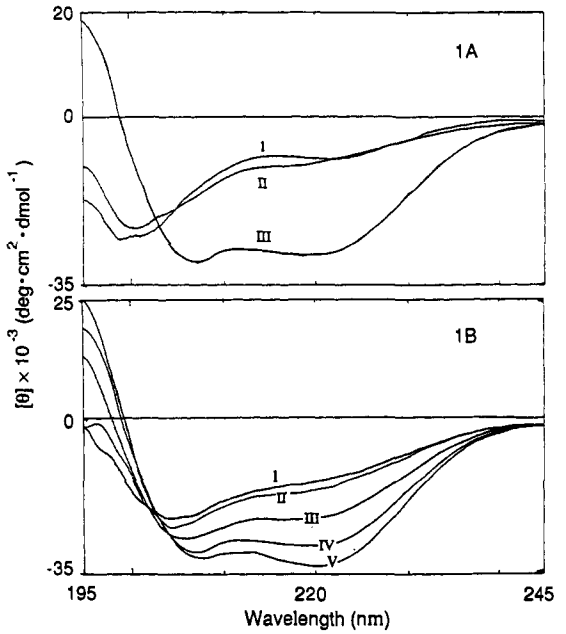

Figure 1. (A) Peptide **5** (20 μM) in H_2O (0.1% TFA) at 20 °C: I, Acm-protected; II, reduced with mercaptoethanol; III, oxidized with iodine. (B) Oxidized peptide **5** (20 μM) in H_2O (0.1% TFA): I, 80 °C; II, 60 °C; III, 40 °C; IV, 20 °C; V, 0 °C.

by reversed-phase C_{18} HPLC.[12-14] The thiol groups were then deprotected and oxidized with excess iodine in 1:1 trifluoroethanol/water, and the products were isolated by HPLC.[15]

The α-helicity of both the Acm-protected and oxidized forms of each peptide[16] was determined in water containing 0.1% trifluoroacetic acid at 0 °C by measuring ellipticity at 222 nm [the CD spectra of the reduced peptides are comparable to those of the Acm-protected peptides (Figure 1A)]. All peptides show a large increase in α-helicity upon oxidation (Table I). *Even the eight amino acid peptide* ***3***, which shows negligible helicity in the Acm-protected form, could be locked into a helical conformation using this approach. Moreover, the fact that both 16 amino acid peptides **2** and **5** show high helical content at 0 °C in their oxidized forms suggests that the disulfide bridge not only locks two turns of the helix but also efficiently propagates the helix to neighboring residues.

All four oxidized peptides retain a significant degree of α-helical character at 60 °C, whereas the Acm-protected forms are largely random coil (Table I). The temperature dependence on α-helicity was measured for peptide **5** between 0 and 80 °C (Figure 1B). A plot of $[\theta]_{222nm}$ versus temperature reveals low cooperativity in the melting behavior. This behavior, in contrast to that of typical α-helices, suggests that, upon heating, the ends of the peptide relax to a random-coil conformation while the residues within the disulfide bridge retain at least partial helicity.

In order to test the necessity of using the D enantiomer of amino acid **1** in the first position of the peptide, the corresponding L,L and L,D analogues of peptide **3** were synthesized and oxidized. The L,L analogue of **3** shows a marginal increase in helicity in the oxidized form (31% vs 11% for the oxidized and protected forms, respectively, at 0 °C) while the oxidized L,D analogue resembles a random coil/β sheet conformation.

These results demonstrate the utility of using intramolecular disulfides to stabilize helical conformations in short peptides. Disulfide bond formation can be carried out under a variety of conditions with peptides containing a wide variety of functional groups. We are currently using 2-D NMR techniques to more accurately define the structure of these peptides as well as applying this strategy to the design of α-helical peptide ligands for proteins and DNA.

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(12) Peptides were synthesized with an ABI 431A automated synthesizer on FMOC-aminomethyl Rink resin using HBTU FMOC-amino acid activation in the presence of HOBT and diisopropylethylamine.[13] Cleavage and deprotection were accomplished with reagent K.[16] Crude peptide (80–92% purity) was purified to >98% purity by reversed-phase C_{18} HPLC (CH_3CN/H_2O/0.1% TFA). Purity and structure were confirmed by electrospray ionization mass spectrometry.

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(15) The Acm-protected peptides were dissolved in 1:1 trifluoroethanol/water (5–10 mM), and 5 molar equiv of iodine was added via addition of a 1 M stock solution in methanol. The mixture was stirred for 2 h at room temperature and concentrated. The residue was dissolved in water (0.1% TFA) and purified by reversed-phase C_{18} HPLC (CH_3CN/H_2O/0.1% TFA). Structures were confirmed by electrospray ionization mass spectrometry.

(16) Helicity was independent of peptide concentration over the range 5–50 μM.

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Conformation and Stereoselective Reduction of Hapten Side Chains in the Antibody Combining Site

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We have reported multinuclear NMR analyses of the mode of interactions of ε-dansyl-L-lysine (**1**) with an anti-dansyl monoclonal antibody (**2**) raised against dansyl keyhole limpet hemocyanin.[1,2] Table I summarizes the association constants for antibody **2** with a variety of dansyl haptens with different lengths of the side chain. This result indicates that the side chain attached to the dansyl ring is recognized by the *hydrophobic subsite*[3] of antibody **2** and significantly contributes to the antigen binding. A transferred nuclear Overhauser effect (TRNOE) measurement[4] has confirmed that the lysine side chain is folded when compound **1** is bound to antibody **2**. On the basis of these results, attempts were made to use the *hydrophobic subsite* of antibody **2** as a chiral auxiliary for stereoselective organic reactions. Here we describe the result of reduction by $NaBH_4$ of 5-(dansylamino)levulinic acid (**3**) in the presence of antibody **2**. Compound **3** binds to antibody **2** with an association constant of 4.5×10^7 M^{-1}. TRNOE data have shown that the side chain of compound **3** is also folded in antibody **2**. As shown in Chart I, the reduction product was 5-[(dansylamino)methyl]-2-oxotetrahydrofuran (**5**). The *R* and *S* enantiomers of compound **5** were separated and identified on

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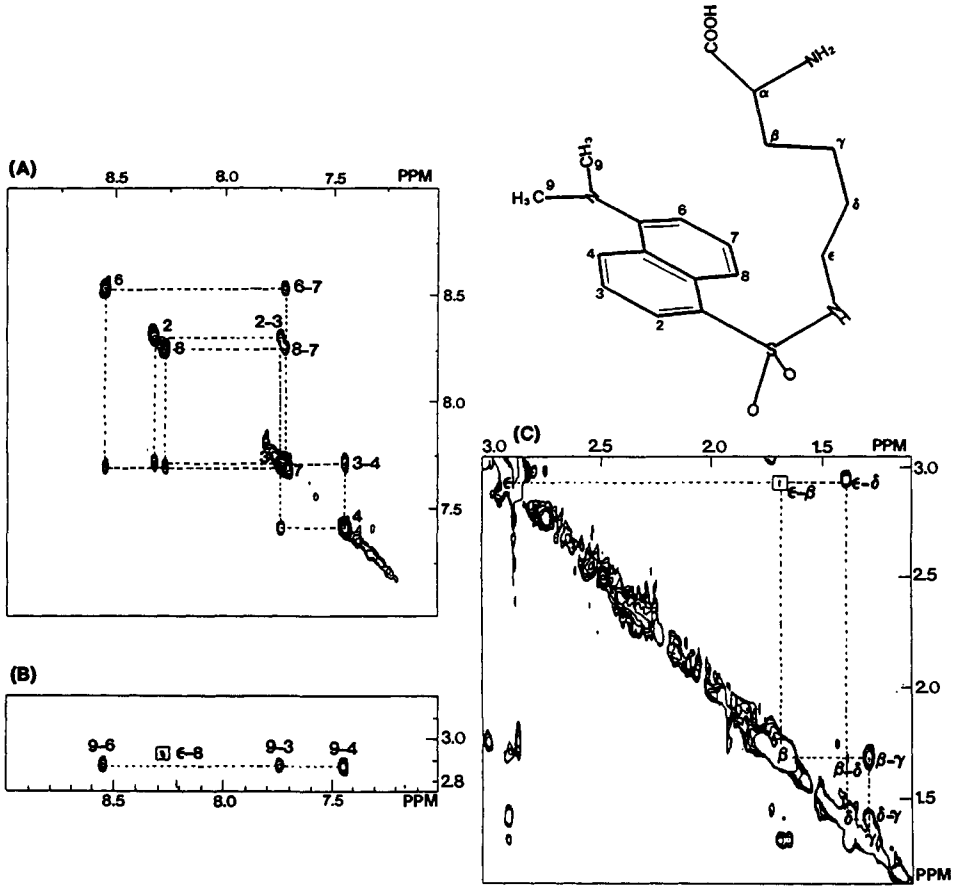


Figure 1. Two-dimensional TRNOE difference spectrum of compound **1** in the presence of antibody **2**: (A) the aromatic region, (B) the region between the aromatic and aliphatic protons, and (C) the aliphatic region. The two-dimensional TRNOE difference spectrum was obtained by subtracting the NOESY spectrum of antibody **2** from that of antibody **2** in the presence of a 10-fold excess of compound **1**. The NOESY spectra were recorded at 45 °C on a JEOL JNM-GSX500 spectrometer. A mixing time of 50 ms was used for the measurement. It was confirmed that a similar result was obtained with a shorter mixing time of 20 ms. Chemical shifts are reported in parts per million relative to DSS. Concentrations: compound **1**, 2.22 mM; antibody **2**, 0.22 mM. Buffer, 5 mM sodium phosphate, 200 mM NaCl, pH 7.4.

Table I. Association Constants of Dansyl Haptens to Antibody **2**

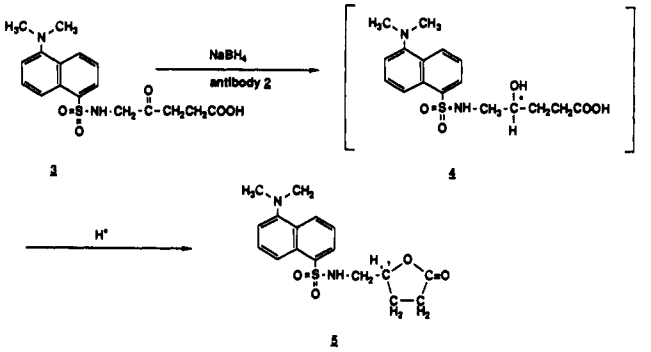
hapten	K_a, M^{-1}
dansylglycine	3.1×10^6
dansyl-β-alanine	6.3×10^6
4-(dansylamino)butyric acid	4.0×10^7
5-(dansylamino)valeric acid	1.3×10^8
6-(dansylamino)caproic acid	1.1×10^8
ε-dansyl-L-lysine	9.0×10^7

a chiral HPLC column. The maximum enantiomeric excess obtained was 36%.

Compound **3** was prepared from dansyl chloride and 5-aminolevulinic acid by following the procedure reported by Chimiak and Polonski.[5] Antibody **2** was separated from ascites according to the standard procedure and purified on an Affi-Gel protein A column (Bio-Rad).

Figure 1 shows a two-dimensional TRNOE difference spectrum of compound **1** observed in the presence of antibody **2**. NOE cross peaks between adjacent protons of compound **1** except between $C_\alpha H$ and $C_\beta H$ were negative in intensity. This indicates that the dansyl ring and the side chains from C_β to C_ϵ of compound **1** are tightly bound to antibody **2**. Other NOE cross peaks were observed between $C_\beta H$ and $C_\epsilon H$ and between $C_\epsilon H$ and H-8 (dansyl ring). A weak NOE cross peak was also observed between $C_\beta H$ and H-4 (data not shown). These results show that the side chain of compound **1** is folded in the binding site of antibody **2**. A similar TRNOE experiment was performed using compound **3**. Negative NOEs were observed between all adjacent protons including between $C_\alpha H$ and $C_\beta H$ and between $C_\alpha H$ and $C_\delta H$ of compound **3**. This result indicates that the entire molecule of compound **3** is also folded in the binding site of antibody **2**.

Chart I



Compound **3** and antibody **2** were dissolved at a molar ratio of 2:1 in 120 mM sodium phosphate buffer containing 150 mM NaCl, pH 7.4, and incubated in the presence of $NaBH_4$ at room temperature for 3 days. The reaction mixture was treated with 6 M urea and 50 mM sodium acetate at pH 5.1, 30 °C, and acidified to pH 1 by hydrochloric acid. Compound **5** obtained from compound **3** through the cyclization reaction of the intermediate, 5-(dansylamino)-4-hydroxypentanoic acid (**4**) (Chart I), was subjected to HPLC on a Chiral Pak AS column (Daicel Chemicals Co.). As Figure 2 shows, the *R* and *S* enantiomers of compound **5** can be separated completely on this column. An authentic sample of the *S* enantiomer of compound **5** was prepared using (*S*)-(+)-5-(aminomethyl)-2-oxotetrahydrofuran,[6] which was

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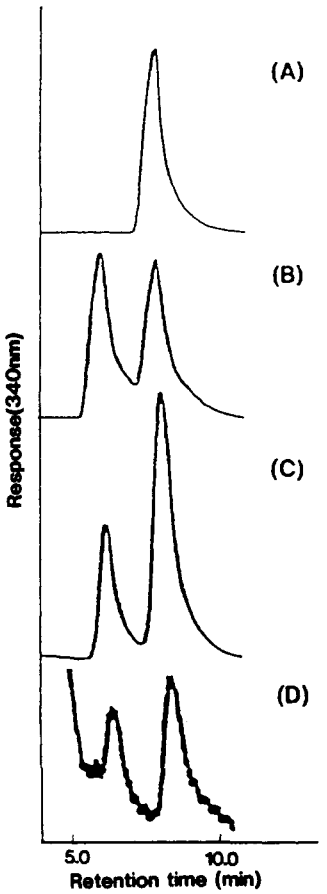

Figure 2. HPLC profiles on a chiral HPLC column (Chiral Pak AS, Daicel Chemicals Co.) for compound **5**: (A) authentic *S* enantiomer, (B) a 1:1 mixture of *S* and *R* enantiomers, (C) a 1:1 mixture of *S* and *R* enantiomers with authentic *S* enantiomer added, and (D) a reaction mixture obtained with an (antibody **2**):(compound **3**):$NaBH_4$ ratio of 1:2:60. Chromatography conditions were as follows: mobile phase, *n*-hexane–ethanol (50:50, v/v); flow rate, 1.0 mL/min; UV detection at 340 nm.

kindly provided by Professor C. Herdeis, Universitat Wurzburg. It has been confirmed by using the authentic sample of (*S*)-**5** that the peak with the longer retention time is due to the *S* enantiomer. See Figure 2C. Enantiomeric excesses were determined by measuring the peak intensity for the *R* and *S* enantiomers. A maximum enantiomeric excess of 36% was obtained with a substrate:$NaBH_4$ molar ratio of 1:30.

Saturation of the antibody combining site with compound **1**, which possesses a stronger affinity to the antibody **2** than compound **3**, abolished the enantiomeric excess. This result indicates that the stereoselective reduction has actually occurred in the antibody combining site.

Use of the active site of proteins as chiral auxiliaries for stereoselective reactions has attracted considerable attention. It was reported that use of pyruvate kinase for the stereoselective reduction by $NaBH_4$ of pyruvate to lactate in the presence of Mg^{2+} as a cofactor resulted in a stereoselective reduction of pyruvate, giving a 19% enantiomeric excess of D-lactic acid.[7] The present method is more general in that the dansyl group may be used as an *anchor* for other types of stereoselective reactions in the *hydrophobic subsite* of the same antibody. In addition, it was possible to isolate and identify the reduction product on an HPLC chiral column. The stereoselective reaction reported in the present communication is stoichiometric rather than catalytic in nature. However, detailed analyses of the conformation and the mechanism of stereoselective reactions of hapten side chains will become of increasing importance, for example, for the design of catalytic antibodies.[8]

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Statistical Incorporation of $^{13}C_2$ Units into C_{60}

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Partially ^{13}C enriched C_{60} (buckminsterfullerene) can be prepared by the laser[1] or contact-arc[2] vaporization of heterogeneous mixtures of solid ^{12}C and ^{13}C. Mass spectral analysis of the partially enriched C_{60} suggests that the ^{13}C is statistically distributed among the clusters.[1,3] Rather than $^{12}C_{60}$ and $^{13}C_{60}$ being formed predominately, $^{13}C_n{}^{12}C_{(60-n)}$ mixtures form with a distribution consistent with isotopic scrambling via C_m units where *m* is small. While the mass spectrum indicates the isotope distribution *between* the carbon clusters, it does not address the isotope distribution *within* the carbon clusters. ^{13}C NMR can potentially indicate the distribution of ^{13}C's within a carbon cluster by the degree of ^{13}C–^{13}C coupling.[4,5] The 1D ^{13}C NMR spectrum of enriched C_{70} qualitatively implies carbon atom mixing on an atomic scale during fullerene formation in that the spectrum consists of "predominantly singlets", rather than "predominantly multiplets due to coupling of adjacent ^{13}C atoms."[5] In this paper, we quantitatively examine the ^{13}C distribution within enriched C_{60}. Buckminsterfullerene cannot be analyzed directly, however, as $^{13}C_n{}^{12}C_{(60-n)}$ shows a single ^{13}C NMR peak[6] and therefore no coupling information. In contrast, osmylated buckminsterfullerene[7] resolves into 17 peaks and the associated couplings.[8] Here, we measure the degree of ^{13}C–^{13}C coupling for four types of carbons in $C_{60}(OsO_4)(4\text{-}tert\text{-butylpyridine})_2$, calculate the expected degrees of ^{13}C–^{13}C coupling for these carbons given a random distribution of ^{13}C's within the clusters, and find that the two sets of numbers agree within experimental error.

Natural-abundance carbon rods were cored, packed with 99% ^{13}C powder, and converted to C_{60} in a Smalley-type contact arc

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