygen. The concentration of available oxygen thus acted to directly affect the observable DNA photocleavage yield. On the other hand, the triplet state sapphyrin is much longer lived. As a result, under the conditions of most of the experiments the actual O_2 concentration is rarely yield-limiting in terms of DNA photocleavage yield.

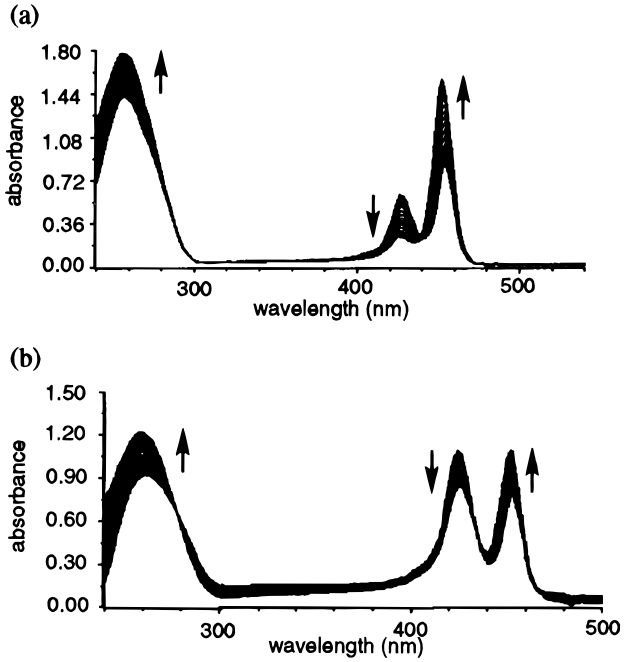


Figure 4. (a) Overlay spectra of conjugate **9** and target **11** (4 μM each) in 10 mM bis-tris, 100 mM NaCl aqueous buffer, pH 7.0, recorded over the temperature range of *ca.* 10−55 °C. (b) Overlay spectra of conjugate **9** and poly(A) (55.0 μM phosphates each) in 10 mM bis-tris, 500 mM NaCl aqueous buffer, pH 7.0, recorded over the temperature range of *ca.* 10−55 °C. (Arrows show the direction of change in absorbance.)

While the above aspects are readily understood, it is important to appreciate that the mechanism of photomodification mediated by sapphyrin is complex. It probably involves electron transfer as well as singlet oxygen activation. In any event, the net result of photoirradiation at wavelengths >620 nm is a combination of cross-linking and alkali-labile sites that together result in a significant per-photon DNA target modification.

Melting Temperature Studies of Conjugate 9 with Target 11. Melting temperature studies were carried out to determine whether the presence of a sapphyrin moiety on an oligonucleotide would augment binding to the appropriate complementary strand. In fact, the melting temperature of the complex formed between conjugate **9** and target **11** was 26.0 °C, whereas that of the relevant control system ($(dT)_{12}$ (**10**) and target **11**) was 23.3 °C (Table 1). Thus, sapphyrin does increase the binding affinity of a sapphyrin-bearing oligonucleotide to a complementary oligonucleotide.

In carrying out these studies, it was found, interestingly, that not only the absorbance band at 260 nm (corresponding to the oligonucleotide) but also the Soret-like bands of the sapphyrin moiety at *ca.* 423 and 452 nm could be monitored as a function of temperature (Figure 4a). These sapphyrin-based spectral changes are of interest since they yield insight into how the local environment of the sapphyrin macrocycle changes.[15a]

As discussed previously, the absorbance bands at λ_{max} of *ca.* 452 nm and 423 nm may be assigned to the sapphyrin moiety of **9** in its monomeric and noncovalent dimeric forms, respectively.[15a,26] Further proof of this model came from studies involving conjugate **9**. Conjugate **9** alone exhibits absorption bands at 428 and 452 nm, with the band at 452 nm being the dominant spectral feature (Figure 1). Under various salt

(39) Merkel, P. B.; Nilsson, R.; Kearns, D. R. *J. Am. Chem. Soc.* **1972**, *94*, 1030−1031.

(40) Hasty, N.; Merkel, P. B.; Radlick, P.; Kearns, D. R. *Tetrahedron Lett.* **1972**, *1*, 49−52.

(41) Cleavage at the 5′ end of the substrate may also be attributed to triple helix formation, although this mechanism is not favored; see the text.

(42) The triplet state lifetime of sapphyrin **8** is 60 ± 5 μs in N_2-saturated acetonitrile.[38a] The triplet lifetime of lutetium(III) texaphyrin **2** in N_2-saturated water is 17.3 ± 1 μs: Sessler, J. L.; Dow, W. C.; O'Connor, D.; Harriman, A.; Hemmi, G.; Mody, T. D.; Miller, R. A.; Qing, F.; Springs, S.; Woodburn, K.; Young, S. W. *J. Alloys Compd.*, in press.

concentration conditions (e.g., 500 mM NaCl or 5 mM NaF), however, the conjugate exhibits an absorbance band at 428 nm that is increased over that at 452 nm. On the other hand, when these same samples are diluted, the proportion of the absorbance at 428 nm decreases while that of the 452 nm band increases until this latter band becomes the dominant spectral feature once again. Such behavior is consistent with the sapphyrin in question at higher concentrations undergoing dimerization; this is a process known to be favored at high salt concentrations.[43] To the extent these spectroscopic assignments are correct, they serve to confirm further the idea that it is the dimeric and monomeric (in sapphyrin) forms of **9** that are the two species of interest in these melting temperature studies.

The dimeric and monomeric sapphyrins may bind nucleic acids by phosphate chelation and/or *via* hydrophobic interactions. In previous studies, three modes of interaction between sapphyrin **3** and DNA were described.[15a] The first mode, phosphate chelation, involves specific chelation of a phosphorylated nucleic acid oxyanion by the protonated core of the sapphyrin *via* Coulombic interactions. This mode is thought to involve both electrostatic and/or hydrogen bonding interactions. A second mode involves hydrophobic associations between sapphyrin units and the nucleobases present in single-stranded polymeric nucleotides. This hydrophobic interaction, which can be considered a type of "pseudointercalation", allows a sapphyrin moiety to bind among or between exposed nucleobases and thus be excluded from the aqueous media. Pseudointercalation has been proposed with other DNA-binding molecules such as proflavine[44] and porphyrin.[45] The final mode of sapphyrin−DNA binding is observed only at high sapphyrin-to-DNA phosphate ratios and involves the highly aggregated forms of sapphyrin **3** bound to the surface of double strand DNA. In the present instance, involving work with conjugate **9** and its complementary nucleic acids, it appears that only the more site-isolated sapphyrin species, i.e., dimeric and monomeric forms, are of relevance; on the basis of the above spectroscopic findings, these are expected to be bound primarily *via* phosphate chelation and/or pseudointercalation.

Melting Temperature Studies in the Presence of Different Salt Concentrations. Studies involving various salt concentrations were carried out to address the question of whether or not phosphate chelation plays a significant role in enhancing the observed conjugate-to-target interactions. Phosphate chelation, as it relates to sapphyrin−DNA binding, is largely an electrostatic event. Accordingly, changes in buffer ionic strength should affect the extent to which this kind of interaction influences observed hybridization processes. Such variations have been previously observed in the case of oligonucleotides bearing cationic functionalities.[12a−c] In the case of sapphyrins, this would lead to the prediction that increasing the ionic strength should decrease the melting temperature.

The first experiments involved different concentrations of sodium chloride. Melting temperature studies were performed using the complexes formed between conjugate **9** and poly(A) as well as $(dT)_{12}$ (**10**) and poly(A), and it was found that in general the melting temperatures of the former complex were the highest (Figure 4b and Table 2).[46] Furthermore, conjugate **9** was found to exhibit the largest increases in melting temperature (again relative to controls) at the *highest* NaCl concentrations. This result is consistent with phosphate chelation playing only a minor role (if any) in enhancing the binding interactions between sapphyrin-containing conjugates and their complementary oligonucleotide targets.

Table 2. Melting Temperatures (°C) of the Complex of Conjugate **9** or $(dT)_{12}$ (**10**) with Poly(A)[a]

	27.5 μM phosphates[b], 50 mM NaCl	55.0 μM phosphates[b], 50 mM NaCl	27.5 μM phosphates[b], 500 mM NaCl	55.0 μM phosphates[b], 500 mM NaCl
conjugate **9**/ poly(A)	21.7 ± 1.4	23.1 ± 0.4	42.1 ± 0.9	43.9 ± 0.7
$(dT)_{12}$ (**10**)/ poly(A)	19.3 ± 0.5	20.6 ± 0.5	38.7 ± 0.2	40.6 ± 0.5

[a] All studies were done in 10 mM bis-tris buffer, pH 7.0. [b] Concentration of each component.

Other experiments, involving sodium phosphate and sodium fluoride, were carried out to address further the possible role phosphate chelation plays in moderating the observed conjugate-to-target interactions. These anions were chosen because they display particularly high affinities for sapphyrins.[47] As a result, they would be expected to "lock up" the phosphate chelation binding site under the conditions of the experiments and thus effectively "cancel out" any increases in the melting temperature that might be due to phosphate chelation.

In spite of the above expectations, the recorded melting temperatures were not found to decrease upon addition of phosphate or fluoride anions. In the presence and absence of 250 mM sodium phosphate, conjugate **9** was found to exhibit similar increases in melting temperature relative to controls (Table 1). Furthermore, conjugate **9** (bound to target **11**) was found to exhibit a *greater* increase in melting temperature relative to the control in the presence of 5 mM NaF (Table 1). A similar trend was observed even when the target nucleic acid was poly(A). Specifically, for the complex formed between conjugate **9** and poly(A), the melting temperature was found to be 47.4 °C (10 mM bis-tris, 5 mM NaF, 500 mM NaCl buffer, pH 7.0). This is a 7.1 °C increase over that observed for the control ($(dT)_{12}$ (**10**) and poly(A)) under the same conditions. These quantitative results thus lend further credence to the conclusion that it is not interstrand sapphyrin−phosphate chelation that is responsible for the increased stability observed for complexes formed between conjugate **9** and its oligonucleotide targets.

The above results, especially the ones obtained from studies involving NaF, do, however, support the conclusion that the increased stabilization observed in the case of sapphyrin conjugate **9** is due to enhanced interstrand hydrophobic binding. When bound to fluoride anion (in particular), the charge of the monoprotonated sapphyrin becomes neutralized. This results in a flat, hydrophobic complex that should be well poised for interaction with its target strand *via* pseudointercalation.

Melting Temperature Studies Using Various Oligonucleotide Targets. To study the influence of different target sequences, oligodeoxyribonucleotides **14**−**20** were investigated (Table 3). Preferential binding was observed for conjugate **9** (over $(dT)_{12}$ (**10**)) with targets **15** and **16** which have a single-stranded "overhanging" oligonucleotide available for binding at the 3′ end of the targets (studies 3−6). Enhanced binding for conjugate **9** (again, over $(dT)_{12}$ (**10**)), however, was not observed with targets **17** and **18** in which no single-stranded oligonucleotide is available at the 3′ end of the target (studies 7−10). These results are consistent with the proposed hydro-

(43) Valdes-Aguilera, O.; Neckers, D. C. *Acc. Chem. Res.* **1989**, *22*, 171−177.

(44) Dourlent, M; Hogrel, J. F. *Biopolymers* **1976**, *15*, 29−41.

(45) Pasternack, R. F.; Brigandi, R. A.; Abrams, M. J.; Williams, A. P.; Gibbs, E. J. *Inorg. Chem.* **1990**, *29*, 4483−4486.

(46) Poly(A) was used in these studies to illustrate how sapphyrin conjugates might be useful in binding RNA substrates, common single-stranded oligonucleotide therapeutic targets.

(47) Shionoya, M.; Furuta, H.; Lynch, V.; Harriman, A.; Sessler, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 5714−5722.

Table 3. Melting Temperatures (°C) of the Complex of Conjugate **9** or $(dT)_{12}$ (**10**) with Various Targets[a]

study	complex	melting temp (°C)	study	complex	melting temp (°C)
1	conjugate **9**/**14**	27.9 ± 0.1	2	$(dT)_{12}$ (**10**)/**14**	27.1 ± 0.2
3	conjugate **9**/**15**	31.4 ± 0.3	4	$(dT)_{12}$ (**10**)/**15**	23.6 ± 0.1
5	conjugate **9**/**16**	33.6 ± 0.5	6	$(dT)_{12}$ (**10**)/**16**	23.9 ± 0.1
7	conjugate **9**/**17**	25.5 ± 0.5	8	$(dT)_{12}$ (**10**)/**17**	25.3 ± 0.3
9	conjugate **9**/**18**	25.1 ± 0.2	10	$(dT)_{12}$ (**10**)/**18**	22.9 ± 0.6
11	conjugate **9**/**19**	26.0 ± 0.4	12	$(dT)_{12}$ (**10**)/**19**	24.0 ± 0.1
13	conjugate **9**/**20**	26.4 ± 0.3	14	$(dT)_{12}$ (**10**)/**20**	25.5 ± 0.2

[a] All studies were done in 10 mM bis-tris, 100 mM NaCl buffer pH 7.0. The concentration of each oligonucleotide component was 4 μM.

phobic binding mode, as such an overhanging single-stranded oligonucleotide on the target constitutes the necessary binding site.

5'-AA-AAA-AAA-AAA-A-3' **14**

5'-AA-AAA-AAA-AAA-ACC-CCC-C-3' **15**

5'-AA-AAA-AAA-AAA-AGG-GGG-G-3' **16**

5'-CC-CCC-CAA-AAA-AAA-AAA-A-3' **17**

5'-GG-GGG-GAA-AAA-AAA-AAA-A-3' **18**

5'-AA-AAA-AAA-AAA-ATG-AGT-GAG-T-3' **19**

5'-AAC-CGT-GAG-TAA-AAA-AAA-AAA-A-3' **20**

Further studies were carried using targets **19** and **20**. These oligonucleotides contain sequence pieces which are analogous to the original target **11**. Preferential binding was observed when the overhanging single-stranded oligonucleotide was at the 3′ end of the target (studies 11−14). These results were, again, consistent with the proposed hydrophobic binding mode of the sapphyrin conjugate **9**. Interestingly, the binding enhancement for the homopolymer containing targets **15** and **16** was much greater than that of the mixed sequence containing target **19**. While the rationale for this effect is not known, it is clear that the binding is sequence dependent.

Conclusion

The present results have served to show that it is possible to generate oligonucleotide conjugates, such as **9**, that produce site-directed photodamage on complementary oligonucleotide targets (e.g., **11**) upon irradiation at wavelengths above 620 nm. This photomodification displays characteristics of both electron transfer and singlet oxygen modification pathways. Melting temperature studies revealed that an attached sapphyrin moiety provides binding enhancement for oligonucleotide targets *via* hydrophobic interactions. Of equal importance is the fact that the synthetic procedures detailed in this paper should be amenable to the synthesis of other sapphyrin conjugates. In particular, the free hydroxy group of the conjugated sapphyrin could be used to effect functionalization with either nucleotides or additional sapphyrin subunits. Thus, prototype **9** represents just one of many conceivable sapphyrin−oligonucleotide conjugates that could possibly be prepared either by starting with a range of different oligonucleotides, using a different number of sapphyrins, or by varying the topology of attachment with regard to the oligonucleotide backbone. We are currently working to explore some of these synthetic possibilities.

Acknowledgment. This work was supported by NIH Grant No. AI 33577 to J.L.S., a NSF-PYI award (Grant CHE-9157440) to B.L.I., and a Howard Hughes Medical Institute grant to V.K. and J.L.S. P.I.S. thanks the University of Texas for a University Graduate Fellowship. Helpful discussions with Dr. Darren Magda (Pharmacyclics, Inc.) are also gratefully acknowledged. We thank Dr. Klaus Linse for the MALDI-MS studies.

Supporting Information Available: A listing of pBR322 gels, a NaCl dependent site-directed cleavage gel, a representative melting temperature analysis, and visible spectra of conjugate **9** recorded under differing salt conditions (8 pages). See any current masthead page for ordering and Internet access instructions.

JA961672L