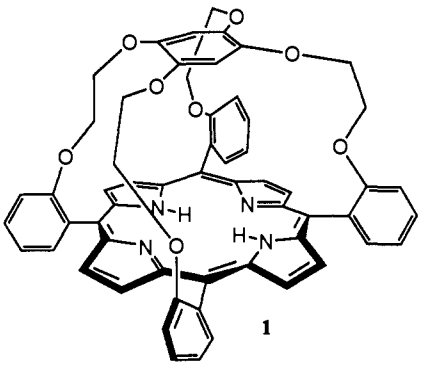
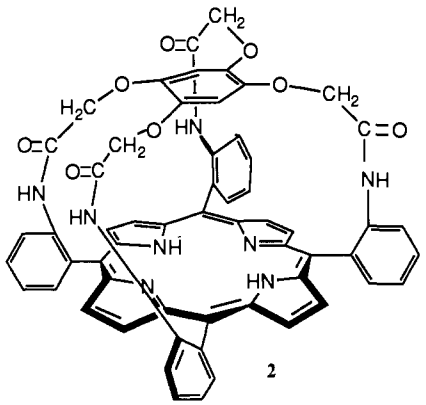


1



2

is 3.96 Å in $H_2(C_2\text{-Cap})$[15] (five-atom linkage) and 3.49 Å in $Co(C_3\text{-Cap})$[16] (a more flexible six-atom linkage). In $Fe(C_2\text{-Cap})(CO)(1\text{-MeIm})$[12] this distance is 5.57 Å for molecule 1 and 5.67 Å in molecule 2 (where the Fe-C-O angles are 172.9 (6)° and 175.9 (6)°, respectively). Thus, if either of the present porphyrins as an Fe^{II}(base) derivative is to accommodate an essentially linear Fe-C-O linkage, the cap must move approximately 1.8 Å further away from the porphyrin plane; less movement is required to accommodate the bent Fe-O-O linkage or the hypothetical bent Fe-C-O linkage. Although model building is of limited use in the prediction of structures of elaborated porphyrins,[10] it does suggest a maximum cap-to-porphyrin distance of about 4.7 Å in the three-atom-bridged pocket porphyrin and 6.0 Å in the present four-atom-bridged porphyrins. In the structure of Fe(PocPiv)(CO)(1,2-Me_2Im) the 1,3,5-linked cap has moved out of the way of the essentially linear Fe-C-O linkage.[10] In the present 1,2,4,5-linked systems the cap cannot move completely out of the way. It would thus appear that the present porphyrins as their Fe^{II}(base) derivatives present a cavity very near the limit to accommodate a linear Fe-C-O linkage. Indeed, absorbance measurements of CO and O_2 binding to **1**·Fe in 1 M 1-MeIm/toluene are isosbestic and afford at 26 °C $P_{1/2}$ values of 100 and 280 Torr, respectively. The resultant M value of 2.8 is the lowest to be measured directly in a model compound[4c] and is a clear indication of pronounced steric inhibition of CO binding. The value of 2014 cm^{-1} for the C=O stretch is substantially greater than that in other model compounds in the same solvent system[11,17] or in the native proteins[11,17] and is indicative of significantly reduced Fe back-bonding and hence of a weaker Fe-CO bond. By contrast, **2**·Fe shows no evidence of CO or O_2 binding. These marked differences between **1** and **2** could arise from the more constrained OCH_2CONH linkage in **2** or possibly from a strongly bound water molecule inside the cap[18] of **2**·Fe. Additionally, neither **1**·Fe nor **2**·Fe shows any sign of binding CO in 1 M 1,2-dimethylimidazole/toluene; of course, 1,2-Me_2Im as compared with 1-MeIm as base is known to decrease CO binding by about a factor of 40-80 in capped systems.[17] Further investigations of CO and O_2 binding to **1**·Fe and **2**·Fe with more axial bases are in progress as are attempts to obtain crystals of any CO adducts suitable for X-ray study.

Acknowledgment. This research was kindly supported by the National Institutes of Health (HL-13157).

Supplementary Material Available: Table SI giving positional and thermal parameters for **1** and **2** (5 pages). Ordering information is given on any current masthead page.

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Disulfide Cross-Linked Oligonucleotides

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Site-specific cross-linking of DNA is a promising tool for the study of genetic structure and function. However, known cross-links either are difficult to target,[2-6] are unstable,[3,7] or disrupt native DNA secondary structure.[6,8] Here we report chemistry that overcomes these difficulties by using an alkane disulfide as the interstrand cross-link. In the present study, our previously reported *convertible nucleoside approach*[9,10] has been extended to the synthesis of dA-tethered oligonucleotides.[11] In nucleoside model studies,[12] we observed quantitative aminolysis of O^6-phenyl-2′-deoxyinosine[13] (ϕdI, cf. Scheme I) to N^6-alkyl-dA. ϕdI was therefore converted to the corresponding "phosphoramidite"[14,15] for use in the synthesis of the decanucleotide 5′-

(1) Searle Scholar, 1990-1993; Eli Lilly Fellow, 1990-1992; Sloan Fellow, 1991-1994.
(2) Known bis-electrophile cross-linking agents generally show no greater than dinucleotide sequence selectivity, often produce mixtures of monoadducts and cross-links, and produce interstrand and intrastrand cross-links.[3-6]
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(12) In these studies, O^6-(2,4,6-trimethylphenyl)-dI was found to be extremely resistant to aminolysis, unlike the previously reported dC-convertible nucleoside O^4-(2,4,6-trimethylphenyl)-2′-deoxyuridine.[9,10] Ferentz, A. E.; Verdine, G. L., manuscript in preparation.
(13) ϕdI was synthesized by the trimethylamine-mediated phenol displacement of 3′,5′-diacetyl-O^6-[(triisopropylphenyl)sulfonyl]-2′-deoxyinosine (O^6-TIPS-dI-diAc), followed by deacetylation (K_2CO_3/MeOH).[14] The displacement was carried out according to an analogous published procedure: Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1982**, *23*, 2253. O^6-TIPS-dI-diAc was synthesized by the route described for the corresponding 3′,5′-diisobutyl ester: Seela, F.; Herdering, W.; Kehne, A. *Helv. Chim. Acta* **1987**, *70*, 1649.
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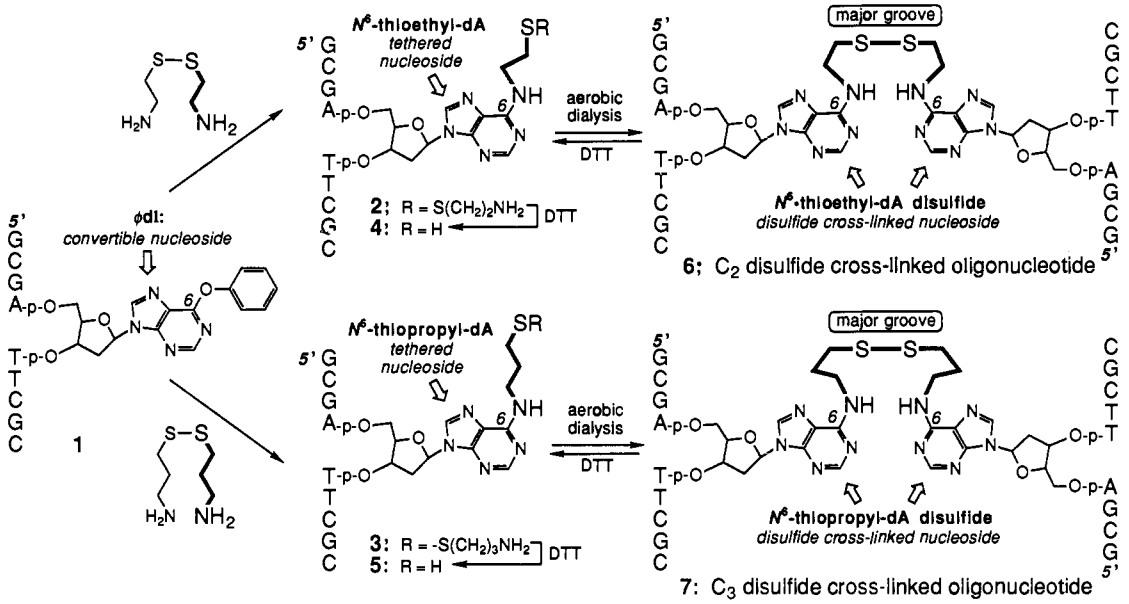
Scheme I



DMT-d(GCGA-φI-TTCGC).[16] The protecting groups[17] and linkage to the solid support were removed by mild ammonia treatment,[18] and the crude oligonucleotide was purified by HPLC and detritylated to yield the precursor d(GCGA-φI-TTCGC) (**1**, Scheme I).[14] Precursor **1** was then treated with the disulfide of aminoethanethiol or aminopropanethiol[10,14] to convert quantitatively *φdI* to an N^6-thioalkyl-dA (protected as a mixed disulfide) containing two (**2**) or three (**3**) methylene units.[19] Oligonucleotides **2** and **3** are self-complementary and thus can dimerize to form a duplex in which the thiol-tethered adenines (A) are located on opposite strands in consecutive base pairs (below).

5'-d(GCGAATTCGC)-3' ⟶ 5'-d(GCGAATTCGC)-3'
3'-d(CGCTTAAGCG)-5'

The mixed disulfides **2** and **3** were reduced with dithiothreitol (DTT) to the free thiols **4** and **5**[9,20] and then dialyzed aerobically to form a disulfide bond. Denaturing polyacrylamide gel electrophoresis revealed complete conversion to new, high molecular weight species, which could be reconverted to monomers by treatment with DTT; nucleoside composition analysis[19] of the high molecular weight oligonucleotides confirmed the presence of a disulfide-linked nucleoside in each. Finally, nondenaturing gel electrophoresis confirmed that the high molecular weight species were dimeric (duplex) in solution and not higher order multimers. These results are consistent with the reversible formation of disulfide-linked oligonucleotide dimers **6** and **7**.

Energy-minimized molecular models[14] indicated that the dithiobis(ethane) (**6**) and dithiobis(propane) (**7**) tethers, which are located in the major groove, would cause little change in DNA secondary structure relative to d(GCGAATTCGC). Consistent with the models, **6** and **7** exhibited circular dichroism and ^{31}P NMR spectra characteristic of B-DNA; furthermore, their ^1H NMR spectra indicated that the cross-linked adenines are Watson–Crick base-paired.[14] In variable-temperature ^1H NMR experiments, cross-links **6** and **7** melted by the same pathway as d(GCGAATTCGC), further evidence that the cross-links do not perturb DNA structure.[21,22]

Thermal denaturation experiments in 1 M NaCl indicated that cross-linked molecules **6** and **7** melt cooperatively at temperatures more than 18 °C above T_m of the unmodified decamer;[23] at a more physiological salt concentration (100 mM NaCl), the cross-linked oligonucleotides are stabilized at least as much, dissociating at temperatures approximately 30 °C above T_m of the native 10-mer.[24] A similar degree of stabilization has been observed in proteins only after introducing *three* nonnative disulfide bonds.[25]

This site-specific incorporation of a derivatizable (amine, **2**/**3**; thiol, **4**/**5**) dA into an oligonucleotide opens a new avenue for attaching reporter and effector groups to DNA.[8c,26] Using this method we have engineered the first disulfide-bonded tethers in

(16) All DNA sequences are presented in the conventional 5′ → 3′ sense reading from left to right. Italics denote the presence of protecting groups and linkage to the solid support. Presence or absence of 5′-DMT is shown explicitly.

(17) This synthesis employed base-labile protecting groups on all exocyclic amines: N^6-phenoxyacetyl-dA, N^4-isobutyryl-dC, N^2-phenoxyacetyl-dG. Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *15*, 397. Commercially available under the name PAC phosphoramidites from Pharmacia (Piscataway, NJ).

(18) Concentrated NH_4OH, room temperature, 4 h, followed by freezing and lyophilization to dryness. Removal of the base-protecting groups prior to conversion of φdI with alkylamines is necessitated by side reactions involving protected dC residues. See ref 6 for a discussion.

(19) Enzymatic digestion by snake venom phosphodiesterase and alkaline phosphatase followed by HPLC analysis of component nucleosides[14] showed the presence of a single N^6-alkyl-dA in each of **2** and **3** and the presence of disulfide-linked nucleosides in **6** and **7**. The identities of the nonnatural nucleosides were confirmed by HPLC, UV, and high-resolution mass spectral comparisons with authentic standards. Neither unreacted φdI nor dI (hydrolysis product) was observed in the digestion mixtures.

(20) Alternative methods for the tethering of thiol groups to oligonucleotides in conjunction with automated synthesis afford attachment only at the 5′ and 3′ ends of the DNA and so are not suitable for use in the present study. Connolly, B. A.; Rider, P. *Nucleic Acids Res.* **1985**, *13*, 4485. Zuckermann, R. N.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 6592. Gupta, K. C.; Sharma, P.; Sathyanarayana, S.; Kumar, P. *Tetrahedron Lett.* **1990**, *31*, 2471.

(21) The terminal two base pairs fray prior to cooperative denaturation of the central six-base-pair region.[14]

(22) The structure of a related dodecamer bearing methyl groups at the positions occupied by the alkanethiol tethers in **2**–**7**, d[CGCGA(N^6-methyl-A)TTCGCG], has been solved by X-ray crystallography; the structure is essentially superimposable on that of the unmodified decamer,[21] establishing that attachment of an alkyl group on the central adenine does not significantly perturb DNA structure. Frederick, C. A.; Quigley, G. J.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J.; Rich, A. *J. Biol. Chem.* **1988**, *263*, 17872.

(23) Melting temperatures (T_m values ±0.1 °C) are as follows: unmodified decamer, 54.2; cross-link **6**, 75.2; cross-link **7**, 72.3. Samples with initial OD_{260} ~ 0.4 AU were prepared in 1 M NaCl, 10 mM KH_2PO_4 (pH 7.0). Measurements made at this high salt concentration have a lower margin of error than those recorded at 100 mM NaCl.

(24) Preliminary thermochemical measurements indicate that the duplex stabilization results from entropic destabilization of the denatured state, and that the cross-linked molecules are destabilized enthalpically relative to the unmodified decamer. This enthalpic destabilization largely results from the requirement to form Watson–Crick base pairs in the disulfide-cross-linked duplex via the less stable anti rotamer about the adenine C6–N^6 bond (shown in Scheme I). Thus far, the ΔH and ΔS values for melting of **6** and **7** have not been sufficiently accurate to permit accounting for the origin of their slightly different T_m's.

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DNA and conclude that (i) an interstrand disulfide cross-link can significantly stabilize duplex DNA while causing little structural distortion; (ii) disulfide cross-links, unlike psoralen,[6b] do not perturb base pairing and the denaturation pathway of DNA; and (iii) it may be possible to drive structural transitions in DNA and to rationally engineer non-ground-state DNA structures by exploiting the favorable energetics associated with disulfide bond formation. Since these unstrained, intramolecular disulfide bonds are both kinetically and thermodynamically resistant to reduction,[27] such cross-linked oligonucleotides should facilitate studies of enzyme-mediated unpairing processes such as transcription, replication, and recombination.

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Supplementary Material Available: Complete experimental details for the synthesis of ϕdI phosphoramidite and oligonucleotides **1**–**7**, details and results of gel electrophoresis and the nucleoside composition analyses, selected CD, ^{31}P NMR, and ^{1}H NMR spectra of cross-linked oligonucleotides **6** and **7** and the unmodified decamer, and energy-minimized molecular models of disulfide cross-linked oligonucleotides **6** and **7** (15 pages). Ordering information is given on any current masthead page.

(27) In experiments to be reported elsewhere, we have determined that the disulfide bond of oligonucleotide **6** (74 μM) is virtually unaffected by 1 mM 2-mercaptoethanol, 25 °C, overnight. This thiol concentration is sufficient to maintain the enzymatic activity of most proteins. It should be noted, however, that **6** and **7** do not form stable duplex DNA at 25 °C, a factor that *facilitates* disulfide reduction. The corresponding cross-linked 12-mer, 5'-d(CGCGAATTCGCG), is completely resistant to 25 mM 2-mercaptoethanol.

Structure of a Free, Unassociated Alkyl-Substituted α-Sulfonyl Carbanion: Isolation and X-ray Crystal Structure Analysis of the Inclusive Lithium Cryptate (Me_2CSO_2Ph)(Li·[2.1.1]cryptand)†

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Recently we disclosed the enantioselective synthesis of a lithium α-sulfonyl carbanion salt which is optically stable at low temperatures.[1] In view of the new mechanistic and synthetic possibilities offered thereby, a deeper knowledge of the structure of α-sulfonyl carbanions and the Li^+ gegenion effect is desirable. Work from our laboratories and elsewhere has shown that alkali-metal salts of α-sulfonyl carbanions exist in the crystal[1-4] and in THF solution[1,2,5] as dimeric and monomeric contact ion pairs which are associated via the sulfonyl O atoms. We have previously probed the free, unassociated α-sulfonyl carbanion[6] and the gegenion effect in the case of the phenyl-substituted species $[PhCH_2(Ph)CSO_2CF_3]^-$ by determining inter alia the crystal structure of its tetrabutylammonium and lithium salt.[2] Surprisingly, here only a small static and dynamic Li^+ gegenion effect was found. Since the free α-sulfonyl carbanion is also of significant theoretical interest,[7] the attainment of the lithium salt of an alkyl-substituted α-sulfonyl carbanion with complete ion separation was an attractive goal. In this communication we report the isolation of the novel title compound (Me_2CSO_2Ph)(Li·[2.1.1]-cryptand) (**1**) and the determination of its crystal structure; that of the solvated dimeric O–Li contact ion pair $[(Me_2CSO_2Ph)$-Li·diglyme$]_2$ (**2**) is already known.[3c]

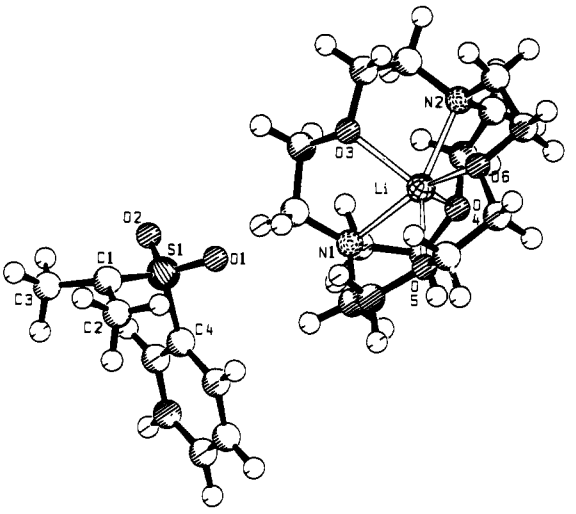

Figure 1. Molecular structure of **1** showing the atom-numbering scheme.[10] Selected bond lengths (Å) and angles (deg) of **1** and of **2** (values following the oblique lines): S1–O1 1.449 (2)/1.462 (2), S1–O2 1.456 (2)/1.454 (2), S1–C1 1.625 (3)/1.640 (3), S1–C4 1.795 (3)/1.794 (3), O1–S1–O2 116.7 (1)/116.6 (1), C1–S1–C4 111.3 (1)/111.8 (1), C3–C1–C2 116.7 (3)/115.5 (3), C3–C1–S1 117.6 (2)/115.7 (2), C2–C1–S1 117.5 (2)/115.3 (2), C1–S1–O1–O2 −128.7 (4)/−128.7 (4), C4–S1–C1–C3 −75.8 (4)/−72.8 (4), C4–S1–C1–C2 71.7 (4)/66.3 (4), O1–S1–C1–C2 42.2 (4)/48.0 (4), O2–S1–C1–C3 −38.6 (4)/−41.3 (4), C3–S1–C1–C2 147.5 (4)/139.2 (4).

Compound **1** was isolated as orange crystals by addition of an equimolar amount of [2.1.1]cryptand[8] to a solution of (Me_2CSO_2Ph)Li in THF and recrystallization of the solid formed from THF. A view of the molecular structure of **1** is depicted in Figure 1.[9] **1** is an inclusive cryptate with discrete

† Dedicated to Professor Dr. H. Prinzbach on the occasion of his 60th birthday.

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