the reaction of the analogous cuprous complex with dioxygen,[2] since the latter reactions give hydroxylation of the ligand. We are still unable at this time to distinguish between a reaction pathway in which the olefin reacts directly with an iodosylbenzene complex as opposed to one in which iodobenzene dissociates and the olefin traps a reactive high-valent oxo intermediate. However, we are able to conclude that the binuclear nature of complexes **1-3** plays an important role in determining the reactivity of the active species. Future studies will include attempts to observe and isolate intermediates in these reactions and to determine the relationship of our results to oxygenation reactions catalyzed by copper enzymes with binuclear active sites.

Acknowledgment. We are grateful to Professor Stephen J. Lippard of MIT for providing a sample of the macrocycle used in **3**. Financial support from the National Science Foundation is also gratefully acknowledged.

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Sequence-Specific Chiral Recognition of Right-Handed Double-Helical DNA by (2*S*,3*S*)- and (2*R*,3*R*)-Dihydroxybis(netropsin)succinamide

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We report a class of chiral sequence-specific DNA binding molecules[1,2] based on two netropsin analogues and the enantiomers of *dl*-tartaric acid: (2*R*,3*R*)- and (2*S*,3*S*)-dihydroxybis(netropsin)succinamide-EDTA [(*R*,*R*)- and (*S*,*S*)-**1**, respectively] (Figures 1 and 2).[3] The enantiomeric *threo*-(*R*,*R*)-**1** and -(*S*,*S*)-**1** should afford diastereomeric complexes at common binding sites on double-helical DNA (Figure 1). Attachment of EDTA to one terminus of the crescent-shaped hexamides allows use of the affinity cleaving method[4,5] to determine the differences between (*R*,*R*)-**1**·Fe^{II} and (*S*,*S*)-**1**·Fe^{II} with regard to relative binding affinities, sequence specificities, and orientations by analysis of the DNA-cleavage patterns on a ^{32}P end-labeled DNA restriction fragment using high-resolution gel electrophoresis.[6] For comparison to the enantiomers of **1**, bis(netropsin)succinamide-EDTA

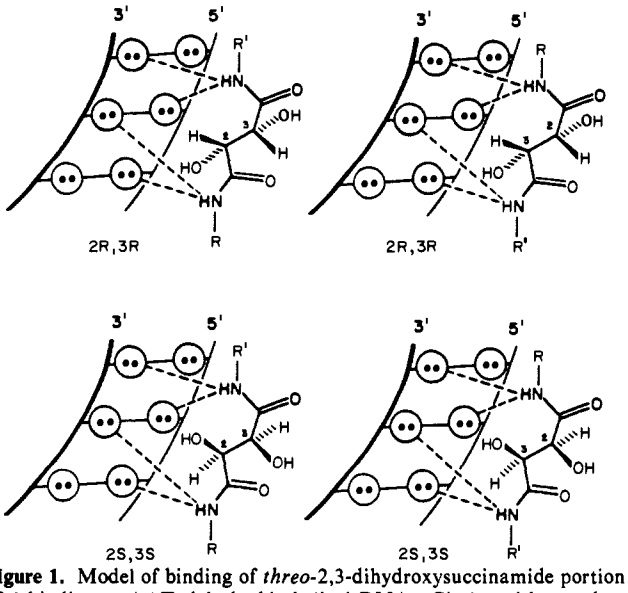

Figure 1. Model of binding of *threo*-2,3-dihydroxysuccinamide portion of **1** binding to A+T-rich double-helical DNA. Circles with two dots represent lone pairs of electrons of N3 of adenine (A) and O2 of thymine (T) at the edges of the base pairs on the floor of the minor groove of the right-handed B DNA helix. (Top) Two diastereomeric binding orientations of (*R*,*R*)-**1** on A+T-rich double-helical DNA. (Bottom) Two diastereomeric binding orientations of (*S*,*S*)-**1** on A+T-rich double-helical DNA.

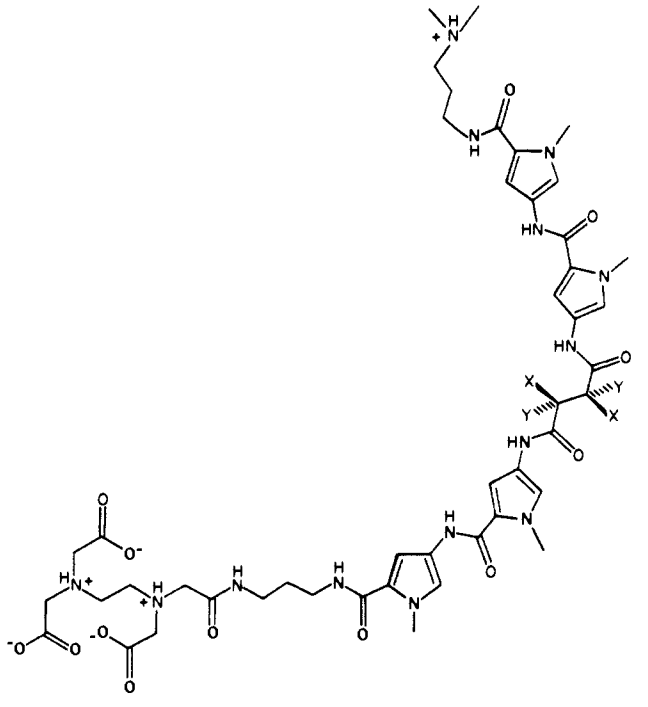

Figure 2. For (*S*,*S*)-**1**: X = OH, Y = H. For (*R*,*R*)-**1**: X = H, Y = OH. For **2**: X = H, Y = H.

(**2**) was synthesized (Figure 2).

A 517-base-pair restriction fragment from plasmid pBR322 DNA, labeled with ^{32}P on the 3' end,[4b,5] was allowed to react with a 100-fold range of concentrations of (*R*,*R*)-**1**·Fe^{II}, (*S*,*S*)-**1**·Fe^{II}, and **2**·Fe^{II} under the same conditions (5 mM dithiothreitol, 5mM NaOAc, pH 7.9, 1.5 h, 37 °C). The DNA cleavage sites were visualized on a DNA sequencing gel (Figure 3). Densitometric analysis of the bottom half of the autoradiogram reveals cleavage flanking two sites, seven-base-pairs in size, 5'-TTTTTAT-3' and 5'-TAATAAT-3', for all three molecules (Figure 4). The binding locations, site sizes, and orientation preferences for the three compounds are similar. However, the *concentrations* of (*R*,*R*)-**1**, (*S*,*S*)-**1**, and **2** required to achieve comparable cleavage efficiency at these seven-base-pair A·T-rich sites vary by *2 orders of mag-*

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(3) (*R*,*R*)-**1** was synthesized from optically pure (>99%) (2*R*,3*R*)-dihydroxysuccinic acid (L-tartaric acid) obtained from Aldrich $[\alpha]^{D}_{23}$ +15.6° (*c* 20, H_2O). (*S*,*S*)-**1** was synthesized from optically pure (99%) (2*S*,3*S*)-dihydroxysuccinic acid (D-tartaric acid) obtained from Aldrich $[\alpha]^{D}_{23}$ -15.2° (*c* 20, H_2O). The NMR, IR, UV, and mass spectral data are consistent with the structural assignments. Synthetic details will be published elsewhere.

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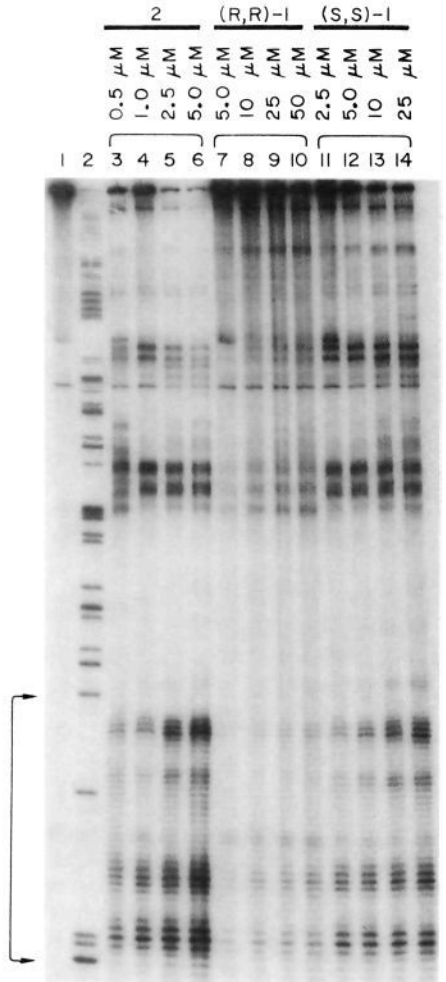

Figure 3. Autoradiogram of ^{32}P end-labeled DNA restriction fragment labeled at the 3' end, 517 bp in length (*EcoRI*/*RsaI*) from plasmid pBR322 (base paris 3848-0002) on a high-resolution denaturing polyacrylamide gel. Lane 1, intact DNA; lane 2, Maxam–Gilbert chemical sequencing G reaction; lanes 3–6, bis(netropsin)succinamide–EDTA·Fe^{II} (**2**·Fe^{II}) at 0.5–5.0 μM concentrations; lanes 7–10 (*R*,*R*)-**1**·Fe^{II} at 5.0–50 μM concentrations, (*S*,*S*)-**1**·Fe^{II} at 2.5–25 μM concentrations. Bracket on lower left side of the gel is sequence left to right in Figure 4.

nitude. Bis(netropsin)succinamide–EDTA·Fe^{II} (**2**·Fe^{II}) cleaves DNA (100 μM in base pairs) efficiently at concentrations of 0.5 μM. (*S*,*S*)-**1**·Fe^{II} must be present at 5.0 μM concentration to afford the same amount of cleavage while (*R*,*R*)-**1**·Fe^{II} is less efficient even at 50 μM concentration.

According to the "$n + 1$ rule", hexamides in the minor groove should bind seven base pairs.[5] Observation of seven-base-pair binding in the absence of four-base-pair binding suggests that the dipeptide moieties for **1** and **2** bind simultaneously.[6] The fact that **1** must be present in at least 10-fold greater concentration than **2** in order to achieve equal cleavage efficiency demonstrates an overall decrease in binding affinity upon introduction of the threo-hydroxy groups. Since *threo*-dihydroxysuccinamide is bulkier than succinamide, reduced binding affinities of **1** might be explained on steric grounds in light of an X-ray crystallographic study which shows that the minor groove of a B-form DNA dodecamer is forced open by 0.5–2.0 Å upon binding of netropsin.[8] The observation that (*S*,*S*)-**1** cleaves DNA sequence specifically at concentrations one-tenth that required for its mirror image

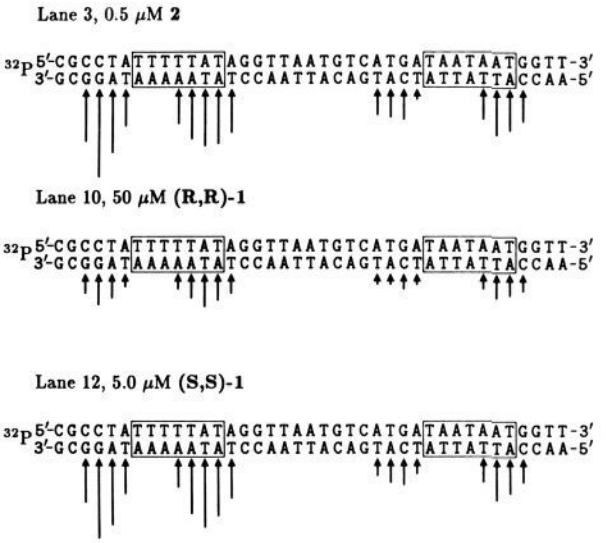

Figure 4. Histograms of common cleavage patterns for (*R*,*R*)-**1**, (*S*,*S*)-**1**, and **2** on 517 bp DNA restriction fragment (base pairs 4334–4296 on plasmid pBR322) from high-resolution denaturing gel (Figure 3). Arrows represent amount of cleavage resulting in removal of indicated base. Boxes define binding site location and size based on model described in ref 4 and 5.

(*R*,*R*)-**1** at identical locations demonstrates differences in stabilizing and/or destabilizing binding interactions in the minor groove for the diasteromeric complexes, (*R*,*R*)-**1**/DNA vs. (*S*,*S*)-**1**/DNA.[7]

One reason for the enantiomeric selectivity could be differential destabilization of the (2*R*,3*R*)- and (2*S*,3*S*)-dihydroxysuccinamides from their lowest energy conformations upon binding to DNA. Crystal structures of tartaric acid derivatives reveal that the *S*,*S* enantiomer is twisted in a right-handed sense, and the *R*,*R* enantiomer is twisted in a left-handed sense.[9] If the conformations which give rise to this handedness persist in solution,[10] then our data suggests that the right-handed twist of (*S*,*S*)-**1** better matches the minor groove of right-handed DNA than does the left-handed twist of the enantiomer, (*R*,*R*)-**1**.

This result bears on the general issue of helical screw sense recognition patterns for DNA complexation. For protein/DNA binding, model building based on crystal structures of proteins that regulate gene expression suggests that sequence specific recognition is mediated by a right-handed polypeptide α-helix in the major groove of right-handed B-DNA.[13] With regard to small molecules binding in the major groove of DNA, Barton and coworkers have found that for intercalation by $(phen)_3Ru^{2+}$ the right-handed isomer,[2a,b] which has the same helical screw sense as right-handed B DNA, is preferred, while metalation by $(phen)_2RuCl_2$ seems to require the left-handed configuration, a structure complementary to the B DNA helix.[2c] The work reported here for a chiral minor groove binder appears to be an example of similar helical screw sense recognition.

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Registry No. (*R*,*R*)-**1**, 103150-36-3; (*S*,*S*)-**1**, 103150-37-4; **2**, 103150-38-5; netropsin, 1438-30-8.

(7) In a formal sense, the iron-bound EDTA moiety is a stereogenic center, but for the purposes of this discussion, this aspect is assumed to be unimportant with regard to the enantiomeric discrimination due to presumed lack of intimate association of this moiety with the DNA helix.

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(10) NMR[11] and VCD[12] data suggests that the conformations observed in the solid state are the most important in solution (aqueous[11] and CCl_4[12]) and may be stabilized by intramolecular five-membered ring hydrogen bonds between the amide carbonyls and adjacent hydroxyl groups.

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