are the resonances of the carbon atoms directly bonded to nitrogen. The two aromatic resonances at δ 138.43 and 138.32 (each with an integration of 2) are broadened and are attributable to the starred 5- and 6-membered-ring carbon atoms (see **2**, structure). Efforts to clarify the structure of **A**[8] are underway.

We have shown above that azafulleroids and not fullerene aziridines are formed as the ultimate product of addition of a number of organic azides to C_{60}. A combination of ^{15}N and ^{13}C NMR was employed to elucidate the structure. The azafulleroids are more electronegative than their carbon analogs but not as electronegative as unsubstituted C_{60}, as determined by cyclic voltammetry.

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Supplementary Material Available: Spectroscopic data (IR, UV–vis, FAB-MS, and ^1H NMR) for azide **1a** and azafulleroids **2a–d**, ^{13}C NMR data for **2a**, and a table of cyclic voltammetry data (4 pages). Ordering information is given on any current masthead page.

(8) The ^{13}C NMR spectrum shows a resonance as low as 159.8 ppm coupled to ^{15}N which is clearly incompatible with a triazoline, the structure which was expected from the normal mode of 1,3-dipolar addition of an alkyl azide with C_{60}.

Sequence-Specific DNA Binding by a Geometrically Constrained Peptide Dimer

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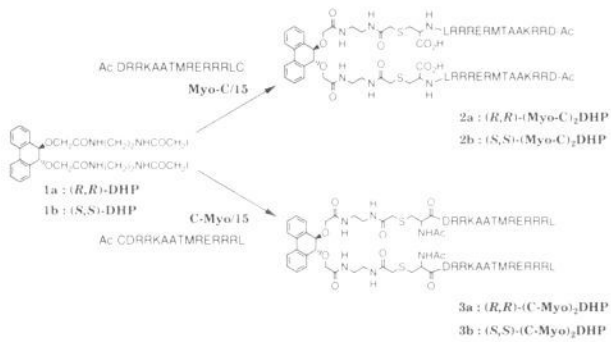
Recent structural and functional analyses of eukaryotic transcriptional regulatory proteins have indicated that the sequence-specific DNA recognition activities lie in the structural motifs containing a relatively small number of amino acid residues, such as helix–turn–helix,[1-3] leucine zipper,[4-6] at least three types of zinc fingers,[7,8] and helix–loop–helix (HLH).[9,10] At the same time, these studies have revealed a common feature of the sequence-specific DNA-binding proteins. That is, many DNA-binding proteins bind DNA as dimers. In the native dimeric proteins, the chemical structure of the dimerization motif determines the geometry of each monomer subunit. This constrained positioning of the DNA-binding regions would facilitate the direct interaction between amino acid residues of the protein and the nucleic acid base pairs.

Scheme I



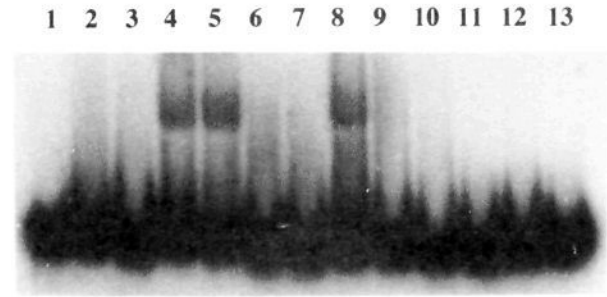


Figure 1. DNA binding of dimeric peptide models to the oligonucleotide containing the MyoD binding sequence. Gel mobility shift assays (14) indicate that (R,R)-(Myo-C)$_2$DHP shows an affinity to MCK probe. Lane 1, no peptides; lanes 2, 6, and 10, 2.5 μM (R,R)-(C-Myo)$_2$DHP; lanes 3, 7, and 11, 2.5 μM (S,S)-(C-Myo)$_2$DHP; lanes 4, 8, and 12, 2.5 μM (R,R)-(Myo-C)$_2$DHP; lanes 5, 9, and 13, 2.5 μM (S,S)-(Myo-C)$_2$DHP. The concentration of the dimeric peptide was determined with an extinction coefficient at 270 nm (ϵ_{270}) of 17 000 M^{-1} cm^{-1}. Nonspecific competitor DNA (calf thymus DNA) was added to the binding mixture such that the final concentration is 20 (lanes 1–5) or 40 μM (lanes 6–9). A specific competitor (non-radiolabeled MCK25) was added to the binding mixture to a final concentration of 500 nM (duplex) (lanes 10–13). Binding solutions contained in 10 μL: 20 mM Tris (pH 7.6), 25 mM NaCl, 10 000 cpm ($\sim$0.01 pmol) 5′-^{32}P-labeled MCK25 (double stranded), and 2.5 μM dimeric peptides when present. Non-radiolabeled duplex MCK25 was added where indicated. The binding mixtures were incubated at 4 °C for 30 min. After addition of 1 μL of 40% glycerol, the mixtures were loaded onto 10% PAGE gel (29:1 acrylamide/bisacrylamide), run in TAE buffer (7 mM Tris, 3 mM sodium acetate, and 1 mM EDTA) at 4 °C, and analyzed by autoradiography.

We describe a system to represent such constrained arrangements of DNA-binding motifs by using enantiomeric and C_2-symmetric templates derived from 9,10-dihydrophenanthrene-9,10-diol ((R,R)-DHP and (S,S)-DHP). In the simplest case, there are four constraints possible for dimeric peptide motifs, right-handed or left-handed arrangement of each peptide, and two orientations for the polarity of the peptides (an N-terminus to N-terminus or C-terminus to C-terminus arrangement). Four differently constrained dimeric peptides are synthesized by using oligopeptides derived from the HLH protein, MyoD. It is shown that a dimer with right-handed and C-terminus to C-terminus arrangements of the peptide binds specifically to the native MyoD binding site.

The chiral templates (R,R)- and (S,S)-DHP were synthesized from enantiomers of *trans*-9,10-dihydrophenanthrene-9,10-diol with well-defined absolute configurations at C-9 and C-10.[11] The $(9R,10R)$ isomer was used to achieve the right-handed geometry

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of peptide dimers, and the (9S,10S) isomer was used to obtain the left-handed geometry. We have used a 14-amino acid sequence for a DNA-binding peptide, which is derived from the basic region on the N-terminal side of the helix-loop-helix region of MyoD. Two oligopeptides possessing a unique cysteine residue either at the N-terminus (C-Myo/15) or C-terminus (Myo-C/15) of the 14-amino acid sequence were synthesized by Fmoc chemistry.[12] The 15-mer peptide C-Myo/15 was used to achieve the N-terminus to N-terminus arrangement and Myo-C/15 for the C-terminus to C-terminus arrangement. The peptides were covalently attached to enantiomerically pure templates (*R*,*R*)- and (*S*,*S*)-DHP through a specific reaction of the iodoacetyl group with the unique SH group of the peptides.[13] Subsequent purification with gel filtration and reversed-phase HPLC yielded four types of dimeric peptide models: (*R*,*R*)- and (*S*,*S*)-(C-Myo)$_2$DHP and (*R*,*R*)- and (*S*,*S*)-(Myo-C)$_2$DHP (Scheme I).

Sequence-specific DNA binding of these four dimeric peptides was tested by gel mobility shift assays,[14] using a 25-bp ^{32}P-end-labeled oligonucleotide (MCK25) containing the native MyoD binding sequence.[15] Both (*R*,*R*)-(Myo-C)$_2$DHP and (*S*,*S*)-(Myo-C)$_2$DHP at the concentration 2.5 μM afford mobility-shifted DNA bands in 20 mM Tris-HCl/25 mM NaCl buffer (pH 7.6) at 4 °C (Figure 1, lanes 4 and 5). However, only (*R*,*R*)-(Myo-C)$_2$DHP binds to the ^{32}P-end-labeled probe upon increasing the concentration of the nonspecific competitor calf thymus DNA (Figure 1, lanes 4 and 8). Furthermore, the distinct retardation band disappears due to competition with the unlabeled MCK25 probe in the binding mixture of (*R*,*R*)-(Myo-C)$_2$DHP, suggesting the formation of a specific peptide-DNA complex (lane 12 of Figure 1).[16] No retardation band was detected for monomeric peptides bearing the chiral template with the MCK25 probe even at 8-fold excess concentration of the (*R*,*R*)-(Myo-C)$_2$DHP.[17]

In summary, the restricted C-terminus to C-terminus arrangement is necessary, and the right-handed geometry of each peptide favors the sequence-specific binding in the dimeric peptide derived from the basic region of MyoD.[18,19] Dimer formation of the basic region is necessary for the sequence specific binding.[9,10] These results indicate that the DNA-binding activity of MyoD lies entirely in the basic region of the HLH motif. The C_2-symmetric template described in this work can provide not only a convenient way to restrict the relative orientation of peptides favoring the dimer formation but also has the potential to be used for the design and study of sequence-specific DNA-binding peptides.

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(15) A 25-bp oligonucleotide derived from muscle creatine kinase (MCK) enhancer sequence is 5'-GATCCCCCCAA*CACCTG*CTGCCTGA-3' and its complementary strand (ref 9a).

(16) The binding mixture of (*R*,*R*)-(Myo-C)$_2$DHP containing a ^{32}P-end-labeled oligonucleotide without bearing MCK enhancer sequence showed no retardation band under the same conditions. (*R*,*R*)-(Myo-C)$_2$DHP shows sequence-specific protection at the MyoD binding sequence from the DNase I digestion (see the supplementary material). As measured by titration of the gel shift, (*R*,*R*)-(Myo-C)$_2$DHP binds MCK25 with a dissociation constant of $\sim 6 \times 10^{-7}$ M at 4 °C. MyoD homodimer binds to the same sequence with a dissociation constant of 1.6×10^{-14} M^2 (see ref 10c).

(17) A binding mixture of the monomeric 15-mer peptides Myo-C/15 and C-Myo/15 even at 50 μM concentration did not afford any mobility-shifted DNA band that indicates the formation of a specific binding complex with MCK25.

(18) Disulfide-bonded peptide dimer (C-Myo/15)$_2$ or (Myo-C/15)$_2$ showed no distinct mobility-shifted band under the same conditions. The observed weak binding of (*S*,*S*)-(Myo-C)$_2$DHP indicates that the flexible tethers would not completely overcome the constraints of the chirality of the attachment points of the peptides. (*R*,*R*)-(Myo-C)$_2$DHP at 2.5 μM binds to the same probe in 100 mM NaCl, however, no mobility-shifted DNA band was observed in the binding mixture of (*S*,*S*)-(Myo-C)$_2$DHP under the same conditions.

(19) Our result on the geometry of basic regions in the dimeric form of MyoD is consistent with the recent proposals for the dimer structures of MyoD (ref 10d); however, we do not have data available to speculate on the topology of the helix-loop-helix region.

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Supplementary Material Available: Listings of experimental data including autoradiograms of DNase I footprinting of dimeric peptide models and gel mobility shift assays of the monomeric peptides (16 pages). Ordering information is given on any current masthead page.

Highly Selective and Operationally Simple Synthesis of Enantiomerically Pure β-Amino Esters via Double Stereodifferentiation

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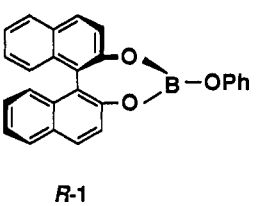
Largely stimulated by the synthesis of β-lactam antibiotics, there have been several investigations into the stereochemical aspects of imine condensations, especially those exhibiting diastereofacial selectivity with an imine containing a chiral auxiliary.[1-8] Initial efforts used α-methylbenzylamine as the auxiliary, but the resulting enantiomeric excesses were moderate (33-78% de).[2,3] Other auxiliaries are more efficient for diastereoselective condensation reactions; however, there are certain practical difficulties in removing or preparing these auxiliaries.[4-8] Described herein are the results of our initial investigation that demonstrates high enantioselectivities with α-methylbenzylamine as the auxiliary by using double stereodifferentiation.

The chiral boron reagent **1** (or its enantiomer)[9] was used to convert imines to β-amino esters. The reagent, derived from equimolar amounts of (*R*)- or (*S*)-binaphthol and triphenyl borate (formed in 1 h at room temperature in CH_2Cl_2), promoted smooth condensation of the imine **2a** and the ketene acetal at −78 °C over 8 h to afford the β-amino ester **3a** in good yield after aqueous workup. The binaphthol was efficiently recovered for reuse.[10] The reaction with (*R*)-**1** produced the (*R*) adduct **3a**[11] in >92% de, whereas the reaction with (*S*)-**1** gave the adduct in 74% de. In a similar way, aliphatic imine **2b** can be converted to the β-amino ester **3b**[11] with 94% de by using (*R*)-**1** (Scheme I).



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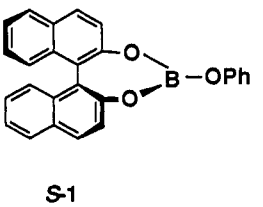

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