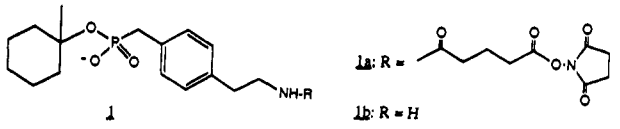
Chart I. Hapten with Activated Linker (**1a**) and Inhibitor (**1b**) Used in Assay Described in Table I



Scheme I. Simplified Mechanistic Picture for Reaction Catalyzed by Antibodies in Table I

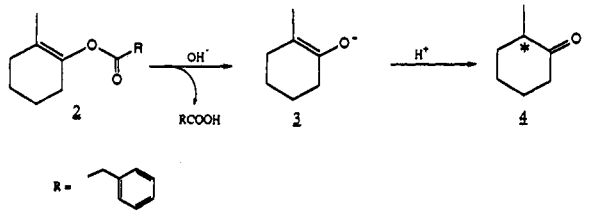


Table I. Kinetic Parameters for the Hydrolysis of Enol Ester **2** by Antibodies (27B5, 7F9, and 32B11)[a]

antibody	K_m, 10^{-6} M	k_{cat}, min^{-1}	k_{cat}/k_{uncat}[b]	K_i,[c] 10^{-6} M	% ee[d]	config[e]
27B5	994	0.01	300	4.3	42	*R*
7F9	909	0.02	680	6.5	<5	*R*
32B11	436	0.01	300	15.2	<5	*R*

[a] Conditions: Assay conditions [pH 9.0, 10% DMSO/ATE (0.1 M ACES, 0.052 M Tris, and 0.052 M ethanolamine), 20 μM antibody, 25 °C]. Hydrolysis rates were measured by following the generation of phenylacetic acid via HPLC on a RP-C18 column eluting with water–acetonitrile (85:15) at a flow rate of 1 mL/min with UV detection at 260 nm. [b] Background hydrolysis rate 3.4×10^{-5} min^{-1}. [c] Inhibition data determined using **1b** as the inhibitor. [d] Determined by GC analysis with the chiral cyclodextrin column. Retention times: (*S*)-**4**, 36 min; (*R*)-**4**, 37 min. Oven temperature 65 °C, He_2 gas. An authentic sample of (*S*)-**4** was prepared by the method described by Meyers:[12] $[\alpha]^{21}_D$ −45.6° (*c* = 2.3, MeOH). [e] The configuration of that fraction of product which is in enantiomeric excess.

An initial screen for simple hydrolytic activity was accomplished by HPLC (high-pressure liquid chromatography). The reaction consisted of 20 μM highly purified antibody[10] and 1500 μM 1-(phenylacetoxy)-2-methylcyclohexene (**2**)[11] (Scheme I) in 10% DMSO/ATE buffer (ACES 0.1 M, Tris 0.052 M, ethanolamine 0.052 M), pH 9.0, 25 °C. From 26 monoclonal antibodies screened, three were found to accelerate the hydrolysis of **2**. These three IgG antibodies were studied as a function of variable concentrations of **2** (50–1500 μM). All exhibited saturation kinetics (Table I) and could be completely inhibited by the addition of free hapten **1b** (Chart I). Next we studied whether any of these abzymes could direct protonation of the hydrolytic reaction product, enolate **3** (Scheme I), in an enantioselective manner.

Determination of the enantioselectivity of this reaction was accomplished through the use of a microcapillary gas chromatograph column (Chrompack, CD-(optically pure)-cyclodextrin-B-236-M-19), which completely resolved both enantiomers. The *S* isomer of ketone **4** was synthesized using the procedure of Meyers.[12] Each of three catalytic antibodies (20 μM) was reacted with 500 μM enol ester **2**. When 70 μM product **4** had formed, the assay mixture was extracted with diethyl ether and concentrated, and the enantiomeric enrichment was determined. One of the three abzymes (27B5) provided an optically enriched mixture of *R* ketone **4** (Table I).[13] Although the optical purity obtained was less than that achieved by natural enzymes for some substrates,[4] it is the *first demonstration of an antibody which influences chirality via enantiofacial protonation.*

In asymmetric induction by antibody catalysis, the enantiomeric excess can be a function of the interaction between complex variables. These may include the extent of solvent exclusion from one face of the substrate, the off rate of the enolate leaving group from the protein, and the availability of a proton or its rate of transfer in the antibody binding site.[14] Since antibodies are highly diverse and molecules from individual clones will bind substrates differently, we can expect to improve the enantiomeric excess by selecting antibodies with a more favorable balance between controlling parameters.

Future studies will include screening of additional antibodies and the design of new haptens for the induction of abzymes capable of retarding the escape of **3** into bulk solvent during protonation. This may be a first step toward a rational approach for improving abzyme-mediated asymmetric induction.

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(13) No racemization of ketone **4** was observed (assay conditions reported, Table I) when (*S*)-**4** was allowed to stand overnight. No asymmetric induction was detected when racemic ketone **4** (50 μM) was incubated with antibody 27B5 (50 μM). This result rules out the possibility that antibody 27B5 binds the *S* isomer of racemic product **4** to generate an enantiomeric excess.

(14) While we have implied the reaction mechanism to proceed through a stepwise process (i.e., enol/enolate), we cannot rule out a concerted mechanism of hydrolysis–protonation.

Solution and Solid-State Structures of a Cyclic ψ[CH_2SO] Pseudopentapeptide

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Amide-bond surrogates within peptides can lead to effective enzyme inhibitors, enzyme-resistant peptide mimetics, or occasionally, potent hormone antagonists.[1] We have been interested in defining the conformational effects of various backbone replacements systematically in order to apply backbone modifications more judiciously. Our previous NMR studies have shown that the ψ[CH_2S][2,3] and ψ[CSNH][4] replacements are generally compatible with reverse turn structures[5] when incorporated into the model cyclic pentapeptide cyclo[Gly-Pro-Gly-D-Phe-Pro].[6-12]

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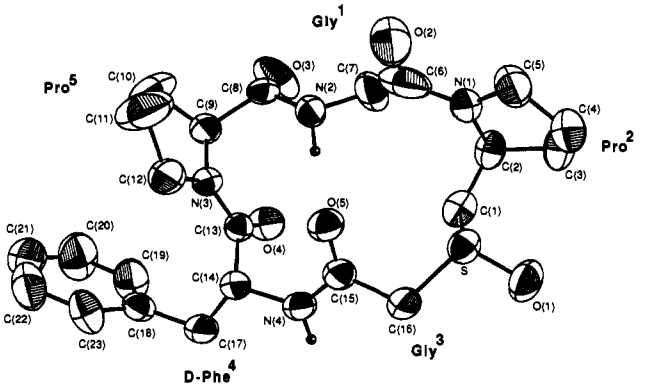

Figure 1. ORTEP perspective view of the molecule including H atoms involved in hydrogen bonding. Only one set of disordered carbonyl atoms [C(6)–O(2) and C(8)–O(3)] is shown for clarity. Selected torsion angles (ϕ, ψ, ω) in degrees: Gly[1] (163.3, 120.0, −179.6), Pro[2] (−76.3, −57.5, 13.5), D-Phe[4] (62.3, −126.4, 175.2), Pro[5] (−81.1, −40.3, −175.3), C(1)–S–C(16)–C(15) = −69.9, S–C(16)–C(15)–N(4) = 161.7, C(2)–C(1)–S–C(16) = 166.0. Selected bond lengths (Å) involving the pseudoamide bond: C(1)–S = 1.811 (3), S–O(1) = 1.496 (3), S–C(16) = 1.817 (4).

The simple oxidation of the thiomethylene ether to the *R* and *S* sulfoxides generates a new amide surrogate that (1) contains a new chiral center, (2) provides a strong H-bond acceptor, which could in principle lead to highly modified H-bonding patterns, and (3) retains enzyme stability, which is a desirable feature of most amide replacements. Hereby we report our latest findings on the crystalline and/or solution conformations of both sulfoxide isomers of cyclo[Gly-Proψ[CH_2-(*R*,*S*)-SO]Gly-D-Phe-Pro],[13] including the first configuration assignment of a backbone sulfoxide surrogate.

NMR results revealed that both sulfoxides showed a single major conformer in $CDCl_3$ but two conformers in DMSO-d_6. Neither diastereomer was comparable to the ψ[CH_2S] precursor in its NMR characteristics in $CDCl_3$, but the temperature coefficients and diagnostic proline carbon-13 chemical shifts were quite similar to each other. These results suggest that in $CDCl_3$ both sulfoxides form new structures that involve altered patterns of hydrogen bonding and an unexpected role for the sulfoxide that does *not* involve H-bond formation. The data is consistent with the existence of a single intramolecular hydrogen bond stabilizing a γ-turn centered about Pro[5]. In DMSO-d_6 the major conformer of each sulfoxide has an all-trans arrangement of amide bonds, but the minor conformer adopts a cis Gly–Pro bond and type II′ β-turn centered around Gly-D-Phe-Pro-Gly.

Following numerous attempts, crystals of the faster eluting sulfoxide isomer I suitable for X-ray analysis were obtained from a DMSO solution by slow evaporation at room temperature.[15] In the crystal state (see Figure 1), cyclo[Gly-Proψ[CH_2SO]Gly-D-Phe-Pro] contains a cis amide bond between Gly[1] and Pro[2] with the carbonyl oxygen located in two positions [$\omega_i(C_i'–N_i)$ = 1.4 (2)° and −12.3 (7)°]. A type II′ β-turn is observed, involving the residues Gly[3]-D-Phe[4]-Pro[5]-Gly[1] with the turn stabilized by a 1 ← 4 intramolecular hydrogen bond between the CO of Gly[3] and NH of Gly[1]. Among related pentapeptides, the only other reported example of a type II′ β-turn in the solid state was cyclo[Gly-Pro-Ser-D-Ala-Pro], involving the Pro-Gly-Pro-Ser residues.[10] Unlike the sulfoxide pseudopeptide, this Ser peptide contained all-trans amide bonds.

Dihedral angles for the D-Phe[4]-Pro[5] residues of the title peptide are also fully consistent with a type II′ β-turn (see supplementary material for details of X-ray and NMR data). The distance between N(2)–H and O(5) is 2.16 Å, indicating the formation of a relatively strong H bond. In contrast, the D-Phe[4] oxygen is 2.73 Å away from the amide proton of Gly[1], thereby making a γ-turn H bond unlikely. Pro generally is found in the i + 1 position of β-turns, but can be accommodated in the i + 2 position as demonstrated before[16] and observed here.

The pentapeptide crystallizes in a lattice with chains of peptides connected by intermolecular H bonding. No solvent was involved in the crystal packing. Both amide hydrogens available are involved in either intra- or intermolecular H bonding.[17] NH of Gly[1] forms an intramolecular H bond with the carbonyl oxygen of Gly[3], thus stabilizing the β-turn. NH of D-Phe[4] forms an intermolecular H bond with the oxygen of Gly[1] at the position (x, y − 1, z), thus forming an infinite chain of rings along the crystallographic b axis. A parallel chain related by a 2-fold screw axis is also present. No other H bonds are present, and the sulfoxide oxygen is not involved as an H-bond acceptor.

The geometry of –CH_2SOCH_2– in the cyclic peptide is almost identical with that of DMSO.[18] Relative to the three other sites of known chirality, X-ray analysis establishes the absolute configuration of sulfoxide isomer I as the *S* isomer. By inference, isomer II represents the *R* sulfoxide. The more hydrophilic compound, isomer I, has its polar sulfoxide oxygen oriented outside the five-residue ring, where it presumably interacts less strongly with the hydrophobic C-18 reversed-phase HPLC substrate.

The X-ray structure actually correlates with the minor DMSO-d_6 conformer. In contrast, the major DMSO conformer (apparently identical with the single $CDCl_3$ conformer) represents an all-trans backbone structure which retains only one intramolecular hydrogen bond, a γ-turn involving residues D-Phe[4]-Pro[5]-Gly[1]. It thus appears that the ψ[CH_2SO] pseudopeptide analogue induces conformational changes that are distinctly different from its ψ[CH_2S] precursor. That the ψ[CH_2S] replacement is conformationally more similar to its amide parent than it is to ψ[CH_2SO] should be further tested to establish the generality of this finding. Nevertheless, in several opioid pseudopeptide analogues, the ψ[CH_2SO] replacements were more potent than their ψ[CH_2S] congeners in various bioassays.[19]

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Supplementary Material Available: Tables SI–SV containing X-ray parameters including atomic coordinates and thermal parameters, bond distances, and bond angles, an appendix providing a detailed account of the ^1H and ^{13}C NMR data of cyclo[Gly-Proψ[CH_2-(*R*,*S*)-SO]Gly-D-Phe-Pro], and Figure S1 showing the crystal packing perspective along the a axis (28 pages); Table SVI containing observed and calculated structure factors (11 pages). Ordering information is given on any current masthead page.

(13) The parent pseudopeptide, cyclo[Gly-Proψ[CH_2S]Gly-D-Phe-Pro], was synthesized by preparation of the linear precursor on a Merrifield resin using the pseudodipeptide Boc-Proψ[CH_2S]Gly followed by coupling with Boc-Gly, Boc-Pro, and Boc-D-Phe. Cyclization was effected using diphenyl phosphorazidate as previously described.[3,14] The purified cyclic product was oxidized with hydrogen peroxide in acetic acid, and the two diastereomeric sulfoxides were separated by reversed-phase HPLC and fully characterized by amino acid analysis, FABMS, and NMR spectroscopy.

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