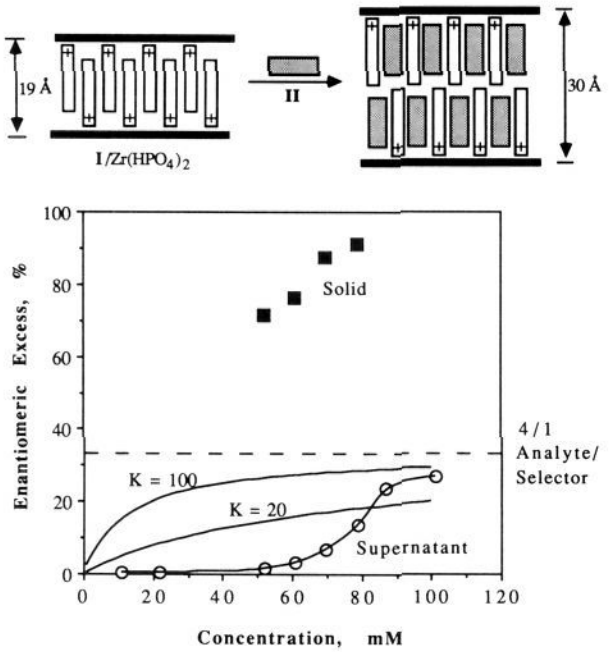

Figure 1. Top: Schematic representation of layer expansion in the intercalation of zirconium phosphate/I by II. Bottom: Enantiomeric excess vs concentration of racemic II in acetonitrile. The dashed line represents the maximum theoretical solution ee for a 4/1 molar ratio of II/I. For comparison, two calculated Langmuir isotherms with $K = 20$ and 100 M^{-1} are shown. The composition of the 19-Å solid phase before intercalation was $Zr[(TBA)_{0.138}(I)_{0.093}H_{0.77}PO_4]_2 \cdot xH_2O$.

there being essentially no intercalation below 50 mM, and almost stoichiometric reaction to form the I·II complex at 100 mM concentration. The experiments shown were carried out with a 4/1 molar ratio of II/I, to allow comparison of the binding curve, expressed as enantiomeric excess (ee),[19] to the Langmuir adsorption isotherms calculated for (*S*,*S*)-I·II complex formation constants of 100 and 20 M^{-1}. This formation constant, measured previously for the same diastereomeric complex lacking the remote quaternary ammonium group, is approximately 100 M^{-1} in fluid solution.[20] While the binding at low concentration is clearly non-Langmuirian, at high concentrations of analyte the observed solution ee's approach the 100 M^{-1} curve. We postulate that the observed concentration threshold for intercalation reflects the work needed to separate the layers from a 19-Å spacing, which represents an interdigitated arrangement of chiral selector molecules, to 30 Å for the I·II intercalated phase.

The intercalation of II is completely and rapidly reversed by simply exposing the solid to pure acetonitrile, and analysis of these solutions shows that the ee exceeds 90% for the fully loaded solid. This result is consistent with chromatographically measured separation factors (α) in excess of 10 for similar molecules[21] and demonstrates that the enantioselectivity of the chiral selector is unimpaired by intercalation into zirconium phosphate; therefore, it is reasonable to expect that other chiral selectors should retain their activity as well as in this host solid. Since the internal surface area of zirconium phosphate and similar layered ion exchangers is on the order of 1000 m^2/g, approximately an order of magnitude higher than that of commercially available chiral stationary phases, these materials may potentially be of interest for preparative chiral separations, operating in either a batchwise or chromatographic mode. These possibilities are currently under investigation.

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(19) Concentrations of (*S*)- and (*R*)-II were measured by HPLC, using a Regis DNB-phenylglycine Pirkle column with UV detection at 242 nm. Enantiomeric excess (ee) is defined as

$$ee = \left|\frac{[R] - [S]}{[R] + [S]}\right| \times 100\%$$

At the 4/1 molar ratio used to generate the plot in Figure 1, 33% ee is the maximum solution ee attainable. Solid ee's were determined by reversing the intercalation reaction in acetonitrile and measuring the concentrations of (*R*)- and (*S*)-II in the supernatant solutions.

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Tentacle Porphyrins: DNA Interactions

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Cationic porphyrins exhibit diverse binding modes with nucleic acids.[1-3] Initial interest in porphyrin–DNA interactions arose in conjunction with photodynamic therapy,[4] but more recent interest in porphyrin–nucleic acid interactions stems from porphyrin antiviral (including HIV-1)[5-8] and anticancer activity[9] and from their utility as nucleic acid structural probes; e.g., metallo derivatives selectively cleave nucleic acids.[10-12] Thus, a greater understanding of the fundamental factors that influence porphyrin–nucleic acid binding not only has value in elucidating the relationship of structure to nucleic acid binding but may also be useful in developing therapeutic agents and biological probes.

Considerable evidence exists that porphyrins can intercalate into nucleic acids;[1,2,13-17] the projection of substituents from the

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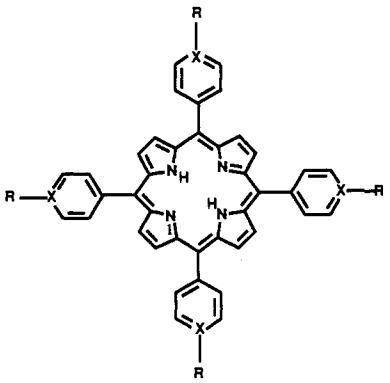

TθOPP : X-R = C-O-CH_2-CH_2-CH_2-$N^+(Me)_3$

TθpyP : X-R = N^+-CH_2-CH_2-CH_2-$N^+(Me)_3$

Figure 1. Structure of tentacle porphyrin cations (chloride salts).

large porphyrin ring appears to place greater steric demand on intercalative binding compared to classical intercalators. For all known intercalators, the aromatic substituents on at least two adjacent quadrants of the porphyrin have been planar with both ortho positions unsubstituted.[14] Thus, the prototypical porphyrin, TMpyP(4),[18] with four planar *N*-methylpyridinium substituents, exhibits characteristics of intercalative binding;[4,15,16] in contrast, TMAP, with four aromatic substituents bearing the nonplanar (6 Å thick) $N(CH_3)_3^+$ groups, is an outside binder.[15] Likewise, other nonplanar porphyrins such as axially ligated metallo-TMpyP(4) derivatives are outside binders.[1-3] Such steric/binding relationships form the basis of the fundamental tenet that intercalating porphyrins must have limited effective thickness.[1,14,15,19,20] Furthermore, it is clear that porphyrin intercalation occurs in GC-rich regions and outside binding occurs in AT-rich regions,[1,2] although the base pairs are similar in size. Since the porphyrins bear no groups that could impart binding selectivity via H-bonding interactions, it is possible that the unusual sequence selectivity may have an electronic origin as found previously for classical intercalators.[21] However, a clear assessment of the effect of porphyrin ring electronic properties on DNA binding mode is not available.

We have synthesized two new water-soluble "tentacle" porphyrins (TθpyP and TθOPP, Figure 1) to assess the binding mode of two porphyrins of similar size but with cores that differ significantly in electronic properties. (Protonated TθpyP has a $pK_a \approx 1$. The pK_a of protonated TθOPP is 4.6, establishing it as an electron-rich porphyrin, consistent with the greater electron-donor phenoxy aromatic substituents.)

Taking advantage of the great similarity of the chromophores in TθpyP and TMpyP(4), we first compared spectroscopic changes on DNA binding under identical conditions.[22] The Soret band

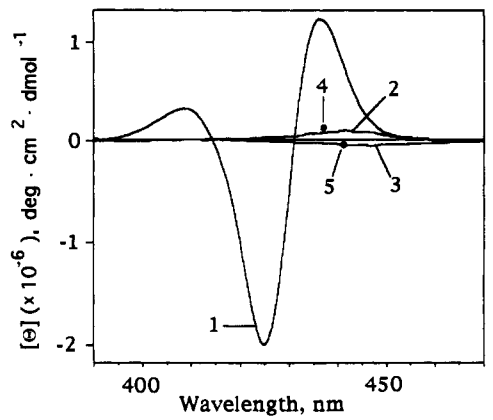

Figure 2. CD spectrum (for $R = 0.05$) of TθOPP-$[poly(dAdT)]_2$ (1), TθpyP-$[poly(dAdT)]_2$ (2), and TθpyP-$[poly(dGdC)]_2$ (3). The dots show the CD maximum for TMpyP(4)-$[poly(dAdT)]_2$ (4) and TMpyP(4)-$[poly(dGdC)]_2$ (5). [Porphyrin] = 7.5 μM.[22]

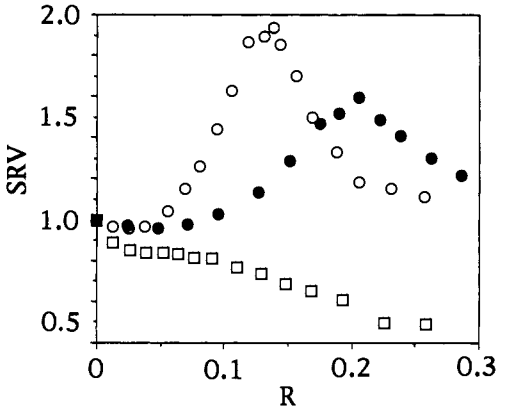

Figure 3. Viscometric titrations of CCS DNA with TMpyP(4) (○), TθpyP (●), and TθOPP (□). $T = 30$ °C, [PIPES] = 10 mM, [NaCl] = 100 mM.

shifts and hypochromicities (H)[23] for TθpyP vs TMpyP(4) were 16 nm (33%) vs 15 nm (28%) for CT DNA, and 21 nm (37%) vs 22 nm (28%) for $[poly(dGdC)]_2$ at $R = 0.05$ (R = [porphyrin]/[DNA base pairs]). Such spectral changes have been attributed to intercalation of TMpyP(4), and the similarity demonstrates the same binding mode for TMpyP(4) and TθpyP. In contrast, for $[poly(dAdT)]_2$ only small red shifts and small intensity changes characteristic of outside binding were observed: 9 nm (13%) for TθpyP vs 8 nm (−10%) for TMpyP(4). Thus, TθpyP and TMpyP(4) have the same binding selectivity.

Intercalative and outside binding modes of TMpyP(4)[17] have been associated with negative and positive induced Soret CD bands, respectively. These CD bands are most clearly observed with synthetic polymers: TθpyP vs TMpyP(4) (cf. Figure 2), $[\Theta]_{446} = -4.8 \times 10^4$ vs $[\Theta]_{442} = -4.0 \times 10^4$ deg·cm^2 dmol^{-1} for $[poly(dGdC)]_2$, and $[\Theta]_{442} = 9.9 \times 10^4$ vs $[\Theta]_{437} = 1.1 \times 10^5$ deg·cm^2 dmol^{-1} for $[poly[dAdT)]_2$. These very similar features further indicate essentially the same binding by TθpyP and TMpyP(4).

Although the similarities in the optical effects were compelling, the intercalation of the large TθpyP appeared to be so remarkable that we conducted viscometric studies. With CCS DNA (pBluescript, KS^+, Stratagene), we found the pronounced increase in SRV on uncoiling and decrease on recoiling characteristic of intercalation (Figure 3). The SRVs of CT DNA and $[poly(dGdC)]_2$ (not shown) were increased to ~1.2–1.3 by TθpyP at $R = 0.25$ and 0.5 M NaCl, whereas that of $[poly(dAdT)]_2$ decreased to ~0.8. For TMpyP(4) at $R = 0.25$, the increase with

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(18) Abbreviations used are as follows: TMpyP(4), *meso*-tetrakis(4-*N*-methylpyridyl)porphine; TMAP, *meso*-tetrakis(4-trimethylaniliniumyl)porphine; TθpyP, *meso*-tetrakis[4-*N*-(3-trimethylaminopropyl)pyridyl]porphine; TθOPP, *meso*-tetrakis[4-[(3-trimethylaminopropyl)oxy]phenyl]porphine; CT DNA, calf thymus DNA; $[poly(dGdC)]_2$, poly(dG-dC)·poly(dG-dC); $[poly(dAdT)]_2$, poly(dA-dT)·poly(dA-dT); PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); CCS DNA, closed circular supercoiled DNA; SRV, solution-reduced viscosity.

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(23) Hypochromicities: $H = [(A_0 - A_s)/A_0] \times 100\%$, where A_0 and A_s are the absorbances at the λ_{max} without and with DNA, respectively.

[poly(dGdC)]$_2$ was similar but the increase with CT DNA and decrease with [poly(dAdT)]$_2$ were somewhat smaller than for TθpyP. The viscosity results reinforce the conclusions that TθpyP and TMpyP(4) DNA binding modes are the same but suggest that the higher charge of TθpyP influences the hydrodynamic properties of the TθpyP-DNA adduct.

Although similar in size to TθpyP and identical in charge to TMpyP(4), TθOPP gave only a gradual *decrease* in SRV for CCS DNA (Figure 3) and for the linear polymers. Therefore, TθOPP does not intercalate. The TθOPP chromophore is rather different from any used in previous studies with DNA. However, with all three linear DNAs, a time-dependent formation of a large conservative CD signal was observed, and the Soret band was red shifted by 7 nm with 20–40% H. The largest CD signal was found with [poly(dAdT)]$_2$ (Figure 2, $[\theta]_{424} = -2 \times 10^6$ and $[\theta]_{436} = 1.3 \times 10^6$). This optical spectroscopic pattern for porphyrins, e.g., TMAP, has been attributed to highly organized outside self-stacking.[13,15] In contrast to previous studies, where the conservative CD curves are induced only in high salt,[13] TθOPP produces the same pattern in *both* high and low salt. Another difference is that the large CD signals often have been found primarily at a high R,[13] but we find such signals at low R (even at 0.001) with TθOPP. At low R values some self-stacking porphyrins become intercalators.[13] However, TθOPP does not intercalate because of the high electron density in the porphyrin core. Most porphyrins studied previously are rigid. The long flexible tentacle arms of TθOPP probably facilitate favorable outside self-stacking interactions while simultaneously permitting near-optimal electrostatic interaction with the DNA phosphate groups. In addition to extending the diversity of potential binding interactions between porphyrins and DNA, the results presented here provide clear evidence that porphyrin intercalation does not require planar traversing groups but does require an electron-deficient porphyrin core.

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The First Determination of Carbonyl Oxide Geometry. Effect of Ring Size on the Stereochemistry of Carbonyl Oxides from Ozonolysis of Cycloalkenes

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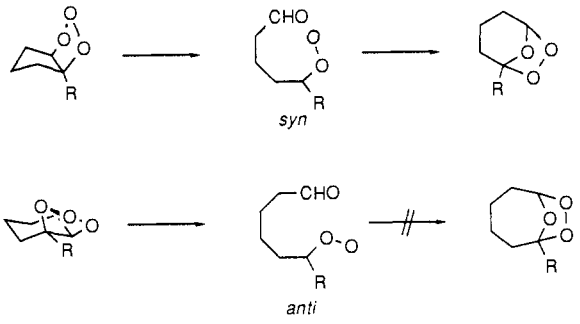
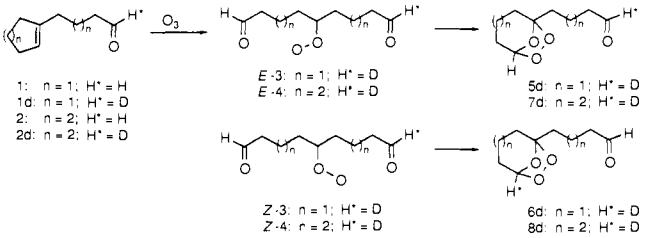
The stereochemistry of 1,2,4-trioxolanes (final ozonides) produced during ozonolysis depends on the geometry of the starting alkene, a result which requires that the intermediate carbonyl oxide is also formed with some stereospecificity.[1] Refinements of the basic Criegee mechanism, which incorporate carbonyl oxide stereoisomerism and can account for the overall stereochemistry of the ozonide formation, have been proposed,[2] but the predictions for carbonyl oxide geometry have not been verified experimentally. The problem is that the final ozonide stereochemistry depends on the combination of the geometry of the carbonyl oxide with the endo–exo topography of its cycloaddition with a carbonyl group. Therefore, deduction of the carbonyl oxide stereochemistry from that of the final ozonide requires knowledge of the particular

Scheme I



Scheme II



cycloaddition transition state, and vice versa.

It occurred to us that this ambiguity could be resolved in the case of intramolecular cycloaddition of a carbonyl oxide. Basically, intramolecular ozonide formation can occur only if the carbonyl oxide has the syn geometry with respect to the tethered carbonyl trap. If the carbonyl oxide stereochemistry is anti, a concerted, intramolecular cycloaddition is sterically impossible, provided that the tether is relatively short (two to four atoms). In this way, the extent of intramolecular trapping can be related to the carbonyl oxide geometry.[3] We now report our initial results, which indicate that intramolecular ozonide formation is indeed highly sensitive to carbonyl oxide stereochemistry.

We chose as a test system the ozonolysis of 1-alkylcyclopentenes and -cyclohexenes. It is well established that cyclopentenes form ozonides in fair to excellent yields (50–90%).[4] This implies that the carbonyl oxide is formed (largely) with a geometry syn to the cognate carbonyl group, so that intramolecular cyclization is facile. Consistent with this idea, semiempirical calculations indicate that the lowest-energy conformation for the primary ozonide of cyclopentene has the 1,2,3-trioxolane ring in an endo-folded envelope.[5] A least-motion fragmentation[2] from this structure would lead to the syn-oriented carbonyl oxide (Scheme I).

On the other hand, the conformation of the primary ozonide of a 1-alkylcyclohexene should be determined largely by the preference for a chair conformation for the six-membered ring with the alkyl substituent in an equatorial orientation. The fused trioxolane ring must then span vicinal axial and equatorial positions, and preferred fragmentation to place the carbonyl oxide on the alkyl-substituted carbon will produce the anti carbonyl oxide, which cannot cyclize intramolecularly. Indeed, cyclohexenes are notorious for producing only miniscule yields of monomeric ozonides, instead forming oligomeric peroxides by intermolecular reaction.[6] This is usually attributed to unfavorable entropy effects in the intramolecular cyclization of the carbonyl oxide–aldehyde pair from a cyclohexene. While that may be a contributing factor,

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(5) Semiempirical calculations with Dewar's Austin model 1 (AM1)[5a] or Stewart's parametric model 3 (PM3)[5b] parameter sets were carried out using the program Mopac:[5c] (a) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909. (b) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 221–264. (c) Stewart, J. J. P. QCPE 455, Vax/VMS Version 5.0.

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