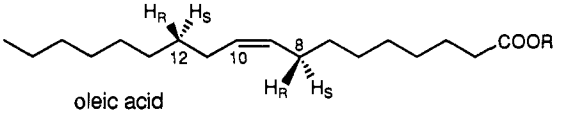
the labeled ester showed a broad *singlet* at 5.4 ppm, assigned to ^2H in natural abundance at the olefinic positions (C-9 and C-10). Thus, there were no intact ^2H–^{13}C units at C-10. Confirmation came from the ^1H-decoupled ^{13}C NMR spectrum of the methyl oleate, since the multiplicity of the 130 ppm C-10 signal was unaffected by ^2H decoupling. Following dilution with unlabeled material, labeled methyl oleate was degraded[14] to octanoic acid (from C-11 through C-18 of oleic acid) and to monomethyl nonanedioate (from C-1 through C-9 of oleic acid, the esterified carboxyl of the derivative corresponding to C-1 of oleic acid). The degradation products were converted to methyl mandelate ester derivatives (**5** and **6**), which were analyzed by ^2H-decoupled HETCOR spectroscopy.



oleic acid

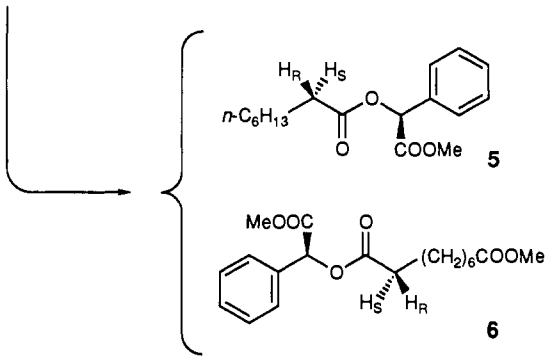
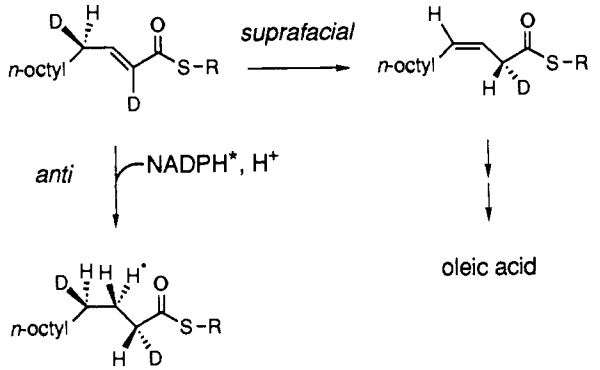


Figure 1 shows the C-2 region of the ^2H-decoupled HETCOR spectrum of labeled **5**. The proton of the CHD group (readily identified by the positive DEPT signal in the ^{13}C dimension) at C-2 is clearly at higher field, corresponding to the *pro*-2*S* position of **5**.[14,18,19] This indicates that ^2H had been incorporated by the *B. ammoniagenes* FAS into the *pro*-12*R* position of oleic acid and therefore into the *pro*-*R* position at all even-numbered carbons on the growing saturated chain.[21] Because C-10 of methyl oleate lacks ^2H, the conversion of **3** to **4** must involve loss of the *pro*-4*R* hydrogen.

To confirm the configuration of acetate-derived ^2H at C-8 of methyl oleate, the ^2H-decoupled HETCOR spectrum of **6** was examined. The proton cross peak of the CHD group at C-2 of **6** was found at lower field (ca. 2.45 ppm vs 2.41 ppm), indicating that ^1H and ^2H are in the *pro*-*R* and *pro*-*S* positions, respectively. Thus, ^2H had been in the *pro*-8*S* position of the labeled oleic acid and in the *pro*-2*R* position of biosynthetic intermediate **4**.

From these experiments, it is clear that the *B. ammoniagenes* FAS-mediated allylic rearrangement is *suprafacial*, suggesting a *stepwise* mechanism involving a single active site acid/base.[5,6] This reaction therefore *conforms* to the stereochemical (hence, mechanistic) trends noted at the beginning of the paper. The basis of the earlier report of antarafacial rearrangement[4] is the assignment of the configuration at C-12 of deuterated oleic acid (from incorporation of [$^2H_2$]malonyl-CoA) opposite[9] to that found in the present work.[24]

The configuration at C-12 of oleic acid indicates the stereochemical course of the NADPH-dependent enoyl reductase reaction. It is now clear that the substrate is protonated at C-2 on the *si* face of the double bond and that the overall stereochemical course of the reduction must be anti.[25] It is interesting that both syn and anti reductions by FAS enoyl reductases are known.[22,26] Further studies are in progress.



Acknowledgment. We thank the National Institutes of Health (Grant GM36286 to J.M.S.) and the Natural Sciences and Engineering Research Council of Canada for generous support. NATO and the Killam Foundation are gratefully acknowledged for fellowships (to T.M.Z.). We also thank Mr. Jerome F. Baker for suggestions pertaining to cell lysis.

Supplementary Material Available: ^1H-decoupled ^2H and ^{13}C NMR spectra of methyl oleate derived from feeding of sodium [2-^{13}C,$^2H_3$]acetate to *B. ammoniagenes* as well as the C-2 region of the ^2H-decoupled HETCOR spectrum of **6** from the degradation of biosynthetically deuterated oleic acid (3 pages). Ordering information is given on any current masthead page.

(18) Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1983**, 83-88.
(19) Parker's method[18] for distinguishing the diastereotopic C-2 protons of carboxylic acids as their methyl mandelate esters appears to be general and highly reliable. The *pro*-2*S* proton of the decanoic acid derivative, for instance, has been shown to resonate at higher field.[3,20]
(20) Schwab, J. M.; Klassen, J. B. *J. Chem. Soc., Chem. Commun.* **1984**, 298-299.
(21) It is accepted that the stereochemical course of fatty acid biosynthesis in a given organism is independent of chain length.[22,23]
(22) Kawaguchi, A.; Seyama, Y. In *Pyridine Nucleotide Coenzymes, Part A*; Dolphin, D., Poulson, R., Avramovic, O., Eds.; John Wiley & Sons: New York, 1987; pp 367-403.
(23) Anderson, V. E.; Hammes, G. G. *Biochemistry* **1984**, *23*, 2088-2094.
(24) A critical discussion of the earlier work[4] has been published.[1]
(25) Kawaguchi, A.; Yoshimura, T.; Saito, K.; Seyama, Y.; Kasama, T.; Yamakawa, T.; Okuda, S. *J. Biochem.* **1980**, *88*, 1-7.
(26) This stereochemical dichotomy has been discussed in the context of molecular evolution.[27,28]
(27) Benner, S. A.; Glasfeld, A.; Piccirilli, J. A. *Top. Stereochem.* **1989**, *19*, 127-207.
(28) Benner, S. A.; Ellington, A. D.; Tauer, A. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7054-7058.

Synthesis, Characterization, Crystal Structures, and CO and O_2 Binding Properties of Novel Four-Atom-Linked Capped Porphyrins

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Frequent reports on the active-site properties of oxygen-carrying hemoproteins[1] and synthetic models[2] continue. Of major interest

(1) (a) Taube, D. J.; Projahn, H.-D.; van Eldik, R.; Magde, D.; Traylor, T. G. *J. Am. Chem. Soc.* **1990**, *112*, 6880-6886. (b) Hauksson, J. B.; La Mar, G. N.; Pandey, R. K.; Rezzano, I. N.; Smith, K. M. *J. Am. Chem. Soc.* **1990**, *112*, 6198-6205. (c) Kobayashi, K.; Hayashi, K. *J. Am. Chem. Soc.* **1990**, *112*, 6051-6053. (d) Gadsby, P. M. A.; Thomson, A. J. *J. Am. Chem. Soc.* **1990**, *112*, 5003-5011. (e) Chatfield, M. D.; Walda, K. N.; Magde, D. *J. Am. Chem. Soc.* **1990**, *112*, 4680-4687. (f) Shiro, Y.; Sato, F.; Suzuki, T.; Iizuka, T.; Matsushita, T.; Oyanagi, H. *J. Am. Chem. Soc.* **1990**, *112*, 2921-2924. (g) Proniewicz, L. M.; Kincaid, J. R. *J. Am. Chem. Soc.* **1990**, *112*, 675-681. (h) Alden, R. G.; Ondrias, M. R.; Shelnutt, J. A. *J. Am. Chem. Soc.* **1990**, *112*, 691-697. (i) Projahn, H.-D.; Dreher, C.; van Eldik, R. *J. Am. Chem. Soc.* **1990**, *112*, 17-22. (j) Kaminaka, S.; Ogura, T.; Kitagawa, T. *J. Am. Chem. Soc.* **1990**, *112*, 23-27. (k) Kaminaka, S.; Ogura, T.; Kitagishi, K.; Yonetani, T.; Kitagawa, T. *J. Am. Chem. Soc.* **1989**, *111*, 3787-3794.

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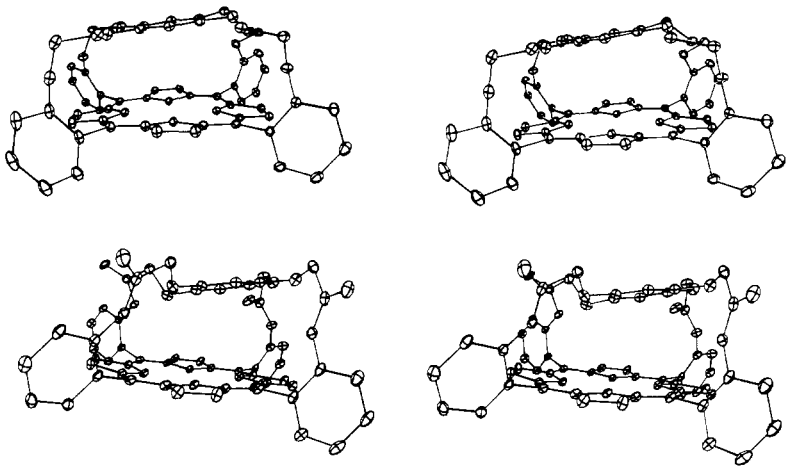
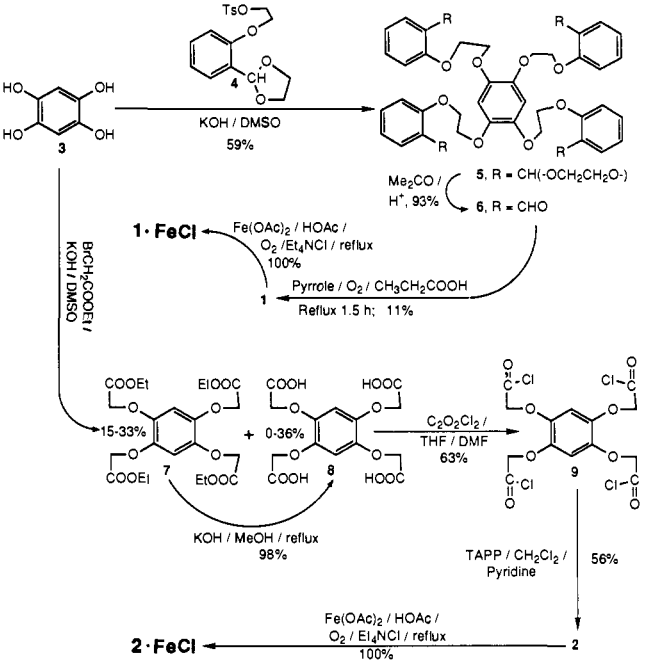


Figure 1. Stereoviews of **1** (top) and **2** (bottom). Ellipsoids are drawn at the 50% probability level. Hydrogen atoms are omitted.

is CO/O_2 discrimination. The basis for the discrimination against CO seen in most natural oxygen-carrying hemoproteins is thought to be steric hindrance,[3] hydrogen bonding,[4] or porphyrin ruffling.[2a] Numerous "picket-fence",[5] "tailed picket-fence",[6] "strapped",[2c,7] "capped",[8] and "pocket"[9] porphyrins, among others,[7b] have been synthesized in an attempt to obtain a system that sterically inhibits CO binding by causing the Fe–C–O bond to bend or tilt or perhaps not form at all. Whether it is "bending" (at the C of the Fe–C–O bond) or "tilting" (of the linear Fe–C–O bond) that occurs in the hemoproteins remains a contentious point.[10,11] To date, none of the X-ray structures of model CO adducts display the distortions reported to occur in some of the natural systems. The "pocket" porphyrin, with a three-atom linkage to a 1,3,5-attached benzene cap, proved too flexible[10] to distort the Fe–C–O linkage significantly. The most compact "capped" porphyrin, namely, a "C_2-Cap" with a five-atom linkage to a 1,2,4,5-attached benzene cap,[8] has a cavity large enough to accommodate the Fe–C–O linkage without much distortion.[12] Thus the synthesis of model compounds that are less flexible, have smaller cavities, and can bind CO or O_2 is crucial.

Scheme I



Here we report the synthesis, crystal structures, and some preliminary CO and O_2 binding studies of two new, four-atom-linked capped porphyrins **1** and **2** whose Fe(II) chelates bind CO and O_2 weakly or not at all. The pathway used to synthesize **1** and **2** is shown in Scheme I. Subsequent reduction of the iron center to Fe(II) was accomplished by shaking a toluene solution of either **1**·FeCl or **2**·FeCl with aqueous $Na_2S_2O_4$ under N_2 and then drying with Na_2SO_4.

The very similar structures of **1**[13] and **2**[14] are shown in Figure 1. In each the benzene cap is slightly off center and modestly tilted with respect to the mean porphyrin plane, the dihedral angles being 8° in **1** and 11° in **2**. The cap atoms average 3.81 Å from the mean porphyrin plane in **1** and 3.90 Å in **2**. This distance

(2) (a) Baltzer, L.; Landergren, M. *J. Am. Chem. Soc.* **1990**, *112*, 2804–2805. (b) Lopez, M. A.; Kollman, P. A. *J. Am. Chem. Soc.* **1989**, *111*, 6212–6222. (c) Gerothanassis, I. P.; Momenteau, M.; Loock, B. *J. Am. Chem. Soc.* **1989**, *111*, 7006–7012. (d) Proniewicz, L. M.; Bruha, A.; Nakamoto, K.; Kyuno, E.; Kincaid, J. R. *J. Am. Chem. Soc.* **1989**, *111*, 7050–7056. Also related: (e) Lee, C.-H.; Garcia, B.; Bruice, T. C. *J. Am. Chem. Soc.* **1990**, *112*, 6434–6435. (f) Gueutin, C.; Lexa, D.; Momenteau, M.; Savéant, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 1874–1880.

(3) (a) Nagai, K.; Luisi, B.; Shih, D.; Miyazaki, G.; Imai, K.; Poyart, C.; De Young, A.; Kwiatkowski, L.; Noble, R. W.; Lin, S.-H.; Yu, N.-T. *Nature* **1987**, *329*, 858–860. (b) Olson, J. S.; Mathews, A. J.; Rohlfs, R. J.; Springer, B. A.; Egeberg, K. D.; Sligar, S. G.; Tame, J.; Renaud, J-P.; Nagai, K. *Nature* **1988**, *336*, 265–266.

(4) (a) Phillips, S. E. V.; Schoenborn, B. P. *Nature* **1981**, *292*, 81–82. (b) Shaanan, B. *Nature* **1982**, *296*, 683–684. (c) Traylor, T. G.; Koga, N.; Deardurff, L. A. *J. Am. Chem. Soc.* **1985**, *107*, 6504–6510. (d) Traylor, T. G.; Tsuchiya, S.; Campbell, D.; Mitchell, M.; Stynes, D.; Koga, N. *J. Am. Chem. Soc.* **1985**, *107*, 604–614.

(5) Collman, J. P.; Gagne, R. R.; Reed, C. A.; Halbert, T. R.; Lang, G.; Robinson, W. T. *J. Am. Chem. Soc.* **1975**, *97*, 1427–1439.

(6) (a) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Sessler, J. L.; Morris, R. M.; Gibson, Q. H. *Inorg. Chem.* **1983**, *22*, 1427–1439 and references therein. (b) Lindsey, J. *J. Org. Chem.* **1980**, *45*, 5215.

(7) (a) Traylor, T. G.; Popovitz-Biro, R. *J. Am. Chem. Soc.* **1988**, *110*, 239–243. (b) A recent review: Morgan, B.; Dolphin, D. *Struct. Bonding (Berlin)* **1987**, *64*, 115–203.

(8) (a) Shimizu, M.; Basolo, F.; Vallejo, M. N.; Baldwin, J. E. *Inorg. Chim. Acta* **1984**, *91*, 247–250. (b) Shimizu, M.; Basolo, F.; Vallejo, M. N.; Baldwin, J. E. *Inorg. Chim. Acta* **1984**, *91*, 251–255. Almog, J.; Baldwin, J. E.; Crossley, M. J.; Debernardis, J. F.; Dyer, R. L.; Huff, J. R.; Peters, M. K. *Tetrahedron* **1981**, *37*(21), 3589–3601.

(9) (a) Sessler, J. L. Ph.D. Dissertation, Stanford University, 1982. (b) Collman, J. P.; Brauman, J. I.; Collins, T. J.; Iverson, B.; Sessler, J. L. *J. Am. Chem. Soc.* **1981**, *103*, 2450–2452.

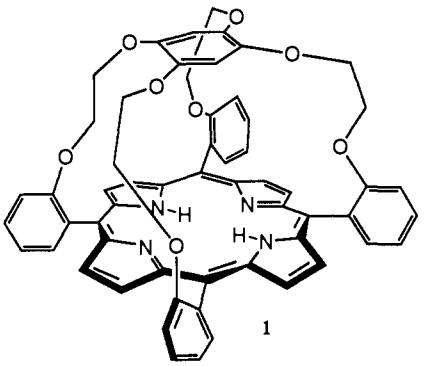
(10) Kim, K.; Fettinger, J.; Sessler, J. L.; Cyr, M.; Hugdahl, J.; Collman, J. P.; Ibers, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 403–405.

(11) Li, X. Y.; Spiro, T. G. *J. Am. Chem. Soc.* **1988**, *110*, 6024–6033.

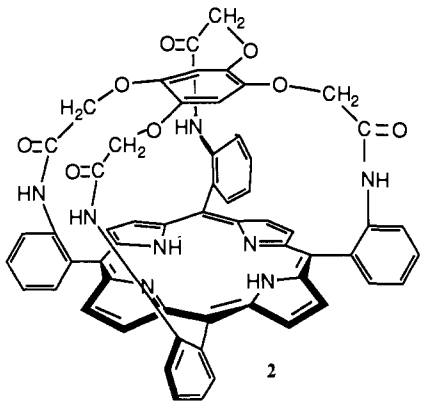
(12) Kim, K.; Ibers, J. A. *J. Am. Chem. Soc.*, in press.

(13) Crystal data for **1**·CH_3COOH: triclinic, $P\bar{1}$, a = 8.859 (3) Å, b = 16.682 (7) Å, c = 16.706 (4) Å, α = 104.01 (2)°, β = 91.41 (2)°, γ = 92.47 (2)°, t = −115 °C; CAD4 diffractometer, Cu radiation, $R(F)$ = 0.075 for 7718 observations and 677 variables. The crystal is 15% contaminated with **1**·Ni·CH_3COOH with no apparent effect on metrical parameters. The solvent, tentatively identified as CH_3COOH, arises from the incomplete metalation reaction.

(14) Crystal data for **2**·0.5$CHCl_3$·0.8$CHCl_3$·CH_3OH: triclinic, $P\bar{1}$, a = 13.385 (3) Å, b = 13.809 (9) Å, c = 17.276 (5) Å, α = 96.98 (4)°, β = 108.25 (2)°, γ = 96.49 (4)°, t = −115 °C; CAD4 diffractometer, Cu radiation, $R(F)$ = 0.11 for 6182 observations and 763 variables. The first $CHCl_3$ molecule is ordered, as is the CH_3OH molecule. The second $CHCl_3$ molecule is badly disordered, and modeling of this disorder continues.



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.

(15) Jameson, G. B.; Ibers, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 2823-2831.
(16) Sparapany, J. W.; Crossley, M. J.; Baldwin, J. E.; Ibers, J. A. *J. Am. Chem. Soc.* **1988**, *110*, 4559-4564.
(17) Hashimoto, T.; Dyer, R. L.; Crossley, M. J.; Baldwin, J. E.; Basolo, F. *J. Am. Chem. Soc.* **1982**, *104*, 2101-2109.
(18) In $Ru(CO)(H_2O)(C_2\text{-Cap})$ the water molecule is inside the cap. Kim, K.; Ibers, J. A., unpublished results.

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]
(3) Ojwang, J.; Gurenberg, D.; Loechler, E. L. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 556. Millard, J. T.; Raucher, S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 2459.
(4) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science* **1987**, *235*, 1204. Teng, S. P.; Woodson, S. A.; Crothers, D. M. *Biochemistry* **1989**, *28*, 3901. Borowy-Borowski, H.; Lipman, R.; Tomasz, M. *Biochemistry* **1990**, *29*, 2999. Weidner, M. F.; Millard, J. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 9270.
(5) Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1985**, *780*, 167. Rahmouni, A.; Leng, M. *Biochemistry* **1987**, *26*, 7229.
(6) (a) Shi, Y.-b.; Gamper, H.; Hearst, J. E. *J. Biol. Chem.* **1988**, *263*, 527. (b) Tomic, M. T.; Wemmer, D. E.; Kim, S.-H. *Science* **1987**, *238*, 1722.
(7) Meyer, R. B., Jr.; Tabone, J. C.; Hurst, G. D.; Smith, T. M.; Gamper, H. *J. Am. Chem. Soc.* **1989**, *111*, 8517.
(8) (a) Cowart, M.; Benkovic, S. J. *Biochemistry* **1991**, *30*, 788. (b) Webb, T. R.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 7661. (c) Matteucci, M.; Webb, T. *Tetrahedron Lett.* **1987**, *28*, 2469.
(9) MacMillan, A. M.; Verdine, G. L. *J. Org. Chem.* **1990**, *55*, 5931.
(10) MacMillan, A. M.; Verdine, G. L. *Tetrahedron*, in press.
(11) The term "oligonucleotides" used throughout the text refers exclusively to oligo-2′-deoxyoligonucleotides.
(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.
(14) See supplementary material for details.
(15) 5′-Tritylation: Weber, H.; Khorana, H. G. *J. Mol. Biol.* **1972**, *72*, 219. 3′-Phosphitylation: Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185.

0002-7863/91/1513-4000$02.50/0