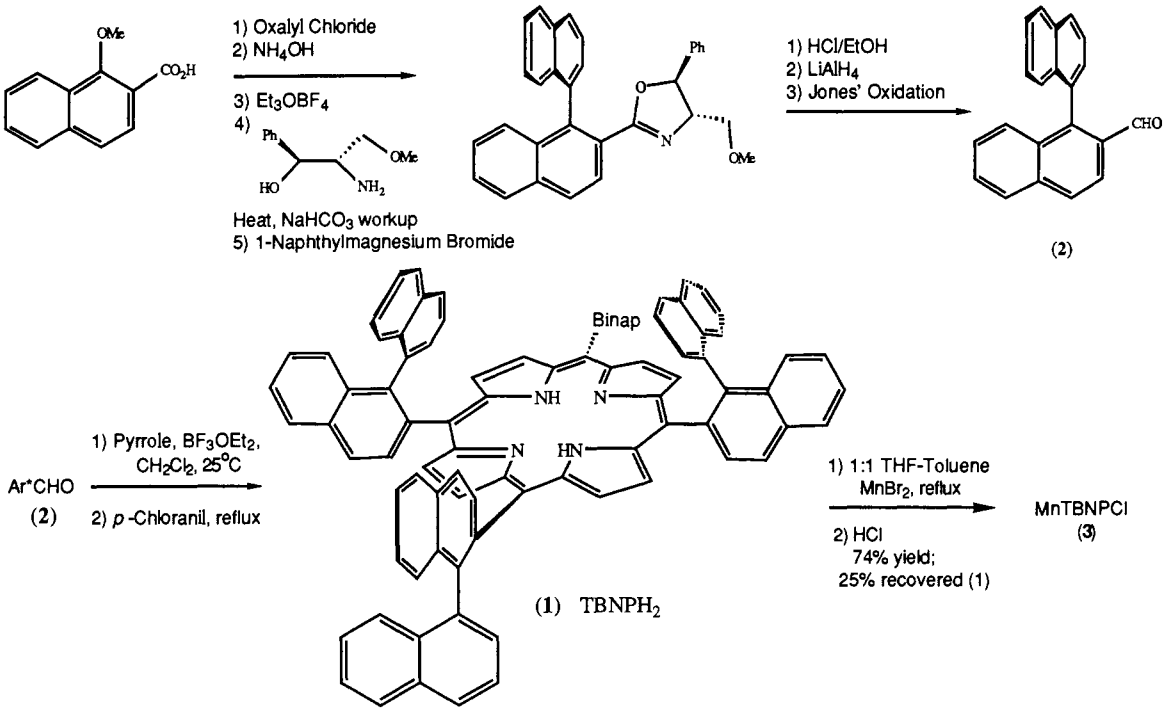

Figure 1. Synthesis of $TBNPH_2$ (**1**) and MnTBNPCl (**3**).

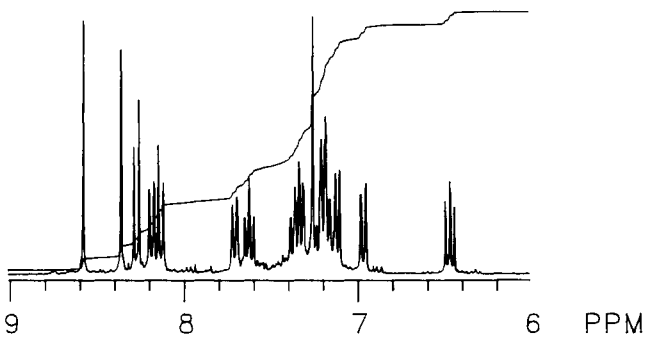

Figure 2. 500-MHz ^1H NMR of $TBNPH_2$ (**1**) in $CDCl_3$ at 22 °C.

"strapped" porphyrin incorporating L-phenylalanine synthesized by Mansuy's group yielded comparable enantioselectivites.[9] Unfortunately these previous porphyrins, though elegant and innovative, suffer from stability problems inherent in their synthetic design, which necessitates the presence of electron-donating substituents on the *meso*-phenyl groups (known to markedly sensitize the porphyrin toward oxidative degradation). Groves and Myers commented that after only 100 catalyst turnovers the enantioselectivity of epoxidation in their system deteriorated significantly. MnTBNPCl, in contrast, is quite robust under oxidation conditions; when catalyst that had been used for a standard styrene run (250 turnovers) was reused, identical rates and ee's were obtained. It is with regard to catalytic efficiency that the chiral wall porphyrin represents a significant advance in asymmetric oxygenation chemistry. By simply increasing the amounts of styrene and bleach we have been able to attain 2800 turnovers in 80 min with little catalyst degradation (examination of the Soret band at 479 nm at identical porphyrin concentrations before and after reaction showed 86% absorbance retained).

We are in the process of further evaluating $TBNPH_2$ as a chiral ligand for metal-mediated oxygenations as well as other types of catalytic reactions. Synthetic work directed toward creating substituted versions of **3** which are more selective epoxidation catalysts is also underway.

Acknowledgment. We thank Robert J. Nick for valuable discussion.

Supplementary Material Available: Synthesis details for **1** and **2** (1 page). Ordering information is given on any current masthead page.

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Introduction of Reporter Groups at Specific Sites in DNA Containing Phosphorothioate Diesters

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Received July 3, 1989

Facile and covalent attachment of reporter groups at specific sites within a DNA sequence would simplify detailed study of the structure and dynamics of unusual DNA forms as well as ligand-DNA or protein-DNA complexes. To date, the covalent introduction of such reporter groups has largely relied upon either (i) the prior de novo synthesis of a modified nucleoside containing the desired probe and then its incorporation into the nucleic acid[1]

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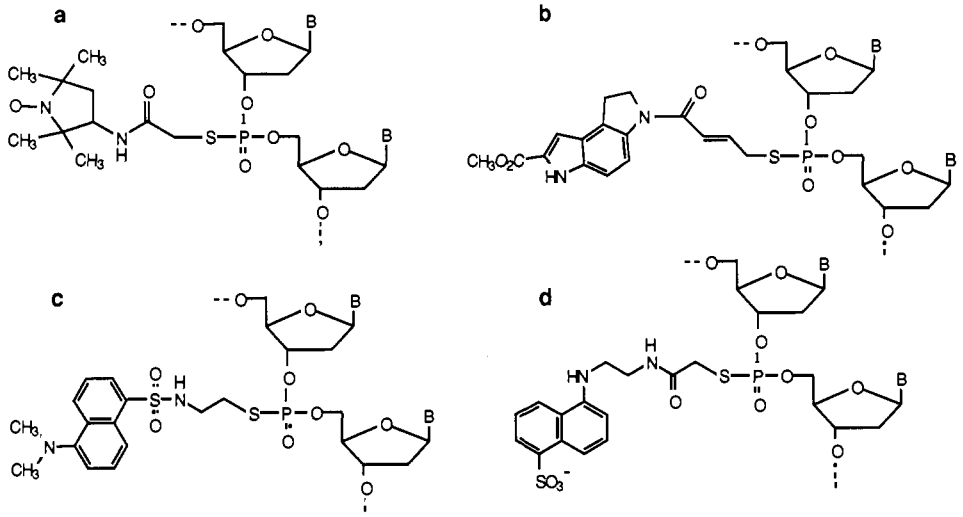

Figure 1. Phosphorothioate triester oligodeoxynucleotides carrying (a) a PROXYL spin label. (b) a derivative of the dihydropyrroloindole subunit of CC-1065, (c) a sulfonamide-linked dansyl fluorophore, and (d) an N-linked dansyl fluorophore. These derivatives were prepared by reaction of the oligodeoxynucleotide with an excess of label for 24 h. Representative reaction mixtures for the dodecamer: (a) 10 mM 3-(2-iodoacetamido)-PROXYL, 0.15 mM d[CGCA(s)AAAAAGCG], pH 8.0 (phosphate) at 50 °C in a solution containing 4% DMF, (b) 5 mM dihydropyrroloindole derivative, 0.07 mM d[CGCA(s)AAAAAGCG], pH 8.0 (Tris) at 50 °C in a solution containing 60% dimethylformamide, (c) 12 mM *N*-dansylaziridine, 0.34 mM d[CGCA(s)AAAAAGCG], pH 8.0 (phosphate) at 25 °C in a solution containing 50% acetonitrile, and (d) 10 mM, 1,5-I-AEDANS, 0.80 mM d[CGCA(s)AAAAAGCG], pH 6.0 (phosphate) at 50 °C in a solution containing 25% DMF. Similar conditions could be employed to label the eicosomer: d[CGTACTAGTT(s)AACTAGTACG].

or (ii) the use of a host of simpler reactions which are confined to the 5′- or 3′-termini of the biopolymer.[2] We now report that phosphorothioate diesters at specific sites within DNA fragments (readily prepared by chemical synthesis) can be employed to direct the covalent attachment of reporter groups such as fluorophores, spin labels, or drug derivatives to the sugar phosphate backbone.

The substitution by sulfur of a nonbridging oxygen in the internucleotidic phosphodiester linkage results in a phosphorothioate diester.[3] DNA containing phosphorothioate diesters is essentially native in structure,[4] and often physical properties, such as T_m values,[5] are indistinguishable from those for unmodified DNA. However, the chemical reactivity of phosphorothioate derivatives has yet to be thoroughly exploited.[6] There are only two reported uses of a phosphorothioate as a site for covalent labeling.[7,8]

In the present report, Tp(s)T was reacted with a number of fluorophores containing a variety of functional groups (typically designed to label the thiol group of cysteine). Three functionalities, γ-bromo-α,β-unsaturated carbonyls, iodo(or bromo)acetamides, and aziridinyl sulfonamides, have been observed to effectively label phosphorothioate diesters and produce the corresponding phosphorothioate triester carrying the desired reporter group. As examples of such reactions, we have site-specifically labeled a dodecadeoxynucleotide and/or an eicosodeoxynucleotide containing a single phosphorothioate diester with fluorophores, a spin label, and a CC-1065 drug analogue (Figure 1).

Synthesis of the oligodeoxynucleotides containing a single phosphorothioate diester was accomplished by altering the oxidation step of the synthetic cycle,[9] resulting in two phosphorus diastereoisomers (R_p and S_p). It is possible to prepare the DNA fragment such that it contains a pure phosphorus diastereoisomer as described.[9a,10]

Oligodeoxynucleotides containing a single covalently bound reporter group are obtained by incubation of the phosphorothioate-containing DNA fragment with the reporter group of choice in aqueous or largely aqueous solutions at pH values from 5 to 8. These reactions are performed at 25 or 50°C and usually proceed with yields greater than 85% after 24 h at 50 °C.[11]

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(11) Compound b in Figure 1 required 48 h at 50 °C or 80 h at 25 °C, at which time it was 70–80% complete.

Resolution of the reaction mixture and isolation of the triester product can be accomplished by using HPLC (4.6 × 250 mm Hypersil-ODS with 0.02 M KH_2PO_4, pH 5.5, and a methanol gradient). Modification of the phosphorothioate was observed to be more efficient for the single-stranded d[CGCA(s)-AAAAAGCG] fragment than the self-complementary eicosomer, d[CGTACTAGTT(s)AACTAGTACG]. This difference in reactivity was partially overcome when the reaction mixture was heated at 50 °C. In the absence of the phosphorothioate diester, control reactions using native oligodeoxynucleotides did not result in any significant labeling.[12]

The unlabeled dodecamer helix, d[CGCA(s)-AAAAAGCG]·d[CGCTTTTTTGCG], exhibited a T_m of 55 °C, and this was indistinguishable from the T_m values obtained for the PROXYL-labeled (a in Figure 1) or drug-labeled (b in Figure 1) helices. The T_m value for the self-complementary eicosomer, d[CGTACTAGTT(s)AACTAGTACG]$_2$, with two labels was also largely unchanged (68.5 °C) in comparison to the unlabeled fragment (T_m = 67 °C).

The hydrolytic stability of the phosphorothioate triesters is an important practical consideration for the value of such derivatives in many studies. Hydrolysis of the triesters proceeded by desulfurization (monitored by HPLC and confirmed by comparison with authentic standards). We have not yet examined the fate of the reporter group in these reactions. No detectable cleavage of the oligodeoxynucleotide at the point of attachment was observed. This agrees with the results of ethylated or hydroxyethylated derivatives, which result in primarily desulfurization and only very minor amounts of chain cleavage.[6]

Less than 5% of the Tp(s)T triester carrying the PROXYL spin label was hydrolyzed after 24 h at pH 7. At pH 8 this increased to 28%, and at pH 10 the triester was completely hydrolyzed within 11 h. With longer fragments, the hydrolytic stability of the triester increased [the labeled dodecamer was hydrolyzed <1%, 30%, and 99% at pH values 7, 8, and 10, respectively; the values for the eicosomer were <1%, 2%, and 63% (24 h)]. The triester prepared from a γ-bromo-α,β-unsaturated carbonyl (b in Figure 1) exhibited stability similar to that of the PROXYL-labeled derivatives while that resulting from reaction with the aziridinyl sulfonamide (c in Figure 1) was more stable [the Tp(s)T-labeled triester was hydrolyzed <1% (pH 7), 5% (pH 8), and 34% (pH 10) after 24 h at ambient temperature].

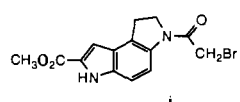
It is noteworthy that the triester produced from 1,5-I-AEDANS and Tp(s)T was significantly less stable than the PROXYL-labeled derivative although the triesters formed both resulted from iodoacetamides.[13] The AEDANS-labeled dimer exhibited 19% (pH 7) and 88% (pH 8) hydrolysis (24 h); it was completely hydrolyzed within 2 h at pH 10. However, the AEDANS-labeled dodecamer (d in Figure 1) exhibited only <1%, 49%, and 99% hydrolysis at the same respective pH values (24 h).

The ability to attach reporter groups (covalently) where desired on the DNA backbone should simplify studies involving protein binding, resonance energy transfer, structural analyses and nucleic acid dynamics. By employment of the appropriate functional group, it should additionally be possible to attach a variety of derivatives including, but not limited to, peptides and proteins, antibiotics, antineoplastics, and antivirals.

Acknowledgment. We acknowledge the technical assistance of Nicole Narekian and thank Prof. Dale Boger and Dr. S. A. Munk, Purdue University, for the preparation of the CC-1065 analogues (compound i[13] and the analogue used to prepare derivative b, Figure 1) and Prof. Fritz Eckstein, Max-Planck Institut, Goettingen, West Germany, for continued scientific discussions. This work was supported by a grant from the National Institutes of Health (GM 37065).

(12) At 50 °C, HPLC analysis of the dansylaziridine reaction indicated the presence of minor products, suggesting some nonspecific reaction with the DNA. Labeling conducted at 25 °C (pH 8.0) proceeded more slowly, but we were unable to detect the presence of any species other than the desired product and starting materials. However, we cannot exclude the possibility of some nonspecific modification of the DNA even at 25 °C.

(13) An additional dodecamer was labeled with the bromoacetamido derivative i. Although the three acetamido-linked adducts are similar in structure, that prepared from i proved to be more stable than either a or d (Figure 1) (only 13% of the triester formed from i was hydrolyzed after 24 h at pH 8.0). This variation in stability of the acetamido–linked probes is under continuing study.



i

Cyclopropyl Anion as an Allyl Anion Synthon. Novel Synthesis of Butadienes by Nickel-Catalyzed Coupling of Cyclopropyl Grignard Reagents with Dithioacetals[1]

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Received August 1, 1989

The synthetic utility of ring-opening reactions of cyclopropyl groups is rich.[3] The rearrangement of cyclopropylcarbinyl organometallic species to homoallylic moieties has recently received much attention.[4] However, the applications of these reactions in the synthesis of butadienes have been rare.[5] To our knowledge, there has been no report with use of the cyclopropyl Grignard reagent as the allyl anion synthon in the synthesis of conjugated dienes. We recently uncovered several useful coupling reactions of dithioacetals with Grignard reagents in the presence of the nickel catalyst[6] to afford the corresponding olefins[7,8] or geminal dimethyl products.[8,9] When cyclopropylmagnesium halide is employed in this coupling reaction, the organometallic intermediate **1** is expected to rearrange[4] to homoallylic organonickel species **2**, which would rapidly undergo β-elimination to give butadiene (eq 1). We

(1) Part 30 of the series Transition Metal Promoted Reactions.

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