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.

Acknowledgment. We thank J. K. Barton, P. S. Kim, and R. Darkow for advice and discussions. We are grateful to the Japan Society for the Promotion of Science for Japanese Junior Scientists for a fellowship to T.M.

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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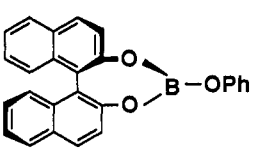
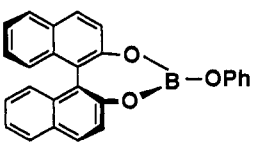
Largely stimulated by the synthesis of β-lactam antibiotics, there have been several investigations into the stereochemical aspects of imine condensations, especially those exhibiting diastereofacial selectivity with an imine containing a chiral auxiliary.[1-8] Initial efforts used α-methylbenzylamine 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).



R-1



S-1

(1)

(12) Atherton, E.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1985**, 165. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; K, Lys; L, Leu; M, Met; R, Arg; and T, Thr.

(13) Bridgen, J. *Biochem. J.* **1972**, *126*, 21.

(14) (a) Gaener, M. M.; Revzin, A. *Nucleic Acids Res.* **1981**, *9*, 3047. (b) Freid, M.; Crothers, D. M. *Nucleic Acids Res.* **1981**, *9*, 6505.

(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.

(1) Review: (a) Kleinnman, E. F. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Heathcock, C. H., Eds.; Pergamon Press: New York, 1991; Vol. 2, Chapter 4.1. (b) Hart, D. J.; Ha, D.-C. *Chem. Rev.* **1989**, *89*, 1447.

(2) Furukawa, N.; Okawara, T.; Noguchi, H.; Terawaki, Y. *Heterocycles* **1977**, *6*, 1323.

(3) Ojima, I.; Inaba, S.-I. *Tetrahedron Lett.* **1980**, 2077. For a similar condensation with a thioester tin enolate, see: Yamada, T.; Suzuki, H.; Mukaiyama, T. *Chem. Lett.* **1987**, 293.

(4) Ojima, I.; Inaba, S.-I. *Tetrahedron Lett.* **1980**, 2081.

(5) More recently, Ojima reported a lithium enolate-imine condensation with optically active 2-phenylcyclohexanol as a chiral control unit. See: Ojima, I.; Habus, I.; Zhao, M. *J. Org. Chem.* **1991**, *56*, 1681 and references cited therein.

(6) Waldmann, H.; Braun, M.; Draeger, M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1468. Waldmann, H.; Braun, M. *J. Org. Chem.* **1992**, *57*, 444.

(7) Pfrengle, W.; Kunz, H. *J. Org. Chem.* **1989**, *54*, 4261.

(8) Midland, M. M.; Koops, R. W. *J. Org. Chem.* **1992**, *57*, 1158.

(9) Reagent **1** has been employed previously as a mediator of an asymmetric hetero-Diels-Alder reaction: Yamamoto, H.; Hattori, K. *J. Org. Chem.* **1992**, *57*, 3264.

(10) Binaphthol can be recovered in >95% yield.

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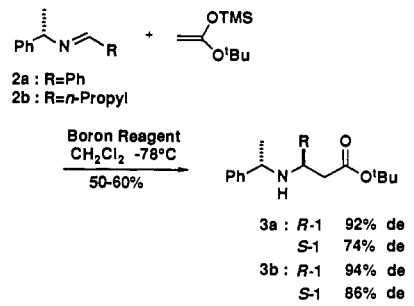
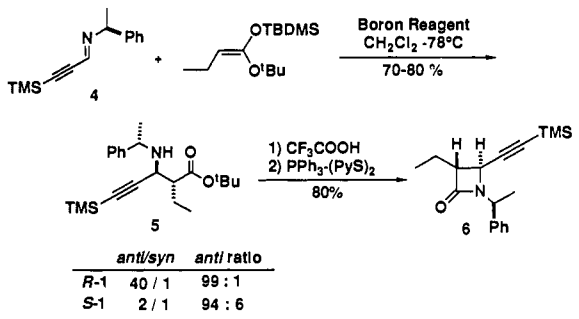
Scheme I



Scheme II



An even more dramatic difference in product ratios was observed in the synthesis of the key intermediate for (+)PS-5, a carbapenem antibiotic active against Gram-positive and Gram-negative bacteria. Acetylenic imine **4** and silyl ketene acetal were exposed to the above conditions (Scheme II). The reaction with (*R*)-**1** as the Lewis acid catalyst produced the aldol adduct **5** with extremely high anti selectivity (anti/syn = 40/1) and diastereoselectivity (97% anti de); **5** was converted to the β-lactam **6** using Ohno's method.[12,13]

The present methodology provided a stereospecific synthesis of the side chain of taxol. The *N*-benzoyl-3-phenylisoserine side chain at C-13 of the taxol molecule is essential for the antitumor activity of taxol.[14] It is noteworthy that the stereoselectivity in this reaction depends on the geometry of the silyl ketene acetal. The reaction of the (*E*)-ketene acetal[15] with (*R*)-**1** produced the anti adduct **7** with high stereoselectivity (anti/syn = 98/2, 92% anti de). In contrast, the reaction of the (*Z*)-ketene acetal[15] with (*S*)-**1** produced the enantiomerically pure syn adduct **9** (syn/anti > 99/1, >99% syn de). Our methodology thus provides the first practical and efficient route for the preparation of both diastereomers of an α-hydroxy β-amino ester. The syn adduct **9** was transformed into the desired *N*-benzoyl-(2*R*,3*S*)-phenylisoserine methyl ester **11**, $[\alpha]_D$ −48° (*c* 0.80, methanol) [lit.[14c] $[\alpha]_D$ −48° (*c* 1.0, methanol)], by hydrogenolysis over a palladium catalyst followed by a Schotten–Baumann reaction (Scheme III).

The diastereoselectivity exhibited here is closely related to the assigned geometry of the intermediate silyl ketene acetals and the

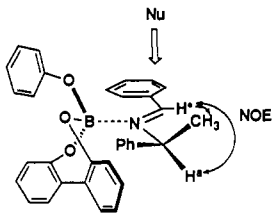
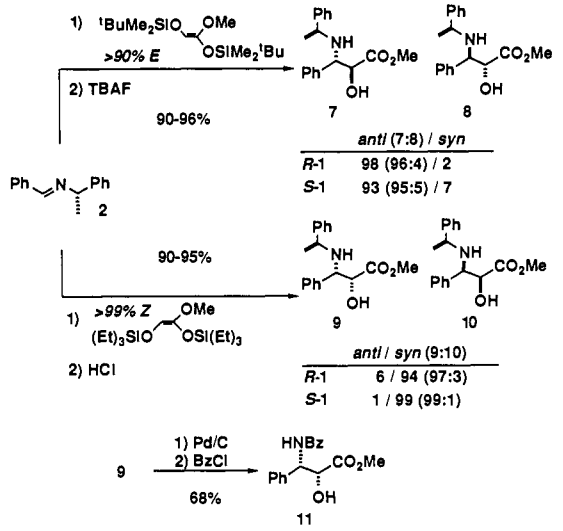


Figure 1. Complex of (*R*)-**1** and **2**.

Scheme III



expected structure of the acyclic transition state.[16] The absolute configuration of the adducts agrees with predictions based on Figure 1. The nucleophile would approach the *re* face of the (*E*)-imine **2**–(*R*)-**1** complex. In a 2D NOESY study of the **2**–(*R*)-**1** complex, an NOE of proton H* was observed with H^a and CH_3; thus, the boron reagent would effectively block the *si* face of the imine.[17] The preferred (*E*) geometry of the imine **2** could clearly be demonstrated by ^1H NMR analysis of the complex of the imine and the chiral Lewis acid **1**.[18] On the other hand, (*E*)-imine **4**, under the influence of the Lewis acid **1**, was gradually rearranged to (*Z*), which could be the actual intermediate in the synthesis of **5**. This would be consistent with the stereochemical results.

Under optimum conditions with a matched pair of reagents, the present method provides almost complete stereocontrol for a variety of aldimine–silyl ketene acetal condensations. The chiral binaphthol ligand is commercially available and easily recovered upon workup, thereby making the method especially attractive for large-scale synthesis.

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Supplementary Material Available: A listing of data on β-amino esters and a general experimental procedure (2 pages). Ordering information is given on any current masthead page.

(11) The absolute configuration was ascertained by converting **3a** to the known 4(*R*)-phenyl-2-azetidinone (Pietsch, H. *Tetrahedron Lett.* **1972**, *27*, 2789) and **3b** to the known 3(*S*)-aminohexanoic acid (Balenovic, S. *Croat. Chim. Acta* **1957**, *29*, 153).

(12) Kobayashi, S.; Iimori, T.; Izawa, T.; Ohno, M. *J. Am. Chem. Soc.* **1981**, *103*, 2406.

(13) Shibasaki synthesized **6** from the imine **4** with a boron enolate and succeeded in transforming it to (+)PS-5 with moderate stereoselectivity. Shibasaki, M.; Ishida, Y.; Iwasaki, G.; Iimori, T. *J. Org. Chem.* **1987**, *52*, 3489.

(14) For the recent synthesis of the side chain of taxol, see: (a) Deng, L.; Jacobsen, E. N. *J. Org. Chem.* **1992**, *57*, 4320. (b) Georg, G. I.; Mashava, P. M.; Akgün, E.; Milstead, M. W. *Tetrahedron Lett.* **1991**, *32*, 3151. (c) Denis, J.-N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1990**, *55*, 1957. (d) Ref 5 and references cited therein.

(15) (*E*)-Ketene acetal can be synthesized >90% pure using 2,2,6,6-tetramethylpiperidine and *n*-BuLi as the base, and (*Z*)-ketene acetal can be synthesized >99% pure using 1,1,1,3,3,3-hexamethyldisilazane and *n*-BuLi. Both reactants were treated with the corresponding α-silyloxy ester at −100 °C in THF. The details of this process will be published in due course.

(16) Colvin, E. W.; Mcgarry, D.; Nugent, M. J. *Tetrahedron* **1988**, *44*, 4157.

(17) The imine proton H* was strongly shielded by the aromatic ring of the boron reagent. Thus, the NMR spectrum of the imine **2** indicated that this proton, originally appearing at δ 8.44, gives rise to a signal δ 8.04 when exposed to (*R*)-**1**.

(18) (*E*,*Z*) geometry of imines was determined by NOE experiments and the ratio of *E*/*Z* by ^1H NMR in CD_2Cl_2 at −60 °C.

	equiv of (*R*)-**1**				
	0	0.3	0.7	1.0	2.0
imine **2**	1/0	1/0	1/0	1/0	1/0
imine **4**	2/1	1.5/1	0.7/1	0.4/1	0/1