Site-Specific Photomodification of DNA by Porphyrin–Oligonucleotide Conjugates Synthesized via a Solid Phase H-Phosphonate Approach

ARTICLE *in* BIOCONJUGATE CHEMISTRY · JANUARY 1997

Impact Factor: 4.51 · DOI: 10.1021/bc960074t · Source: PubMed

5 AUTHORS, INCLUDING:

Olga S Fedorova

Institute of Chemical Biology and Fundamen…

124 PUBLICATIONS **1,040** CITATIONS

Site-Specific Photomodification of DNA by Porphyrin–Oligonucleotide Conjugates Synthesized via a Solid Phase H-Phosphonate Approach

Handong Li,[†] Olga S. Fedorova,[†,‡] William R. Trumble,[§] T. Rick Fletcher,[†] and Leszek Czuchajowski*[,†]

Department of Chemistry and Department of Microbiology, Molecular Biology, and Biochemistry, University of Idaho, Moscow, Idaho 83844. Received July 31, 1996[⊗]

meso-Tris(4-pyridyl)[[(ω-hydroxyhexamethylene)carbamoyl]phenyl]porphyrin was converted to its H-phosphonate derivative and conjugated using solid phase synthesis with the 5′-hydroxyl group of deoxyribonucleotides d(TCTTCCCA) and $d(T)_{12}$. These conjugates were transformed into their (*N*-methylpyridiniumyl)porphyrin analogs in the reaction with methyl iodide. A 532 nm laser beam was utilized to photoactivate both types of the conjugates in the presence of the target 22-mer and 16-mer oligonucleotides. Photoactivation of porphyrin–oligonucleotide conjugates resulted in site-specific DNA modification characterized by a main reaction site size of ∼5 bases.

INTRODUCTION

Antisense oligonucleotides recognize a selected sequence on the target nucleic acid and can inhibit gene expression (*1*–*3*). Introduction of DNA-cleaving functional groups into an antisense oligonucleotide has been shown to generate site-directed damage on the target nucleic acids through chemical or photochemical reactions (*4*–*6*). A variety of chemical molecules have been covalently linked to oligonucleotides which were hybridized to cellular DNA to affect processes such as cellular uptake (*2*), nuclease resistance (*3*), and binding affinity (*6*).

Examples of those reactive molecules are alkylating agents such as aromatic (2-chloroethyl)amino derivatives (*4*–*6*), intercalating agents such as acridinium (*3*), ethidium, and phenazinium (*6*) derivatives, photoactivable cross-linkers such as psoralens (*7*–*10*), azides (*11*–*13*), or porphyrins (*14*–*16*), and artificial endonucleases such as EDTA–Fe(II) (*17*–*19*), Cu(I)–1,10-phenanthroline (*20*–*21*), bleomycin (*22*–*23*) or metalloporphyrins (*24*–*32*). The porphyrin–oligonucleotide conjugates and porphyrinyl–nucleoside derivatives (*33*–*36*) are of special interest to us due to their potential ability to modify nucleic acids either under dark conditions or under irradiation (*37*–*39*).

Sequence-specific modification of nucleic acids by porphyrin–oligonucleotide derivatives was reported using an excitation light wavelength around the porphyrin's Soret band (400–430 nm) (*14*–*15*) or in the case of porphyrin with an expanded structure such as lutetium(III) texatexaphyrin at an excitation wavelength of 732 nm (*16*). Porphyrins are used as anticancer agents in photodynamic therapy (PDT) (*40*). Their application as reactive groups of oligonucleotide derivatives in an antisense approach can result in selective inhibition of both oncogenes and proliferation of tumor cells (*6*).

Hitherto, only a few solution phase synthetic approaches have been reported for the preparation of the porphyrin–oligonucleotide conjugates. Amino derivatives of oligonucleotides were first made and then coupled with activated esters (*15*, *24*–*27*, *29*, *31*, *32*) or bromoalkyl derivatives (*28*) of porphyrin. Another method was based on the reaction between the activated 5′-phosphate group on an oligonucleotide and the amino group on a porphyrin (*14*, *28*). Porphyrin with a hydroxyl group was also used as a monomer in triester solution phase synthesis of oligonucleotide derivatives (*27*).

Since porphyrin derivatives of oligonucleotides activated by light or active in dark conditions can be used as the selective anticancer drugs, simple and efficient synthetic methods for porphyrin–oligonucleotide conjugates are required to increase the availability. It appears that only the use of the porphyrin residue as the monomer in the synthesis of oligonucleotides leads to a high yield of conjugated product (*27*). Therefore, we developed a simple approach for producing porphyrin–oligonucleotide conjugates, based on the commercially available, fully protected resin-bound deoxyribooligonucleotides, synthesized by the phosphoramidite method. The porphyrin monomer was used in the form of the H-phosphonate derivative. The methodology of H-phosphonate coupling (*41*, *42*) was modified to accomplish the porphyrin–oligonucleotide conjugation.

We chose two antisense oligonucleotides for application of this solid phase conjugation approach: a 12-mer of deoxyribothymidylate [$d(T)_{12}$], the complementary sequence to a riboadenylate track of single-stranded (+)-RNA in a wide variety of viruses such as potato virus y and swine vesicular exanthema, and an 8-mer d(TCTTCCCA), the complementary fragment to the selected sequence of RNA of tick born encephalitis virus.

A green laser beam at 532 nm was applied to study the photoactivated modification of target nucleic acids in the presence of porphyrin–oligonucleotide conjugates. We found that photoactivation at porphyrin's Q band region (500–650 nm) was highly effective for modification of DNA. It is possible to suggest that porphyrin–oligonucleotide derivatives can be used as specific drugs in photodynamic therapy because the light source with this longer wavelength is more penetrating through tissue.

* To whom correspondence should be sent.

[†] Department of Chemistry.

[‡] Permanent address: Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, 630090 Novosibirsk, Russia.

[§] Department of Microbiology, Molecular Biology, and Biochemistry.

[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1997.

S1043-1802(96)00074-2 CCC: $14.00

EXPERIMENTAL PROCEDURES

Materials. Fully protected resin-bound oligonucleotides synthesized by the phosphoramidite method were purchased from Bio-synthesis (Lewisville, TX). The target DNA sequences of d[(A_{12})GTGT] (16-mer) and d(TGAATGGGAAGAGGGTCAGGTT) (22-mer) were obtained from GIBCO BRL (Grand Island, NY). Oxidizers (I_2/pyridine/H_2O and H_2O/THF/Et_3N), adamantanecarbonyl chloride, pyridine, and acetonitrile were obtained from Glen Research Co. (Sterling, VA). All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). ^{32}P labeling of the 16-mer and the 22-mer at their 5′-ends was accomplished using T4-polynucleotide kinase obtained from United States Biochemical (Cleveland, OH) and [γ-^{32}P]ATP purchased from New England Nuclear/Dupont (Boston, MA). Labeled oligomers were separated from the nonlabeled oligomers by electrophoresis in denaturing 20% polyacrylamide gels containing 7 M urea and subsequently electroeluted from the gel.

Synthesis of *meso*-Tris(4-*N*-pyridyl)[(methylcarboxy)phenyl]porphyrin 1. 4-Pyridaldehyde (16.1 g, 0.15 mol) and methyl 4-formylbenzoate (7.4 g, 0.05 mol) were dissolved in 600 mL of propionic acid and 10 mL of acetic anhydride in a 2000 mL flask. The solution was heated to 100 °C. Pyrrole (13.4 g, 0.2 mol) in 50 mL of propionic acid was added dropwise to the hot solution over 0.5 h. The solution became darker and was stirred and refluxed for an additional 1 h. At this point, about 600 mL of propionic acid was removed under vacuum. The residue was poured slowly into ice-cold 5% aqueous sodium bicarbonate with stirring. The black precipitate was washed with 5% aqueous sodium bicarbonate and then water until neutral and air-dried. The black solid was dissolved in chloroform, applied to a silica gel column, and eluted with chloroform and chloroform/methanol (50:1). The fifth fraction was collected and identified as compound **1**. The yield was 8%.

^1H NMR ($CDCl_3$, ppm): 9.03 (d, 6H, Py), 8.84 (m, 8H, β-pyrrole), 8.44 (d, 2H, phenyl), 8.27 (d, 2H, phenyl), 8.14 (d, 6H, Py), 4.10 (s, 3H, OCH_3), −2.93 (s, 2H, NH). MS (FAB): *m*/*z* 678 (M + 2). UV ($CHCl_3$, nm): 418 (Soret), 514, 548, 588, 644. MS (FAB): *m*/*z* 678 (M + 2), 647 (M + 2 − OCH_3).

Synthesis of *meso*-Tris(4-pyridyl)[[(ω-hydroxyhexamethylene)carbamoyl]phenyl]porphyrin 2. 1 (67.6 mg, 100 μmol) and 6-aminohexanol (2.0 g, excess) were introduced into a flask. The mixture was stirred at 65−70 °C under nitrogen for 48 h. The sublimated 6-aminohexanol was melted down to the flask with a heating gun during the reaction. Aminohexanol (1.5 g) was recovered by sublimation of the reaction mixture. The residue was introduced into ice/water and stirred for 10 min. The precipitate was collected and washed with ice/water until neutral and then air-dried. The dried purple solid was chromatographed on a silica gel (60−230 mesh) using 100:1 and 10:1 chloroform/methanol as eluent. Fraction 1 contained the starting material. Fraction 2 was identified as **2** with a yield of 80%.

^1H NMR ($CDCl_3$, ppm): 9.03 (d, 6H, Py), 8.82 (m, 8H, β-pyrrole), 8.30 (d, 2H, phenyl), 8.22 (d, 2H, phenyl), 8.15 (d, 6H, Py), 6.60 (t, 1H, NHCO), 3.76 (t, 2H, CH_2O), 3.68 (t, 2H, NCH_2), 1.81 (quint, 4H, CH_2CH_2), 1.74 (quint, 4H, CH_2CH_2), −2.91 (s, 2H, NH). MS (FAB): *m*/*z* 763 (M + 2), 663 [M + 3 − $(CH_2)_6OH$], 617 [M − $CONH(CH_2)_6$-OH]. UV ($CHCl_3$, nm): 414 (Soret), 514, 548, 588, 644.

Synthesis of the H-Phosphonate Derivative of *meso*-Tris(4-pyridyl)[[(ω-hydroxyhexamethylene)-carbamoyl]phenyl]porphyrin 3. 2 (15 mg, 20 μmol) was dried under vacuum and dissolved in 10 mL of CH_2Cl_2. A catalytic amount of triethylamine and 12 mg (21 μmol) of tris(1,1,1,3,3,3-hexafluoro-2-propyl) phosphite were added. The reaction was completed in 10 min. A mixture of 1 M triethylamine bicarbonate (TEAB) and triethylamine (50:1 v:v) was added to the reaction mixture. After 30 min, the product was extracted with chloroform, washed with 1 M TEAB, and dried over anhydrous sodium sulfate. The chloroform layer was evaporated, and the residue was chromatographed on silica gel using chloroform/methanol/triethylamine (from 100:2:0 to 100:10:2). The second fraction was collected and washed with 1 M TEAB and dried with sodium sulfate. The porphyrin H-phosphonate **3** was obtained after evaporation of the chloroform with a 82% yield.

^1H NMR (DMSO-d_6, ppm): 9.05 (m, 6H, Py), 8.90 (m, 8H, β-pyrrole), 8.29 (m, 4H, phenyl), 8.26 (m, 6H, Py), 7.56 (s, 1H, NHCO), 5.67 (s, 1H, HP), 3.68 (m, 2H, CH_2O), 3.42 (m, 2H, CH_2NCO), 3.04 (quart, 2H, CH_2N), 1.68 (m, 4H, CH_2CH_2), 1.44 (m, 4H, CH_2CH_2), 1.15 (t, 3H, CH_3), −3.02 (s, 2H, NH). ^{31}P NMR (DMSO-d_6, ppm): 1.76 (PH). MS (negative FAB): *m*/*z* 824 (M^-). UV ($CHCl_3$/CH_3OH, 1:1, nm): 418 (Soret), 512, 548, 592, 638.

Table 1. UV−Vis Data of Porphyrins, Oligonucleotides, and Porphyrin−Oligonucleotide Conjugates

	UV	visible
compound	λ_{max} (nm), ϵ (M^{-1} cm^{-1})	λ_{max} (nm), ϵ (M^{-1} cm^{-1})
2	260, 4×10^4	406, 2.8×10^5
2-Me	260, 1.2×10^4	422, 2.2×10^5
4	260, 6.78×10^4	
5	260, 6.36×10^4	
6	266	416
7	268	416
8	268	430
9	266	430

Synthesis of Conjugates of *meso*-Tris(4-pyridyl)-[[(ω-hydroxyhexamethylene)carbamoyl]phenyl]-porphyrin with Oligonucleotides d(TCTTCCCA) and d(T)$_{12}$ (6 and 7, Respectively). Fully protected resin-bound oligonucleotide (1 mmol) was packaged in a 1 mL syringe column which was connected to a vacuum line by a switch valve. All reaction agents and vessels were in a dry nitrogen glovebox. A procedure, closely approximating the reactions for automated DNA synthesis, was utilized as follows: (1) Detritylation was done with 2% $Cl_2CHCOOH$ for 2 min. (2) Washing was done with CH_3CN and CH_3CN/pyridine for 2 min. (3) **3** (38 mg, 40 μmol) dissolved in 1 mL of CH_3CN/pyridine and 20 mg (100 μmol) of adamantanecarbonyl chloride was added immediately and applied to the column for 4 min. (4) Washing was done with CH_3CN/pyridine and CH_3CN for 2 min. (5) Oxidation was done with I_2/pyridine/H_2O and Et_3N/THF/H_2O for 5 min. (6) Washing was done with CH_3CN/pyridine and CH_3CN for 2 min. (7) Deprotection was done with concentrated $NH_3 \cdot H_2O$ at room temperature for 12 h. The product was then lyophilized and redissolved in Tris-acetate/EDTA (TAE) buffer (pH 7.5) and purified on a Hewlett-Packard series 1050 HPLC apparatus with a variable wavelength UV detector set at 260 nm, using a Lichrospher 100 reverse phase C18 column (5 μm). The solvent ramp followed a linear gradient from 9% CH_3CN and 91% 0.05 M TEAc (pH 7.5) at the beginning of each run to 81% CH_3CN and 19% 0.05 M TEAc (pH 7.5) at the end, 25 min later. The flow rate was 1.0 mL/min. HPLC profiles (shown in Figure 1) were recorded on a Hewlett-Packard 3396A integrator. The yields of **6** or **7** were about 60−70%. UV data are shown in Table 1.

Conjugates **6** and **7** were also analyzed by dynamic sieving capillary electrophoresis on a Biofocus 3000

Capillary Electrophoresis System (BioRad) using a coated column (length of 24 cm, diameter of 75 μm) in a buffer of 25 mM Tris-borate and 2 mM EDTA at pH 8.3. The absorption profiles (shown in Figure 2) were monitored at 260 nm for oligonucleotide moieties and at 416 nm for porphyrin units of the conjugates.

Concentrations of conjugates were estimated using absorption at 260 nm. The molar absorption coefficient at 260 nm was calculated as the sum of absorption for oligonucleotide and porphyrin moieties. The molar absorption coefficients of oligonucleotides at 260 nm were found using a procedure described in ref 43 to be 6.78×10^4 M^{-1} cm^{-1} for d(TCTTCCCA) and 6.36×10^4 M^{-1} cm^{-1} for $d(T)_{12}$. The molar absorption coefficient of porphyrin **2** was measured and is shown in Table 1.

Synthesis of *meso*-Tris(4-*N*-methylpyridiniumyl)-[[(ω-hydroxyhexamethylene)carbamoyl]phenyl]-porphyrin (2-Me) and Its Conjugates with Oligonucleotides d(TCTTCCCA) and $d(T)_{12}$ (8 and 9, Respectively). Compound **6**, **7**, or **2** was dissolved in DMF. Excess CH_3I was added, and the mixture was kept at room temperature for 3 h. N-Methylation of **2** was chosen as a model reaction for which completeness was monitored by TLC. The product **2-Me** was obtained by removing the excess CH_3I and DMF under vacuum with no need of further purification. The yield was 100%.

^{1}H NMR of **2-Me** (DMSO-d_6, ppm): 9.62 (d, 6H, 3, 5-Py), 9.18 (d, 6H, 2, 6-Py), 8.98 (m, 8H, β-pyrrole), 8.32 (m, 4H, phenyl), 4.72 (s, 9H, NCH_3), 3.48 (m, 4H, CH_2-CO and CH_2O), 1.67 (m, 4H, CH_2CH_2), 1.44 (m, 4H, CH_2-CH_2), −3.02 (s, 2H, porphyrin-NH).

UV [H_2O, λ_{max} (nm)]: 422, 520, 560, 592, 652. The molar absorption coefficient of porphyrin **2-Me** is presented in Table 1.

Compounds **8** and **9** were made in the same way as **2-Me**. Their UV−vis data are shown in Table 1. The red shifts of porphyrin Soret bands after conjugation with oligonucleotides are characteristic and consistent with our previous data (*33*).

Photomodification of Target DNA. Photomodification reactions were carried out in a 20 μL buffer solution containing 10 nM [^{32}P]16-mer (or 22-mer), 0.2 M NaCl, 10 mM Tris-HCl, 1 mM EDTA (pH 7.5), and porphyrinyl derivatives of the 12-mer (or 8-mer) at 25 °C in a polypropylene Eppendorff tube. Tubes were placed in a horizontal position with caps opened in the path of a laser beam and irradiated for 10 min. The laser was a Nd:YAG laser at 532 nm with 25 mJ/pulse and 10 pulses/s.

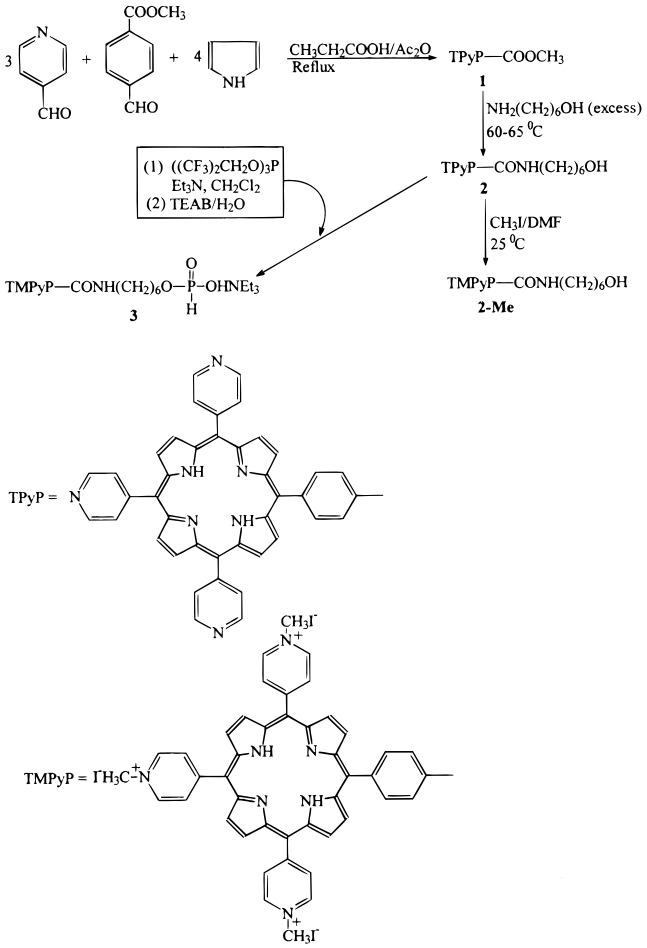
After irradiation, the oligomers were precipitated by the addition of 100 μL of 2% $LiClO_4$/acetone solution. The precipitate was washed three times with 100 μL of acetone and dried under vacuum. If required, the oligomer pellets were suspended in 10 μL of 10% piperidine at 95 °C for 30 min. The solutions were cooled and subjected to a second $LiClO_4$/acetone precipitation. The precipitates were dissolved in 4 μL of water and 3 μL of 95% formamide containing 0.025% bromophenol blue and 0.025% xylene cyanol and loaded onto a 20% denaturing polyacrylamide sequencing gel (19:1 cross-linked, 20 cm × 20 cm × 0.4 mm). The gels were run at 50 W/cm and exposed on Kodak X-ray film. The autoradiograms of the gels were quantitized using an UltroscanXL densitometer (LKB, Sweden). The autoradiograms are shown in Figures 3 and 4. The densitometry data were plotted as shown in Figures 5−7.

RESULTS AND DISCUSSION

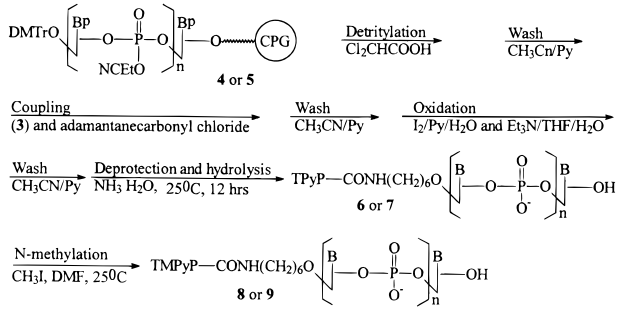
Synthesis of Porphyrin−Oligonucleotide Conjugates. Porphyrin−oligonucleotide conjugates were synthesized by coupling of the H-phosphonate derivative of oligonucleotide with fully protected oligonucleotides. For this purpose, a porphyrin ester **1** was synthesized by the Rothmund−Longo method (*44*). An ω-hydroxylhexamethylene group was introduced through an ester−amide exchange reaction which gave a new porphyrin **2** with a hydroxyl group in a high yield (Scheme 1). A small amount of acetic anhydride was added to the solvent of propionic acid to prevent the hydrolysis of the ester group. An ester−amide exchange reaction was found to be very efficient for derivatizing the porphyrin ester into new porphyrins with different functional groups, such as hydroxyl and amine. This is an easy way to make pure porphyrin derivatives compared with reactions previously reported in the literature that employed activation of porphyrin carboxylic acid in DMSO or DMF and produced a low yield (*28*). The *meso*-trispyridylporphyrin H-phosphonate **3**, as the coupling active compound, was synthesized using an efficient fluorinated agent (*45*). Compound **3** has good solubility in pyridine/acetonitrile solution, which is necessary for the coupling reaction. The H-phosphonate derivative was activated by adamantanecarbonyl chloride and coupled with the 5′-hydroxyl group of deoxyribonucleotides d(TCTTCCCA) **4** or $d(T)_{12}$ **5** bound on the resin (Scheme 2). The H-phosphonate group was oxidized by I_2 to phosphate. Hydrolysis, removing the conjugates from the resin, and deprotection were carried out in one step at room temperature. Conjugates **6** and **7** were purified by HPLC and converted to cationic porphyrin−oligonucleotides **8** and **9** by methyl iodide.

The coupling was allowed to proceed for less than 3 min to avoid the hydrolysis of the previously formed

Scheme 1. Synthesis of the Porphyrin H-Phosphonates



Scheme 2. Solid Phase H-Phosphonate Approach to the Synthesis of Porphyrinyl Oligonucleotides[a]



[a] CPG = controlled pore glass. Bp = (phenylacetyl)deoxyadenosine, deoxythymidine, and (phenoxyacetyl)deoxycytosine. B = T, A, or C. **6** and **8** have an oligonucleotide sequence of TCTTCCCA. **7** and **9** have an oligonucleotide sequence of $(T)_{12}$.

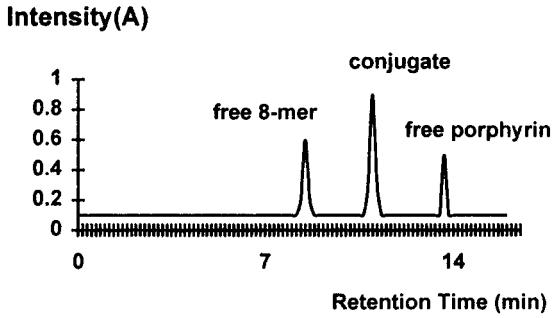


Figure 1. HPLC profile of coupling reaction products from porphyrin **3** and 8-mer **4**.

H-phosphonate diester. Adamantanecarbonyl chloride was chosen instead of pivaloyl chloride to minimize the capping of the oligonucleotide. The aliphatic amide bond on the porphyrin is stable under both room-temperature hydrolysis conditions and deprotection conditions in concentrated ammonium hydroxide.

HPLC and Capillary Electrophoresis. As may be seen in the HPLC elution profiles of conjugates (Figure 1), the free oligonucleotide was eluted first, the conjugate second, and the free porphyrin last. The free porphyrin is neutral and least polar, compared to the free anionic oligonucleotide or the conjugate. The retention time and the polarity are very well correlated on the reverse phase column. Each fraction was confirmed by the UV−vis spectrum.

As compared with acrylamide gel electrophoresis, capillary electrophoresis should provide more precise information. It is very easy to identify the porphyrin−oligonucleotide conjugate by applying two different detector wavelengths: 260 nm for oligonucleotides and 416 nm for porphyrin units. Only free oligonucleotides and porphyrin−oligonucleotide can be detected at 260 nm, while only free porphyrin and porphyrin−oligonucleotide can be detected at 416 nm. The capillary electrophoresis profiles are shown in Figure 2.

UV−Vis Spectra. Soret bands of pyridylporphyrin−oligonucleotide and of pyridiniumylporphyrin−oligonucleotide exhibited a red shift up to 10 nm compared to the free pyridylporphyrin and N-methylated pyridiniumylporphyrin.

Selective Photomodification of Target DNA. Synthesized porphyrin−oligonucleotide conjugates were tested for their abilities to modify the complementary nucleic acid strands under irradiation by visible light in the spectral region of the porphyrin Q-bands at 532 nm. Photomodification reactions were carried out with 5′-^{32}P-labeled targets, d(TGAATGGGAAGAGGGTCAGGTT) (22-mer) and d[$(A)_{12}(GT)_2$] (16-mer), at a concentration of $\sim 10^{-8}$ M. Concentrations of the porphyrin−oligonucleotide conjugates were equal to 4 μM. In these equilibrium conditions, all targets must be in the form of a complex with the conjugate. The complementary complexes have the following structures:

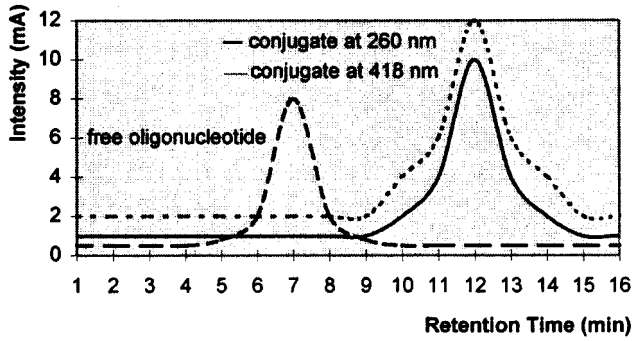


Figure 2. Capillary electrophoresis profile of purified conjugate **6** and pure oligonucleotide **4**.

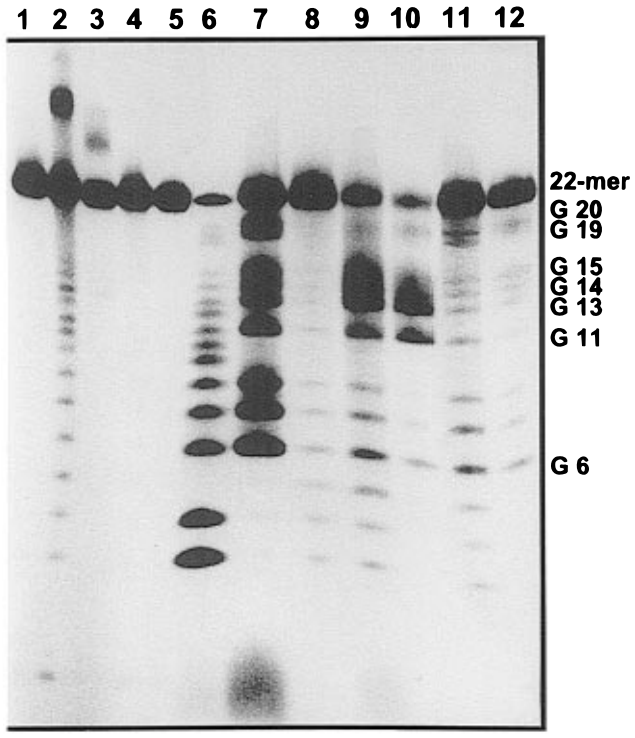


Figure 3. Autoradiogram of a 20% denaturing polyacrylamide gel showing photomodification products produced by irradiation of the 22-mer for 10 min: controls without porphyrinyl conjugates (lanes 1 and 8), in the presence of 4×10^{-6} M conjugates **6** (lanes 2 and 9), **7** (lanes 4 and 11), **8** (lanes 3 and 10), **9** (lanes 5 and 12), A + G (lane 6), and G (lane 7). Samples in lanes 9−12 were treated with piperidine.

(5′) TGAATGGGAAGAGGGTCAGGTT (3′)
(3′) ACCCTTCT-X (5′)
Complex I

(5′) AAAAAAAAAAAAGTGT (3′)
(3′) TTTTTTTTTTTT-X (5′)
Complex II

where

X = -O-P(O)-O-$(CH_2)_6$NH-CO-Porphyrin
O$^-$

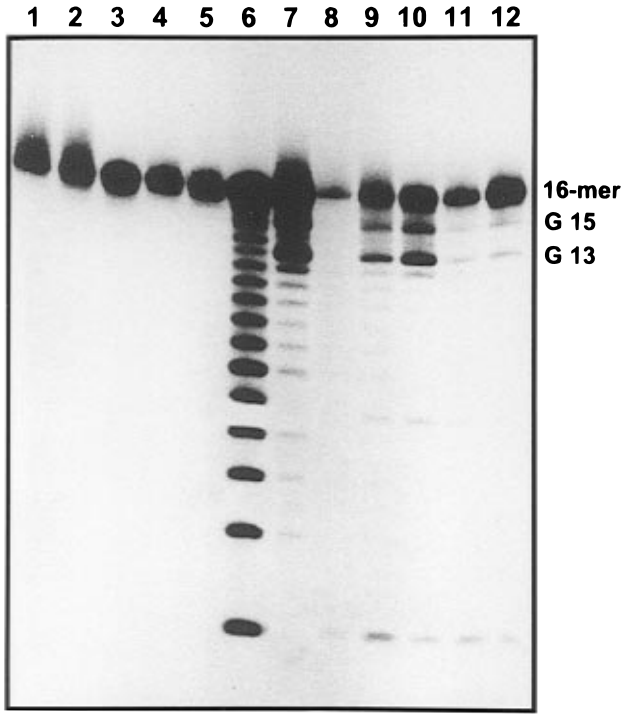


Figure 4. Autoradiogram of a 20% denaturing polyacrylamide gel showing photomodification products produced by irradiation of the 16-mer for 10 min: controls without porphyrinyl conjugates (lanes 1 and 8), in the presence of 4×10^{-6} M conjugates **6** (lanes 4 and 11), **7** (lanes 2 and 9), **8** (lanes 5 and 12), **9** (lanes 3 and 10), A + G (lane 6), and G (lane 7). Samples in lanes 9−12 were treated with piperidine.

The products were separated by 20% polyacrylamide gel electrophoresis under denaturing conditions, in the presence of 7 M urea. Results of control experiments and sequence-specific photomodification of the 22-mer and 16-mer are shown in Figures 3 and 4, respectively. The modification sites were identified after piperidine cleavage from comigrating Maxam−Gilbert sequencing ladders (*46*), namely A + G and G. No photomodification of the targets was seen in the absence of the porphyrin conjugates (lanes 1 and 8). Slow-migrating bands (corresponding to cross-linked products) appeared above the bands corresponding to the starting materials when the 22-mer was irradiated in the presence of **6** and **8** (Figure 3, lanes 2 and 3, respectively). When these targets were irradiated in the presence of conjugates **7** and **9** which are not complementary to the targets, the cross-linked products were absent (see Figure 3, lanes 4 and 5). In the case where the 16-mer was irradiated in the presence of **7** and **9**, the bands of cross-linked products were absent (Figure 4, lanes 2 and 3, respectively). The photomodification sites were assigned after piperidine treatment of the reaction mixture and were observed primarily at G11, G13, G14, and G15 for the 22-mer (Figure 3, lanes 9 and 10). For the case of the 16-mer, specific modification products were observed only after the treatment by piperidine at G13 and G15 (Figure 4, lanes 9 and 10). This suggests that alkaline-labile photomodification occurred.

Densitograms are presented in Figures 5 and 6 for the photomodification of the 22-mer by reagents **6** and **8**, respectively, and in Figure 7 for the modification of the 16-mer by **7** and **9**. Figures 5−7 also show the absolute cleavage yields for each site after piperidine treatment and illustrate the data for both complementary and noncomplementary oligonucleotide conjugates. No specific photomodifications of the target 22-mer and 16-mer were observed in the presence of conjugates which could not hybridize with the target DNA. Modification by conjugates containing an uncharged porphyrinyl group **6** and **7** or a positively charged porphyrinyl group **8** and **9** showed slightly different efficiency and site selectivity (Figures 5−7).

For the 22-mer, the total yield, of cleavage (~77% for

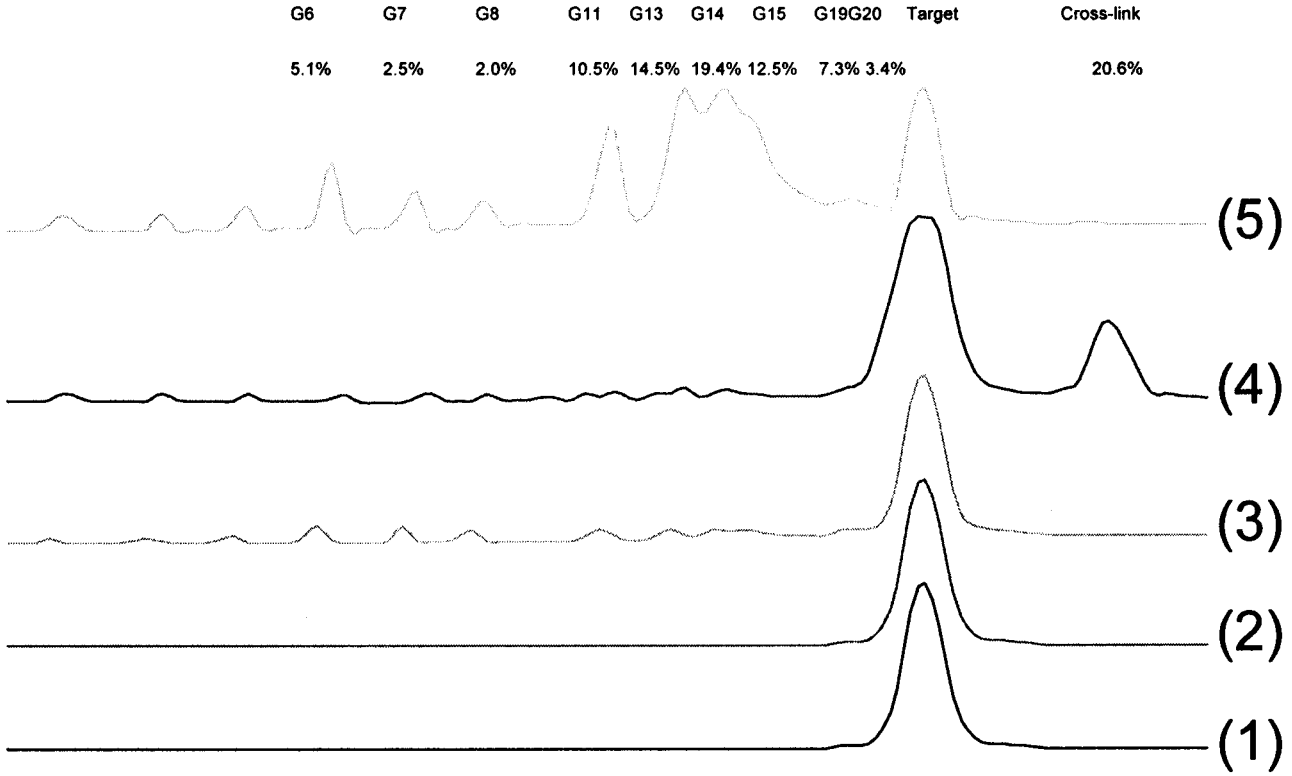


Figure 5. Densitograms of the gel electrophoretic separation of the reaction mixture, containing 22-mer target modified by conjugate **6**; the data for noncomplementary conjugate **7** are presented as the control (line 1, without conjugates; lines 2 and 3, 4 μM **7**, with **6** absent; and lines 4 and 5, 4 μM **6** with **7** absent). Samples for lines 1, 3, and 5 were treated with piperidine.

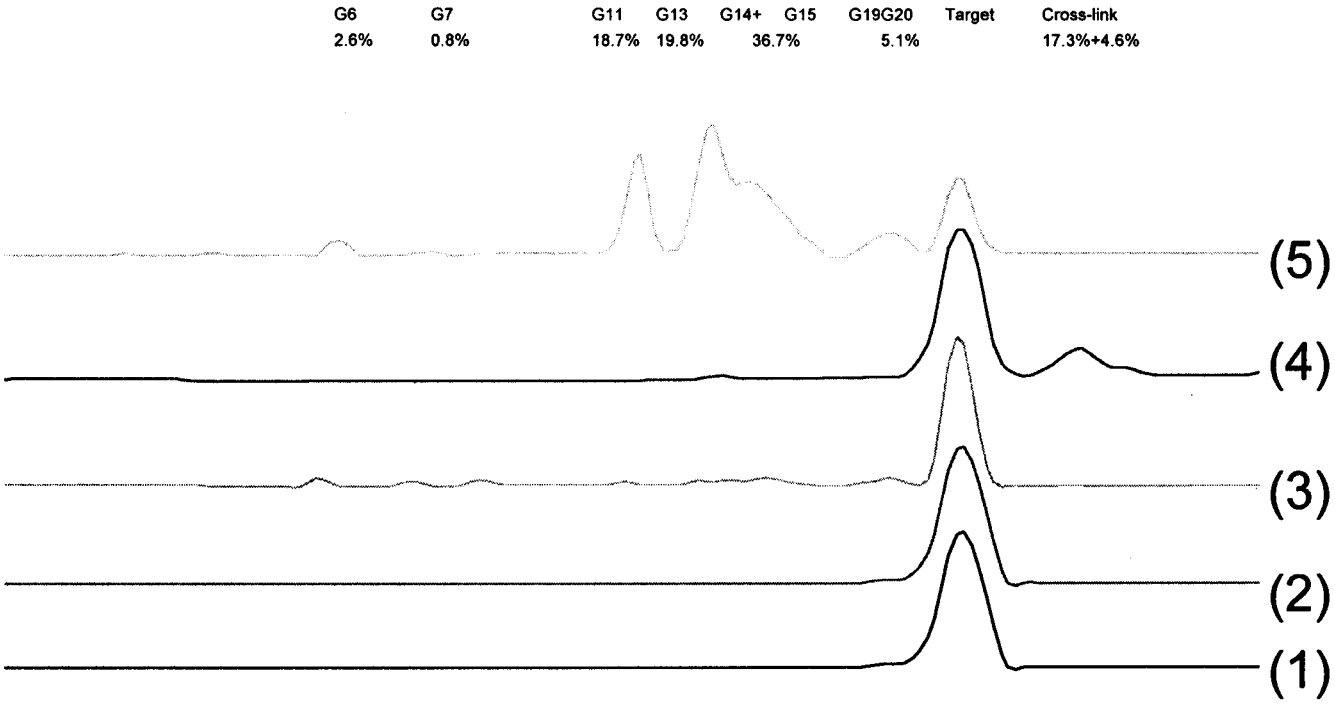


Figure 6. Densitograms of the gel electrophoretic separation of the reaction mixture, containing 22-mer target modified by conjugate **8**; the data for noncomplementary conjugate **9** are presented as the control (line 1, without conjugates; lines 2 and 3, 4 μM **9** and with **8** absent; and lines 4 and 5, 4 μM **8** with **9** absent). Samples for lines 1, 3, and 5 were treated with piperidine.

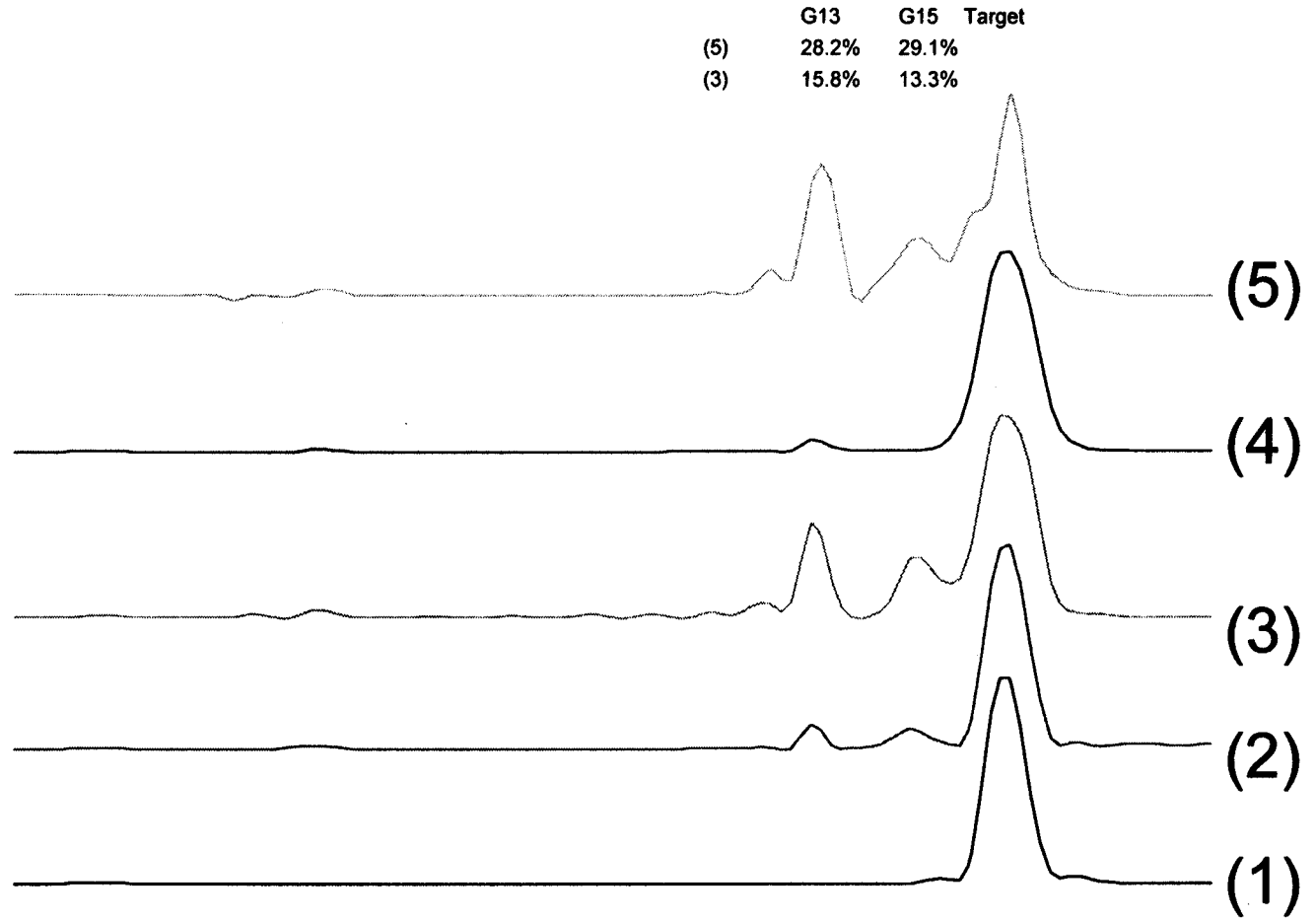


Figure 7. Densitograms of the gel electrophoretic separation of the reaction mixture, containing 16-mer target modified by conjugate **7** or **9**; the data for noncomplementary conjugates **6** and **8** are presented as the control (line 1, without conjugates; line 2, 4 μM **6**; line 3, 4 μM **7**; line 4, 4 μM **8**; and line 5, 4 μM **9**). Samples for lines 1−5 were treated with piperidine.

6 and ~84% for **8**) are higher than the yields of cross-linked products (~20%). Therefore, the alkaline-labile modification of the 22-mer occurred also as in the case of the 16-mer.

The most intensively utilized modification site consisted of about five nucleotides. Two factors may account for this result. First, the dimension of the photoactive center porphyrin molecule is 18 Å (*47*), equivalent to one-half of a DNA helix turn. Second, a singlet molecular oxygen ($^1O_2$)-modifying agent is presumed to form after sensitization by the porphyrin. The lifetime of this species is equal to 1−20 μs in solution (*48*). Therefore, this molecule can migrate a large distance along the polynucleotide chain.

The results of this study demonstrate that the porphyrinyl groups of oligonucleotide conjugates can be activated by a green laser beam with a wavelength in the spectral region of the porphyrin's Q-bands. The photomodification efficiency is high with a total yield around 80% for a specific modification site of five nucleotides. This efficient site-directed photomodification of DNA suggests that porphyrinyl agents may be developed as anticancer or antiviral drugs for use in photodynamic therapy.

Our procedure provide an efficient method for producing porphyrin−oligonucleotide conjugates in sufficient quantity and with sufficient purity to make these compounds readily available for medical and chemical applications.

ACKNOWLEDGMENT

The authors thank V. V. Koval for the preparation of densitometric pictures and to the Idaho EPSCoR and SBOE in Boise, ID, for financial support.

LITERATURE CITED

(1) Cohen, J. S., Ed. (1989) *Oligonucleotides: antisense inhibitors of gene expression*, MacMillan Press, London.
(2) Wickstrom, E., Ed. (1991) *Prospects for antisense nucleic acid therapy of cancer and AIDS*, Willey-Liss. Inc., New York.
(3) Crooke, S. T., and Lebleu, B., Eds. (1993) *Antisense research and applications*, CRC Press, Boca Raton, FL.
(4) Belikova, A. M., Zarytova ,V. F., and Grineva, N. I. (1967) Synthesis of ribonucleotides and diribonucleotides phosphates containing 2-chloroethylamine and nitrogen mustard residues. *Tetrahedron Lett*., 3557−3567.
(5) Knorre, D. G., and Vlassov, V. V. (1989) *Affinity Modification of Biopolymers*, CRC Press, Boca Raton, FL.
(6) Knorre, D. G., Vlassov, V. V., Zarytova,V. F., Lebedev, A. V., and Fedorova, O. S. (1994) *Design and targeted reaction of oligonucleotide derivatives*, CRC Press, Boca Raton, FL.
(7) Sastry, S. S., Spielman, P., Dwyer ,T. J., Wemmer, D. E., and Hearst, J. E. (1992) Recent advances in the synthesis and structure determination of site specifically psoralen-modified oligonucleotides. *J. Photochem. Photobiol. B*, 65−79.
(8) Lee, B. L., Murakami, A., Blake, K. R., Lin, S.-B., and Miller, P. S. (1988) Interaction of psoralen-derivatized oligodeoxyribonucleoside methylphosphonates with single-stranded DNA. *Biochemistry 27*, 3197−3203.
(9) Pieles, U., and English, U. (1989) Psoralen covalently linked to oligonucleotides: synthesis, sequence specific recognition of DNA and photo-cross-linking to pyrimidine residues of DNA. *Nucleic Acids Res. 17*, 285−299.
(10) Woo, J., and Hopkins, P. B. (1991) Template-directed modification of single-stranded DNA by psoralen-tethered oligonucleotides: sites of photoadduct formation analyzed by sequence-specific and sequence-random cleavage. *J. Am. Chem. Soc. 113*, 5457−5459.
(11) Decout, J.-L., Thuong, N. T., Lhomme, J., and Helene, C. (1987) Sequence-specific recognition, photocrosslinking and cleavage of the DNA double helix by an oligo-[α]-thymidylate covalently linked to an azidoproflavine derivative. *Nucleic Acids Res. 15*, 7749−7760.
(12) Levina, A. S., Tabatadze, D. R., Khalimskaya, L. M., Prikhodko, T. A., Shishkin, G. V., Alexandrova, L. A., and Zarytova, V. F. (1993) Oligonucleotide derivatives bearing reactive and stabilizing groups attached to C5 of deoxyuridine. *Bioconjugate Chem. 4*, 319−325.
(13) Koshkin, A. A., Kropachev, K. Yu., Mamaev, S. V., Bulychev, N. V., Lokhov, S. G., Vlassov, V. V., and Lebedev, A. V. (1994) Ethidium and azidoethidium oligonucleotide derivatives: synthesis, complementary complex formation and sequence-specific photomodification of the single-stranded and double-stranded target oligo- and polynucleotides. *J. Mol. Recognit. 7*, 177−178.
(14) Fedorova, O. S., Savitskii, A. P., Shoikhet, K. G., and Ponomarev, G. V. (1990) Palladium(II)-coproporphyrin I as a photoactivable group in sequence-specific modification of nucleic acids by oligonucleotide derivatives. *FEBS Lett. 259*, 335−337.
(15) Mastruzzo, L., Woisard, A., Ma, D. D. F., Rizzarelli, E., Favre, A., and Le Doan, T. (1994) Targeted photochemical modification of HIV-derived oligoribonucleotides by antisense oligodeoxynucleotides linked to porphyrins. *Photochem. Photobiol. 60*, 316−322.
(16) Magda, D., Wright, M., Miller, R. A., Sessler, J. L., and Sanson, P. I. (1995) Sequence-specific photocleavage of DNA by an expanded porphyrin with irradiation above 700 nm. *J. Am. Chem. Soc. 117*, 3629−3630.
(17) Boutorin, A. S., Vlassov, V. V., Kazakov, S. A., Kutiavin, I. V., and Podyminogin, M. A. (1984) Complementary addressed reagents carrying EDTA-Fe(II) groups for directed cleavage of single-stranded nucleic acids. *FEBS Lett. 172*, 43−46.
(18) Chu, B. C. F., and Orgel, L. E. (1985) Nonenzymatic sequence-specific cleavage of single-stranded DNA. *Proc. Natl. Acad. Sci. U.S.A. 82*, 963−967.
(19) Dreyer, G., and Dervan, P. B. (1985) Sequence-specific cleavage of single-stranded DNA. *Proc. Natl. Acad. Sci. U.S.A. 82*, 968−972.
(20) Chen, C. B., and Sigman, D. S. (1986) Nuclease activity of 1,10-phenanthroline-copper: sequence-specific targeting. *Proc. Natl. Acad. Sci. U.S.A. 63*, 7147−7151.
(21) Francois, J.-C., Saison-Behmoaras, T., Chassignol, M., Thuong, N. T., and Helene, C. (1989) Sequence-targeted cleavage of single- and double-stranded DNA by oligothymidylates covalently linked to 1,10-phenanthroline. *J. Biol. Chem. 264*, 5891−5898.
(22) Sergeyev, D. S., Godovikova, T. S., and Zarytova, V. F. (1991) Direct cleavage of a DNA fragment by a bleomycin-oligonucleotide derivative. *FEBS Lett. 280*, 271−273.
(23) Zarytova, V. F., Sergeev, D. S., and Godovikova, T. S. (1993) Synthesis of Bleomycin A5 Oligonucleotide Derivatives and Site-Specific Cleavage of the DNA Target. *Bioconjugate Chem. 4*, 189−193.
(24) Le Doan, T., Perrouault, L., Helene, C., Chassignol, M., and Thuong, N. T. (1986) Targeted Cleavage of Polynucleotides by Complementary Oligonucleotide Covalently Linked to Iron-Porphyrins. *Biochemistry 25*, 6736−6739.
(25) Le Doan, T., Perrouault, L., Chassignol, M., Thuong, N. T., and Helene, C. (1987) Sequence-targeted chemical modifications of nucleic acids by complementary oligonucleotides linked to porphyrins. *Nucleic Acids Res. 15*, 8643−8659.
(26) Ivanova, E. M., Mamaev, S. V., Fedorova, O. S., and Frolova, E. I. (1988) Complementary addressed modification of single-stranded DNA fragment by ferrous-porphyrin derivative of oligonucleotide. *Bioorg. Khim. 14*, 551−554.
(27) Frolova, E. I., Ivanova, E. M., Zarytova, V. F., Abramova, T. V., and Vlassov, V. V. (1990) Porphyrin-linked oligonucleotides. Synthesis and sequence-specific modification of ss-DNA. *FEBS Lett. 269*, 101−104.
(28) Boutorine, A. S., Le Doan, T., Battioni, J. P., Mansuy, D., Dupre, D., and Helene, C. (1990) Rapid routes of synthesis of chemically reactive and highly radioactively labeled α- and β-oligonucleotide derivatives for *in vivo* studies. *Bioconjugate Chem. 1*, 350−356.
(29) Casas, C., Lasey, C. J., and Meunier, B. (1993) Preparation of hybrid "DNA cleaver-oligonucleotide" molecules based on metallotris(methyl-pyridiniumyl)porphyrin motif. *Bioconjugate Chem. 4*, 366−371.
(30) Frolova, E. I., Fedorova, O. S., and Knorre, D. G. (1993) Kinetic study of the addressed modification by hemin derivatives of oligonucleotides. *Biochimie 75*, 5−12.

(31) Mestre, B., Pratviel, G., and Meunier, B. (1995) Preparation and nuclease activity of hybrid "metallotris(methylpyridinium)porphyrin oligonucleotide" molecules having a 3′-loop for protection against 3′-exonucleases. *Bioconjugate Chem. 6*, 466−472.

(32) Magda, D., Miller, R. A., Sessler, J. L., and Iverson, B. L. (1994) Site-specific hydrolysis of RNA by europium(III) texaphyrin conjugated to synthetic oligodeoxyribonucleotide. *J. Am. Chem. Soc. 116*, 7439−7440.

(33) Li, H., and Czuchajowski, L. (1994) Ribofuranosides N-substituted with meso-porphyrin as nucleoside-like compounds. *Tetrahedron Lett. 35* (11), 1629−1630.

(34) Czuchajowski, L., Palka, A., Morra, M., and Wandrekar, V. (1993) Porphyrinyl nucleosides containing fluorinated nucleobases. *Tetrahedron Lett. 34*, 5409−5412.

(35) Czuchajowski, L., Habdas, J., Niedbala, H., and Wandrekar, V. (1992) Synthesis of Porphyrin-Nucleosides. *J. Heterocycl. Chem. 29*, 479−484.

(36) Li, H., Trumble, W. R., and Czuchajowski, L. (1997) Compounds Based on meso-Tris-(4-pyridyl)-p-acrylamidophenylporphyrin able to intercalate with DNA. *J. Heterocycl. Chem*. (in press).

(37) Kochevar, I., Dunn, D. A. (1990) Photosensitized reactions of DNA: cleavage and addition. In *Bioorganic Photochemistry* (H. Morrison, Ed.) pp 273−315, John Wiley & Sons, New York.

(38) Meunier, B. (1992) Metalloporphyrins as versatile catalysts for oxidation reactions and oxidative DNA cleavage. *Chem. Rev. 92*, 1411−1456.

(39) Knorre, D. G., Fedorova, O. S., and Frolova, E. I. (1993) Oxidative degradation of nucleic acids. *Russ. Chem. Rev. (Engl. Transl.) 62*, 65−86.

(40) Dolphin, D. (1994) Photomedicine and photodynamic therapy. *Can. J. Chem. 72*, 1005−1013.

(41) Froehler, B. C., and Matteucci, M. D. (1986) Nucleoside H-phosphonate: valuable intermediates in the synthesis of deoxynucleotides. *Tetrahedron Lett. 27*, 469−472.

(42) Gaffney, B. L., and Jones, R. A. (1988) Large-scale oligonucleotide synthesis by H-phosphonate method. *Tetrahedron Lett. 29*, 2619−2622.

(43) Fasman, G., Ed. (1975) *Handbook of Biochemistry and Molecular Biology. Nucleic Acids*, 3rd ed., p 175, CRC Press, Boca Raton, FL.

(44) Adler, A. D., Longo, F. R., Finarell, J. D., Goldmacher, J., Assour, J., and Korsakoff, L. (1967) A simplified synthesis for meso-tetraporphyrins. *J. Org. Chem. 32*, 476.

(45) Sakatsume, O., Yamane, H., Takaku, H., and Yamamoto, N. (1990) Use of new phosphorylating and coupling agents in the synthesis of oligodeoxyribonucleotides via H-phosphonate approach. *Nucleic Acids Res. 18*, 3327−3331.

(46) Maxam, A. M., and Gilbert, W. (1980) Sequencing end-labelled DNA with base-specific chemical cleavages. *Methods Enzymol. 65*, 499−560.

(47) Ford, K. G., Pearl, L. H., and Neidle, S. (1987) Molecular modelling of the interactions of tetra-(4-methylpyridyl) porphin with TA and CG sites on DNA. *Nucleic Acids Res. 16*, 6553−6562.

(48) Kearns, D. R. (1971) Physical and chemical properties of singlet molecular oxygen. *Chem. Rev. 71*, 395−427.

BC960074T