An Antibody-Catalyzed Allylic Sulfoxide−Sulfenate Rearrangement

Zhaohui S. Zhou,† Alexander Flohr,† and Donald Hilvert*

Departments of Chemistry and Molecular Biology, The Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and Laboratory for Organic Chemistry, Swiss Federal Institute of Technology (ETH), Universitätstrasse 16, CH-8092 Zurich, Switzerland

Received August 17, 1999

Antibodies SZ-*cis*-39C11 and SZ-*trans*-28F8, which were elicited in response to *N*-aryl-3-methoxyphenyl proline derivatives, catalyze the [2,3]-sigmatropic rearrangement of allylic sulfoxides to sulfenates. Reduction of the sulfenates with dithiothreitol in situ yields allylic alcohols as the final product. The antibodies achieve rate accelerations in the range 10^2-10^3 over background and exhibit distinctive hapten-dependent substrate specificity and enantio- and diastereoselectivity. Of particular note is the effective chirality transfer from the sulfoxide center to the product alcohol in the SZ-*cis*-39C11-catalyzed conversion of (*Z*)-2-(4-methoxyphenyl)-but-2-en-1-yl 4-nitrophenyl sulfoxide. These properties can be contrasted with those of bovine serum albumin (BSA) which accelerates the same reactions to a comparable extent but does not discriminate between substrate isomers. Partitioning of substrate from aqueous solution into the less polar environment of the protein pocket can account for much of the observed rate enhancement, whereas specific conformational constraints programmed by the haptens must orient the flexible substrate within the induced antibody-combining sites so as to favor certain reaction pathways over others. These studies thus expand the scope of antibody catalysis to an important new class of pericyclic reactions and illustrate how medium effects can be exploited together with conformational constraint to control reactivity and selectivity.

Enzymes that catalyze pericyclic reactions are rare in nature[1] but can be engineered for mechanistic and synthetic studies via catalytic antibody technology. Examples of antibody-catalyzed Diels−Alder cycloadditions[2] and Claisen[3] and Cope[4] rearrangements are notable in this regard. Ongoing structural and mechanistic studies on several of these catalysts[5−8] are providing valuable information on the ways in which proteins can exploit binding energy to enhance rates and control selectivities.

Antibody catalysts have also been described for [2,3]-sigmatropic eliminations of *N*-oxides[9] and selenoxides.[10] These reactions proceed via five- rather than six-membered pericyclic transition states which are substantially less polar than their respective ground states. Substituted tetrahydrofuran and pyrrolidine rings were used to mimic these transition states, and preliminary analysis[9,10] suggests that the induced antibodies exploit medium effects in addition to conformational constraint to achieve rate accelerations of $\sim 10^3$ over background. In the case of the selenoxide elimination, these factors are sufficient to overcome unfavorable eclipsing interactions in the transition state and allow formation of a *cis*-olefin (Scheme 1).[10]

We have begun to investigate various substrate modifications as a means of mapping the stereoelectronic properties of representative catalysts for the selenoxide elimination. In the course of this work, we have found that these antibodies also promote another [2,3]-sigmatropic process, the allylic sulfoxide−sulfenate (Evans−Mislow) rearrangement (Scheme 2). Because sulfoxide moieties are easily introduced and sulfenate esters readily reduced, the Evans−Mislow rearrangement is widely used for the synthesis of chiral allylic alcohols.[11−13]

* To whom correspondence should be addressed at the Swiss Federal Institute of Technology. E-mail: hilvert@org.chem.ethz.ch.

† These authors contributed equally to this work.

(1) Pindur, U.; Schneider, G. H. *Chem. Soc. Rev.* **1994**, 409−415.

(2) (a) Hilvert, D.; Hill, K. W.; Nared, K. D.; Auditor, M. T. M. *J. Am. Chem. Soc.* **1989**, *111*, 9261−9262. (b) Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 7430−7431. (c) Gouverneur, V. E.; Houk, K. N.; Pascal-Teresa, B.; Beno, K.; Janda, K. D.; Lerner, R. A. *Science* **1993**, *262*, 204−208. (d) Meekel, A. A. P.; Resmini, M.; Pandit, U. K. *J. Chem. Soc., Chem. Commun.* **1995**, 571−572. (e) Yli-Kauhaluoma, J. T.; Ashley, J. A.; Lo, C.-H.; Tucker, L.; Wolfe, M. M.; Janda, K. D. *J. Am. Chem. Soc.* **1995**, *117*, 7041−7047. (f) Pitt, A. R.; Stimson, W. H.; Suckling, C. J.; Marrero-Tellado, J. J.; Vazzana, C. *Isr. J. Chem.* **1996**, *36*, 171−175.

(3) (a) Hilvert, D.; Carpenter, S. H.; Nared, K. D.; Auditor, M. T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4953−4955. (b) Hilvert, D.; Nared, K. D. *J. Am. Chem. Soc.* **1988**, *110*, 5593−5594. (c) Jackson, D. Y.; Jacobs, J. W.; Sugasawara, R.; Reich, S. H.; Bartlett, P. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 4841−4842.

(4) Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1994**, *116*, 2211−2212.

(5) Haynes, M. R.; Stura, E. A.; Hilvert, D.; Wilson, I. A. *Science* **1994**, *263*, 646−652.

(6) Haynes, M. R.; Lenz, M.; Taussig, M. J.; Wilson, I. A.; Hilvert, D. *Isr. J. Chem.* **1996**, *36*, 151−159.

(7) (a) Ulrich, H. D.; Mundorff, E.; Santarsiero, B. D.; Driggers, E. M.; Stevens, R. C.; Schultz, P. G. *Nature* **1997**, *389*, 271−275. (b) Driggers, E. M.; Cho, H. S.; Liu, C. W.; Katzka, C. P.; Braisted, A. C.; Ulrich, H. D.; Wemmer, D. E.; Schultz, P. G. *J. Am. Chem. Soc.* **1998**, *120*, 1945−1958.

(8) (a) Romesberg, F. E.; Spiller, B.; Schultz, P. G.; Stevens, R. C. *Science* **1998**, *279*, 1929−1933. (b) Heine, A.; Stura, E. A.; Yli-Kauhaluoma, J. T.; Gao, C.; Deng, Q.; Beno, B. R.; Houk, K. N.; Janda, K. D.; Wilson, I. A. *Science* **1998**, *279*, 1934−1940.

(9) Yoon, S. S.; Oei, Y.; Sweet, E.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 11686−11687.

(10) Zhou, Z. S.; Jiang, N.; Hilvert, D. *J. Am. Chem. Soc.* **1997**, *119*, 3623−3624.

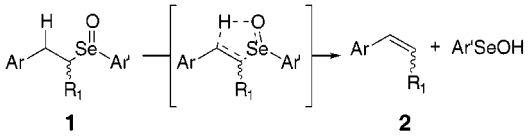
(11) Evans, D. A.; Andrews, G. C. *Acc. Chem. Res.* **1974**, *7*, 147−155.

(12) Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 563−572.

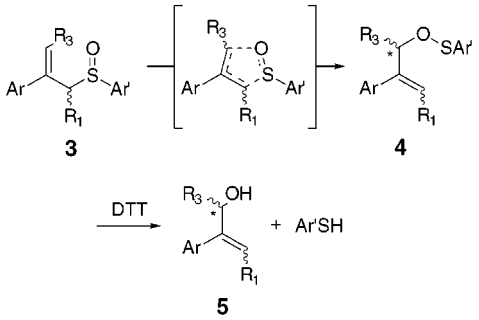
(13) Braverman, D., In *The Chemistry of Sulphones and Sulphoxides*; Patai, S., Rappoport, Z., Stirling, C. J. M., Eds.; John Wiley: London, 1988; Chapter 14.

10.1021/jo991299a CCC: $18.00 © 1999 American Chemical Society
Published on Web 10/13/1999

Scheme 1



Scheme 2



In contrast to selenoxides, which undergo rapid racemization via achiral hydrates,[14,15] sulfoxides are configurationally stable, making possible an investigation of antibody-controlled chirality transfer.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 250 and AMX 400 and 500 instruments. Mass spectra were obtained on a VG ZAB-2VSe double-focusing high-resolution mass spectrometer. HPLC analyses were performed on Merck LiChrosorb RP18 10μ, LiChrospher 100 CH-18/2 (5μ), Rainin Microsorb-MV C18 300 Å and 86−203 (C-18) stationary phases using various acetonitrile in water (0.05% TFA) gradients at elution rates of 1.0 mL min^{-1}. For all kinetic runs, a guard column filled with Whatman Pellicular ODS (C-18) was added upstream of the analytical column. Melting points are uncorrected.

2-(4-Methoxyphenyl)prop-2-en-1-yl 4-Nitrophenyl Sulfoxide (3a). *n*-Butyllithium (2.0 M in hexane, 1.0 mL, 2.0 mmol) and 4-nitrobenzenesulfenyl chloride (398 mg, 2.1 mmol) were added sequentially to a solution of 2-(4-methoxyphenyl)-prop-2-en-1-ol[16,17] (**5a**) (328 mg, 2.0 mmol) in dry THF at 0 °C. After being stirred for 30 min, the reaction was diluted with ethyl acetate, washed with aqueous phosphate buffer (400 mM, pH 6.8) and brine, dried with $MgSO_4$, and concentrated in vacuo. Flash chromatography on silica gel with hexane/ethyl acetate 2:1 afforded 55% of the yellow solid **3a**. Mp: 99.9−100.7 °C. TLC: R_f 0.25 (hexane/ethyl acetate 2:1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.80 (s, 3H), 3.91 (d, 1H, 12.8 Hz), 4.16 (d, 1H, 12.8 Hz), 5.02 (s, 1H), 5.49 (s, 1H), 6.84 (d, 2H, 8.9 Hz), 7.29 (d, 2H, 8.9 Hz), 7.72 (d, 2H, 8.9 Hz), 8.27 (d, 2H, 8.9 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.2, 64.1, 113.9, 118.7, 123.6, 125.4, 127.1, 127.6, 130.5, 135.8, 149.3, 150.8, 159.6. DEPT 135: CH−CH_3, δ 113.9, 123.6, 125.4, 127.1; CH_2, δ 64.1, 118.7. UV_{max} (water): 258.3 nm (ϵ = 10 430 M^{-1} cm^{-1}). LRMS (FAB$^+$, NBA/NaI): *m*/*z* 318 (M + H$^+$). HRMS: calcd for $C_{16}H_{15}N_4O$ 318.0800, found 318.0807.

(*Z*)-2-(4-Methoxyphenyl)but-2-en-1-yl 4-Nitrophenyl Sulfoxide (3b). This compound was obtained from 3-(4-methoxyphenyl)but-3-en-2-ol[18] (**5b**) following the procedure described for the preparation of **3a**. Yellow solid (61%). Mp: 101 °C. TLC: R_f 0.25 (hexane/ethyl acetate 3:2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.58 (d, 3H, 7.1 Hz), 1.69 (d, 0.7H, 7.0 Hz), 3.79 (ms, 3.3H), 3.81 (s, 0.23H), 3.88 (d, 0.23H, 12.6 Hz), 3.99 (d, 1H, 12.8 Hz), 4.26 (d, 1H, 12.8 Hz), 5.71 (q, 0.23H, 7.0 Hz), 6.07 (q, 1H, 7.1 Hz), 6.81 (d, 2H, 8.9 Hz), 6.85 (d, 0.45H, 8.9 Hz), 7.10 (d, 0.45H, 8.9 Hz), 7.19 (d, 2H, 8.9 Hz), 7.71 (dd, 2.45H, 8.9 Hz), 8.25 (d, 2H, 8.9 Hz), 8.34 (d, 0.45H, 8.9 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.7, 15.4, 55.3, 59.3, 68.4, 77.7, 113.9, 114.0, 123.7, 123.9, 125.3, 125.5, 127.2, 128.7, 129.8, 130.2, 131.7, 133.0, 149.4, 151.0, 159.1. DEPT 135: CH−CH_3, δ 14.7, 15.4, 55.3, 77.7, 113.9, 123.7, 123.9, 125.3, 125.5, 127.1, 129.8, 130.1, 131.6; CH_2, δ 59.3, 68.4. UV_{max} (water): 252 nm (ϵ = 16 100 M^{-1} cm^{-1}). LRMS (FAB$^+$, NBA): *m*/*z* 332 (M + H$^+$). HRMS: calcd for $C_{17}H_{18}NO_4S$ 332.0957, found 332.0951.

The product is a 4:1 mixture of the *Z* and *E* isomers as shown by NOE difference measurements ($CDCl_3$, 300 MHz). Irradiation of =C*H*-Me results in large positive NOE's to C-2′, C-5′ of the neighboring aromatic ring and to =CH-*Me*. Irradiation of =CH-*Me* gives small positive NOEs to =C*H*-Me and to C*H*$_2$SO.

(*E*)-2-(4-Methoxyphenyl)but-2-en-1-ol (5c). Stille coupling of (*E*)-2-(tri-*n*-butylstannyl)-2-buten-1-ol[19] (**12**) and 4-iodoanisole using triphenylarsine, dipalladium tris(dibenzylideneacetone), and copper iodide as the catalyst[20] yielded compound **5c** as a colorless oil (35% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.64 (d, 3H, 6 Hz), 3.81 (s, 3H), 4.30 (d, 2H, 6 Hz), 5.78 (q, 1H, 6 Hz), 6.90 (dt, 2H, 9 Hz, 3 Hz), 7.16 (dt, 2H, 11 Hz, 3 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.3, 55.1, 113.6, 123.0, 129.7, 133.5, 140.2, 158.5. UV_{max} (H_2O): 200 nm, 240 nm. LRMS (FAB$^+$, NBA/NaI): *m*/*z* 178 (M$^+$). HRMS: calcd for $C_{11}H_{14}O_2$ 178.0990, found 178.0994.

3-(4-Methoxyphenyl)but-3-en-1-yl) 4-Nitrophenyl Sulfoxide (3c). Compound **5c** was converted to **3c** in 40% yield according to the protocol described for **3a**. Neat sulfoxide **3c** rearranges within minutes to **3b** via a [1,3]-shift. Because of this rapid rearrangement, flash-chromatography fractions containing product were diluted with acetonitrile and concentrated at 0 °C three times, taking care not to evaporate the sample to dryness. The concentration of **3c** in the final acetonitrile solution was determined by integration of the ^{1}H NMR signals against an internal standard. HPLC and NMR indicate a 1:1 mixture of diastereomers. ^{1}H NMR (MeCN-d_3, 400 MHz): δ 1.39 (d, 1.5H, 7 Hz), 1.49 (d, 1.5H, 7 Hz), 3.99 (q, 0.5H, 7 Hz), 4.35 (q, 0.5H, 7 Hz), 5.02 (s, 0.5H), 5.32 (s, 0.5H), 5.43 (s, 0.5H), 5.60 (s, 0.5H), 6.75 (dt, 1H, 9 Hz, 3 Hz), 6.86 (dt, 1H, 9 Hz, 3 Hz), 7.14 (dt, 1H, 9 Hz, 3 Hz), 7.28 (dt, 1H, 9 Hz, 3 Hz), 7.56 (dt, 1H, 9 Hz, 3 Hz), 7.80 (dt, 1H, 9 Hz, 3 Hz), 8.11 (dt, 1H, 9 Hz, 3 Hz), 8.26 (dt, 1H, 9 Hz, 3 Hz). ^{13}C NMR (MeCN-d_3, 125 MHz): δ 12.4, 13.9, 55.9, 63.5, 65.5, 114.5, 114.8, 116.9, 117.1, 124.1, 124.8, 127.1, 127.2, 128.6, 128.7, 133.5, 142.4, 145.0, 149.9, 151.8, 160.6. UV_{max} (MeCN): 256 nm (ϵ 12 300 M^{-1} cm^{-1}). LRMS (FAB$^+$, NBA/NaI): *m*/*z* 332 (M + H$^+$). HRMS: calcd for $C_{17}H_{17}NO_4S$ 332.0957, found 332.0949.

The diastereomers were separated by preparative HPLC on LiChrospher 100CH-18/2 (5 μm) using water/acetonitrile gradients (without TFA). In acetonitrile at 5 °C, **3c** does not rearrange to **3b** over the course of several weeks, but within days, the separated diastereomers reequilibrate to the 1:1 mixture.

***cis*- and *trans*-1-(4-Nitrophenyl)-3-(4-methoxyphenyl)-proline (*cis*-8 and *trans*-8).** A mixture of 3-(4-methoxyphenyl)proline hydrochloride **7**[21] (244 mg, 0.95 mmol), 1-fluoro-4-nitrobenzene (0.53 mL, 5.0 mmol), and potassium carbonate (848 mg, 8.0 mmol) in 3.5 mL of dry DMSO was heated at 120 °C for 23 h. The mixture was then poured into 50 mL of saturated aqueous sodium carbonate solution and stirred at room temperature for 1 h to hydrolyze the 4-nitrophenyl ester. The orange-red solution was washed with ethyl acetate, adjusted to pH 1.5 with concentrated aqueous HCl and then extracted with diethyl ether. The bright yellow organic extract

(14) Davis, F. A.; Reddy, R. T. *J. Org. Chem.* **1992**, *57*, 2599−2606.
(15) Shimizu, T.; Yoshida, M.; Kobayashi, M. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1555−1557.
(16) Gassman, P. G.; Harrington, C. K. *J. Org. Chem.* **1984**, *49*, 2258−2273.
(17) Mickelson, T. J.; Koviach, J. L.; Forsyth, C. J. *J. Org. Chem.* **1996**, *61*, 9617−9620.
(18) Cabri, W.; Candiani, I.; Bedeschi, A.; Santi, R. *J. Org. Chem.* **1992**, *57*, 3558−3563.
(19) Ensley, H. E.; Buescher, R. R. *J. Org. Chem.* **1982**, *47*, 404−408.
(20) Flohr, A. *Tetrahedron Lett.* **1998**, *39*, 5177−5180.

was washed with brine, dried with $MgSO_4$, and concentrated in vacuo. Flash chromatography on silica gel (hexane/ethyl acetate/HOAc 1:1:0.002) afforded *cis*-**8** (47 mg, 15%) as a thick yellow oil. Subsequent elution with more polar solvent (hexane/ethyl acetate/HOAc 1:1:0.003) yielded *trans*-**8** (114 mg, 35%) as a thick yellow oil.

cis-**8**. R_f: 0.35 (hexane/ethyl acetate/HOAc 1:1:0.002). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.36 (m, 1H), 2.78 (m, 1H), 3.41 (m, 1H), 3.78 (s, 3H), 3.84 (m, 3H), 4.51 (d, 1H, J = 8.5 Hz), 6.54 (d, 2H, J = 9.2 Hz), 6.84 (d, 2H, J = 8.7 Hz), 7.21 (d, 2H, J = 8.7 Hz), 8.14 (d, 2H, J = 9.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 47.3, 48.1, 55.2, 65.8, 110.8, 114.0, 126.3, 127.4, 128.9, 138.1, 150.8, 159.1, 174.9. LRMS (FAB$^+$, NBA/NaI) *m*/*z* 343 (M + H$^+$). HRMS: calcd for $C_{18}H_{18}N_2O_5$ 343.1294, found 343.1290.

trans-**8**. R_f: 0.25 (hexane/ethyl acetate/HOAc 1:1:0.002). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.16 (m, 1H), 2.58 (m, 1H), 3.72 (m, 4H), 3.78 (s, 3H), 4.45 (d, 1H, J = 3.4 Hz), 6.56 (d, 2H, J = 9.2 Hz), 6.86 (d, 2H, J = 8.7 Hz), 7.07 (d, 2H, J = 8.7 Hz), 8.15 (d, 2H, J = 9.3 Hz), ^{13}C NMR ($CDCl_3$, 125 MHz): δ, 20.6, 32.0, 47.9, 48.4, 55.3, 67.1, 111.3, 114.3, 126.3, 127.6, 133.5, 138.3, 150.7, 158.8, 177.3. DEPT 135: (CH−CH_3): δ 48.4, 55.3, 67.1, 111.3, 114.4, 126.3, 127.5; (CH_2): δ 32.0, 47.9. HRMS (FAB, NBA/NaI) *m*/*z* 343.1290 (M + H$^+$ $C_{18}H_{18}N_2O_5$ requires 343.1294).

***cis*- and *trans*-1-(4-Nitrophenyl)-3-(4-methoxyphenyl)-proline Trimethylsilylethyl Ester (*cis*-9 and *trans*-9). General Procedure.** 1,3-Dicyclohexylcarbodiimide (DCC) (0.28 mmol) was added to a solution of the desired isomer of **8** (0.23 mmol), 2-trimethylsilylethanol (0.28 mmol), and 4-(dimethylamino)pyridine (DMAP, 0.05 mmol) in 5.0 mL dry acetonitrile at 0 °C. After being stirred at 4 °C for 48 h, the mixture was filtered. The filtrate was diluted with 50 mL of ethyl acetate, washed with 0.1 M aqueous HCl, aqueous $NaHCO_3$, and brine, dried with $MgSO_4$, and concentrated in vacuo. Flash chromatography on silica gel (hexane/ethyl acetate 5:1) afforded the desired ester.

cis-**9**. Pale yellow solid (50%). TLC: R_f 0.30 (hexane/ethyl acetate 5:1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ −0.07 (s, 9H), 0.47 (m, 1H), 0.61 (m, 1H), 2.33 (m, 1H), 2.83 (m, 1H), 3.62 (m, 1H), 3.72 (m, 1H), 3.90 to 3.78 (ms, 6H), 4.47 (d, 1H, J = 8.5 Hz), 6.48 (d, 2H, J = 9.0 Hz), 6.88 (d, 2H, J = 8.7 Hz), 7.22 (d, 2H, J = 8.7 Hz), 8.12 (d, 2H, J = 9.3 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ −1.7, 17.0, 27.5, 47.2, 48.1, 55.2, 63.4, 66.2, 110.7, 113.8, 126.2, 127.9, 129.0, 137.7, 150.9, 159.1, 171.1.

trans-**9**: Brown solid (82%). Mp: 95.5−96.0 °C. TLC: R_f 0.30 (hexane/ethyl acetate 5:1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.02 (s, 9H), 0.97 (m, 2H), 2.15 (m, 1H), 2.57 (m, 1H), 3.68 (m, 4H), 3.79 (s, 3H), 4.24 (m, 2H), 4.39 (d, 1H, J = 3.7 Hz), 6.53 (d, 2H, J = 9.2 Hz), 6.86 (d, 2H, J = 8.7 Hz), 7.07 (d, 2H, J = 8.7 Hz), 8.15 (d, 2H, J = 9.4 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ −1.6, 17.5, 32.0, 47.9, 48.4, 55.3, 64.2, 67.7, 111.2, 114.3, 126.2, 127.6, 133.7, 138.0, 150.9, 158.7, 172.3. LRMS: (FAB$^+$, NBA/NaI): *m*/*z* 454 (M$^+$). HRMS: calcd for $C_{25}H_{34}N_2O_4Si$ 454.2288, found 454.2274.

***cis*- and *trans*-1-(4-[2-Bromoacetylamino]phenyl)-3-(4-methoxyphenyl)proline Trimethylsilylethyl Ester (10). General Procedure.** A mixture of the desired isomer of **9** (26 mg, 0.06 mmol) and palladium on activated carbon (5%, 7 mg) in 3.0 mL of ethanol was stirred under hydrogen (1 atm) at room temperature overnight. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo to give a colorless oil. The oil was dissolved in 5.0 mL of dry CH_2Cl_2, and triethylamine (41 μL, 0.3 mmol) was added. The mixture was then cooled to 0 °C before bromoacetyl bromide (16 μL, 0.18 mmol) was added. After being stirred for an additional 10 min, the reaction mixture was diluted with 30 mL of ethyl acetate, washed with 0.05 M aqueous HCl, 5% aqueous $NaHCO_3$, and brine, dried with $MgSO_4$, and concentrated in vacuo. Flash chromatography on silica gel afforded the desired amide.

cis-**10**. Pale yellow oil (34%). TLC: R_f 0.3 (hexane/ethyl acetate 3:1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ −0.07 (s, 9H), 0.51 (m, 1H), 0.60 (m, 1H), 2.27 (m, 1H), 2.77 (m, 1H), 3.51 (m, 1H), 3.80 to 3.64 (m, 7H), 3.87 (m, 1H), 4.01 (s, 2H), 4.36 (d, 1H, J = 8.5 Hz), 6.51 (d, 2H, J = 8.9 Hz), 6.86 (d, 2H, J = 8.7 Hz), 7.22 (d, 2H, J = 8.7 Hz), 7.33 (d, 2H, J = 8.9 Hz), 7.97 (m, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ −1.7, 17.0, 27.9, 29.6, 47.3, 47.8, 55.2, 62.9, 66.4, 111.7, 113.7, 122.3, 126.4, 129.0, 129.1, 144.4, 158.9, 162.9, 172.5. LRMS (FAB$^+$, NBA) *m*/*z* 532/524 (M$^+$, $C_{25}H_{33}BrN_2O_4Si$ calcd 532/534).

trans-**10**. Pale yellow oil (54%). TLC: R_f 0.3 (hexane/ethyl acetate 2:1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.01 (s, 9H), 0.96 (m, 2H), 2.06 (m, 1H), 2.55 (m, 1H), 3.55 (m, 1H), 3.64 (m, 1H), 3.78 (s, 3H), 4.01 (s, 2H), 4.25 to 4.14 (m, 2H), 4.36 (d, 1H, J = 3.5 Hz), 6.55 (d, 2H, J = 8.9 Hz), 6.83 (d, 2H, J = 8.7 Hz), 7.06 (d, 2H, J = 8.7 Hz), 7.35 (d, 2H, J = 8.9 Hz), 7.97 (m, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ −1.5, 17.4, 29.6, 32.3, 47.5, 48.5, 55.3, 63.5, 67.7, 112.2, 114.1, 122.3, 126.5, 127.7, 134.9, 144.2, 158.5, 163.0, 173.8. LRMS (FAB$^+$, NBA): *m*/*z* 532/524 (M$^+$, $C_{25}H_{33}BrN_2O_4Si$ calcd 532/534).

Coupling of Haptens *cis*-6 and *trans*-6 to Carrier Proteins. The haptens were deprotected immediately prior to coupling. Approximately 5 mg of *cis*-**10** or *trans*-**10** were dissolved in 500 μL of neat TFA. After 1 h at room temperature, the reaction was evaporated to dryness, and crude **6** was redissolved in 200 μL of DMF. The carrier proteins bovine serum albumin (BSA) and thyroglobulin (TG) were modified with 2-iminothiolane (Traut's reagent) in deoxygenated phosphate-buffered saline solution (PBS, 10 mM sodium phosphate, 100 mM NaCl, pH 8.0, containing 1.0 mM EDTA) to convert surface amine residues to thiols. The modified proteins were purified by gel filtration on a Sephadex G-50 column eluted with PBS and then immediately reacted with the DMF solutions of hapten *trans*-**6** or *cis*-**6** under an atmosphere of argon at room temperature for 2 h. The ratio of haptens to thiols in the reaction mixtures ranged from 10:1 to 35:1. The carrier protein−hapten conjugates were purified by gel filtration on a G-50 Sephadex column eluted with PBS. Protein concentration was determined by the BCA method,[22] and thiol concentration was determined by titration with 5,5′-dithiobis(2-nitrobenzoic acid).[23] The epitope density was 8.5 (BSA) and 9.8 (TG) for *cis*-**6** and 8.3 (BSA) and 26.0 (TG) for *trans*-**6**.

Preparation and Purification of Monoclonal Antibodies. Mice (129 GIX$^+$ strain) were immunized with the thyroglobulin conjugates of *cis*-**6** and *trans*-**6**. Serum titer was checked with the corresponding BSA conjugates by enzyme-linked immunosorbent assay (ELISA).[24] Hybridomas were obtained by fusion of spleen cells with SP2/0$^+$ myeloma cells according to standard protocols.[25] High affinity *anti-cis*-**6** and *anti-trans*-**6** antibodies were identified by screening tissue culture supernatants by ELISA. The corresponding hybridomas were subcloned twice and propagated as mouse ascites. Monoclonal antibodies were obtained from ascites fluid by ammonium sulfate precipitation, followed by chromatography on DEAE-Sephacel using NaCl gradients (0 to 500 mM) in 10 mM Tris, pH 8.0. Antibodies were further purified by FPLC on Protein G Sepharose followed by Mono Q HR 10/10. Antibody concentrations were determined by absorbance at 280 nm (l mg mL^{-1} = 1.37 OD, assuming a molecular weight of 160 000 for IgG).

Kinetic Assays. Assays were performed in 100 mM Tris−HCl and 100 mM NaCl (pH 8) in thermostated quartz cuvettes at 20 °C. The substrate sulfoxide (**3**) was incubated with or without antibody, and upon addition of DTT, the production of 4-nitrothiophenol was monitored either spectrophotometrically at 410 nm or by reversed-phase HPLC with *p*-methoxyacetophenone as an internal standard. Initial rates were determined from the linear portion of the time course (typically 5−10% conversion) and were unaffected by the order of

(21) Chung, J. Y. L.; Wasicak, J. T.; Arnold, W. A.; May, C. S.; Nadzan, A. M.; Holladay, M. W. *J. Org. Chem.* **1990**, *55*, 270−275.
(22) Walker, J. M. *Methods Mol. Biol.* **1994**, *32*, 5−8.
(23) Riddles, P. W.; Blakeley, R. L.; Zerner, B. *Methods Enzymol.* **1983**, *91*, 49−60.
(24) Clark, B. R.; Engvall, E. *Enzyme-Immunoassay*; CRC Press: Boca Raton, Florida, 1980.
(25) Harlow, E.; Lane, D. *Antibodies: A Laboratory Manual*; Cold Spring Harbor Lab: New York, 1988.

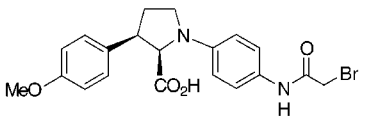
addition of the reagents. *p*-Methoxyacetophenone does not act as an inhibitor or activator of the antibodies, nor is it converted under the assay conditions. To account for substrate depletion during assays, the data were fit to the equation $v_o = [(k_{cat}/2) \times [(E + S + K_m) - [(E + S + K_m)^2 - 4ES]^{1/2}]]$, where v_o is the initial rate, k_{cat} and K_m are the steady-state kinetic parameters, and E and S are the total concentrations of antibody binding site and substrate, respectively.[26] All reaction products were identified by HPLC analysis by co-injection of authentic samples under at least two different separation conditions. The stoichiometry of the reaction was similarly determined by monitoring the total time course of the reaction spectrophotometrically or by HPLC.

Inhibition Studies. Inhibition studies were performed under standard assay conditions in the presence of increasing concentrations of the hapten analogues *cis*-**8** and *trans*-**8**. The ratio of inhibitor to antibody binding site was varied between 0.25 and 4.0. Control experiments with inhibitor in the absence of antibodies showed no affect of the course of reaction.

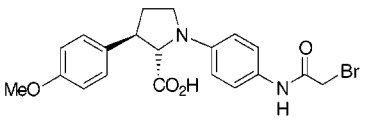
Determination of Enantioselectivity. Substrate **3b** (470 μM) was incubated at 21 °C with antibody (15 μM) and DTT (645 μM) in 640 μL of buffer solution (100 mM Tris−HCl, 100 mM NaCl, pH 8) for 80 min. The reaction was quenched by addition of 50 mM iodoacetic acid sodium salt. After being stirred for an additional 30 min, the aqueous solution was extracted with ethyl acetate. Solvent was then removed with a stream of nitrogen and the crude product redissolved in hexanes/2-propanol (80:20). The enantiomers of the product alcohol and the unreacted substrate sulfoxide were then separated by HPLC on a Chiralcel OD-H column eluted isocratically at 0.5 mL min^{-1} with hexanes/2-propanol (85/15). The product enantiomers (**5b**) eluted with retention times of 11.0 and 11.9 min and the substrate enantiomers (**3b**) at 62.4 and 65.6 min, respectively.

Results

Synthesis. The pyrrolidine derivative **6** was originally designed to mimic the five-membered transition state for the [2,3]-sigmatropic elimination of selenoxides illustrated in Scheme 1.[10] Antibodies elicited in response to this compound were expected to provide a low dielectric environment capable of constraining the flexible substrate in a reactive conformation and desolvating the polar selenoxide moiety. Like the selenoxide elimination, the Evans−Mislow rearrangement of allylic sulfoxides to sulfenate esters proceeds via a five-membered transition state that is less polar then the starting sulfoxide.[11,27,28] Although the precise steric demands of the two reactions differ, it seemed conceivable that antibodies generated against **6** might also promote the reaction shown in Scheme 2.

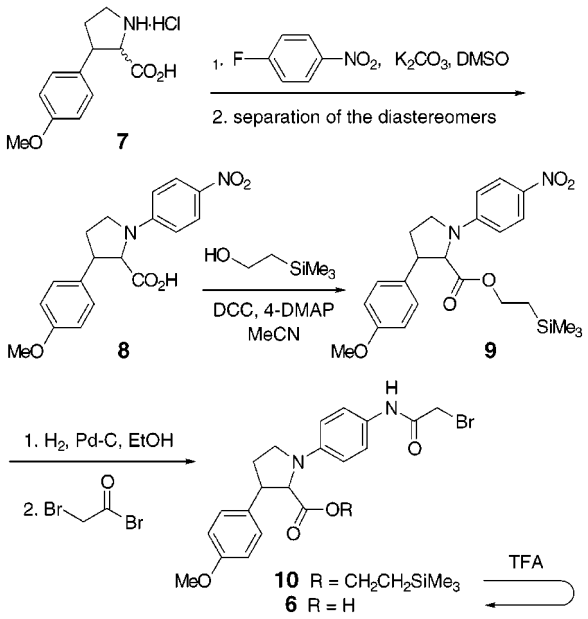


cis-**6**

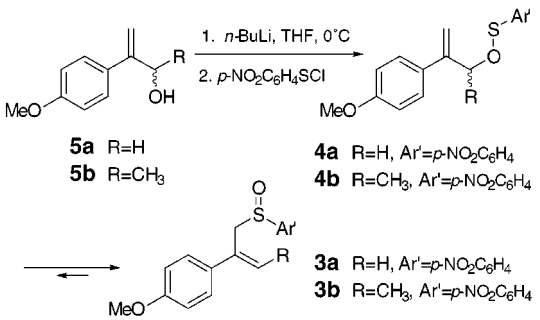


trans-**6**

Scheme 3



Scheme 4



Hapten **6** was synthesized as shown in Scheme 3. 3-(4-Methoxy)proline **7** was prepared by the methods of Chung et al.[21] and Cox et al.[29] and alkylated at nitrogen with 1-fluoro-4-nitrobenzene to give **8** as a 1:2 mixture of diastereomers. The diastereomers were separated by silica gel chromatography and elaborated separately. Protection of the carboxylic acid as a trimethylsilylethyl ester, hydrogenation of the nitro group, and acylation of the resulting amine with bromoacetyl bromide yielded protected haptens *cis*-**10** and *trans*-**10**. Deprotection with TFA afforded *cis*-**6** and *trans*-**6**. The activated bromides were coupled to bovine serum albumin (BSA) and thyroglobulin (TG) that had been previously derivatized with 2-iminothiolane.

All sulfoxide substrates were prepared from the corresponding sulfenate esters. Sulfenate esters **4a** and **4b** were obtained by reacting allylic alcohols **5a**[16,17] and **5b**[18] with *n*-butyllithium and 4-nitrobenzenesulfenyl chloride in THF (Scheme 4). They rearranged spontaneously in solution to afford **3a** and **3b**. Compound **3b** was obtained as a 4:1 mixture of diastereomers that were not separable by HPLC. The major diastereomer was identified by difference NOE-measurements to be the cis isomer (as shown in Scheme 4).

Sulfoxide **3c** was synthesized as shown in Scheme 5. Stille coupling of (*E*)-2-(tri-*n*-butylstannyl)-2-buten-1-ol

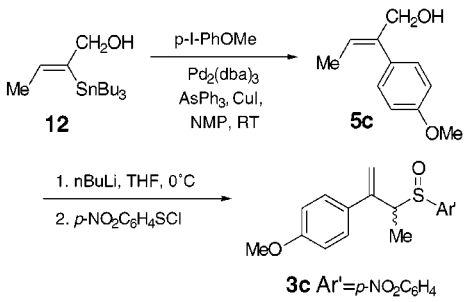
(26) Segel, I. H. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*; Wiley: New York, 1975; pp 72−74.

(27) Bickart, P.; Carson, F. W.; Jacobus, J.; Miller, E. G.; Mislow, K. *J. Am. Chem. Soc.* **1968**, *90*, 4869−4876.

(28) Tang, R.; Mislow, K. *J. Am. Chem. Soc.* **1970**, *92*, 2100−2104.

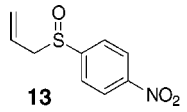
(29) Cox, D. A.; Johnson, A. W.; Mauger, A. B. *J. Chem. Soc.* **1964**, 5024−5029.

Scheme 5



(**12**)[19] with 4-iodoanisole[20] yielded the *Z*-configured allylic alcohol **5c**. Treatment of the latter with *n*-butyllithium and 4-nitrobenzenesulfenyl chloride afforded sulfenate **4c**, which spontaneously rearranged to sulfoxide **3c**. Compound **3c** could not be handled in the absence of solvent because it rapidly undergoes a [1,3]-allyl shift in the solid phase to give **3b** as a 2:1 mixture of diastereomers. α,α-Disubstituted allylic sulfoxides undergo an analogous [1,3]-allyl shift,[11] presumably due to steric crowding near the sulfur atom. Davis et al.[14] have also reported large rate accelerations for racemization of selenoxides in the solid state which they attributed to association of the polar Se−O bond moieties. In solution, however, **3c** is stable for weeks at 5 °C. HPLC and NMR indicate that it is present as a 1:1 mixture of diastereomers. Although the diastereomers could be separated by reversed-phase HPLC, the individual isomers reequilibrate to the 1:1 diastereomeric mixture within days.

Allylic 4-nitrobenzenesulfoxide **13** was used to evaluate the importance of the methoxy-substituted aromatic ring. This compound was synthesized as described by Tang and Mislow.[28]



13

Antibody Catalysis. The TG conjugates of *cis*-**6** and *trans*-**6** were used separately to immunize mice, and monoclonal antibodies were prepared in the usual way.[25] Screening of tissue culture supernatants by ELISA with the corresponding BSA-hapten conjugates yielded 20 antibodies with high affinity for *trans*-**6** and 28 antibodies with high affinity for *cis*-**6**. Hybridomas were subcloned twice and propagated in mouse ascites. Antibodies were purified by ammonium sulfate precipitation and chromatography on DEAE-Sepharose, Protein G, and Mono Q ion exchange columns.

Although the interconversion of allylic sulfoxides **3** and sulfenate esters **4** is reversible, with the equilibrium greatly favoring the sulfoxides in aqueous solution, the sulfenate esters can be trapped with dithiotreitol (DTT) in situ, shifting the equilibrium to the right and releasing product alcohol and an easily detected aryl thiol. All 48 antibodies were consequently screened individually for the ability to accelerate the formation of 4-nitrothiophenol from sulfoxide **3a** in the presence of DTT (Scheme 2). Product formation was monitored at 410 nm. Under the assay conditions neither antibody precipitation nor sulfoxide reduction was observed. Only three antibodies showed significant rate enhancements over background: SZ-*cis*-39C11, SZ-*trans*-28F8, and SZ-*cis*-42F7. These are the same antibodies that were previously found to catalyze the selenoxide elimination reaction.[10] None of the other antibodies showed detectable activity. The most active catalyst, SZ-*cis*-39C11, and SZ-*trans*-28F8 were investigated in greater detail.

The antibody-catalyzed reactions are competitively inhibited by the corresponding transition state analogues. As reported previously,[10] SZ-*cis*-39C11 recognizes only one enantiomer of the hapten analogue *cis*-**8**, as shown by the 2:1 stoichiometry of inhibition (per binding site) by racemic hapten. In contrast, SZ-*trans*-28F8 binds both enantiomers of *trans*-**8** equally well. The rate of the catalyzed reaction is independent of the DTT concentrations used for the assay (>0.3 mM), showing that the rate-limiting step proceeds within the induced binding pocket.

Steady-state kinetic parameters were determined from plots of initial rates versus substrate concentration and are presented in Table 1. The low K_m values and the sensitivity of the antibodies to changes in aromatic substitution pattern (data not shown) suggest that the aryl rings contribute significantly to binding. Compound **13**, lacking the *p*-methoxy-substituted aromatic ring, is not a substrate for either antibody, suggesting that the aromatic rings help to preorganize the pericyclic system for reaction. Rate enhancements over the corresponding uncatalyzed reactions (k_{cat}/k_{uncat}) are in the range 10^2−10^3 as is typical of other antibody-catalyzed sigmatropic rearrangements.[3,4,9,10] Catalytic proficiency [$(k_{cat}/K_m)/k_{uncat}$] provides a measure of the antibody's affinity for the rate-limiting transition state,[30] and the values of 10^6−10^7 M^{-1} obtained here are also typical for these systems.

The chirality of the sulfoxide substrates provides a means of assessing the degree of stereocontrol achieved by the antibodies. In the case of racemic **3a**, the rearrangement is promoted with comparable efficiency but different enantioselectivity by the two catalysts. Both enantiomers are converted to product **5a** in the presence of DTT and antibody SZ-*cis*-39C11, albeit at somewhat different rates, but only one is a substrate for SZ-*trans*-28F8 (Figure 1A). The differences in selectivity are magnified with methyl-containing substrates. Thus, compound **3b**, which has a γ-methyl group, is the poorest substrate for SZ-*trans*-28F8, not being detectibly converted to product. It is the best substrate for SZ-*cis*-39C11, however, subject to a >800-fold acceleration over background. SZ-*cis*-39C11 not only recognizes the chirality of the sulfoxide with 80:20 selectivity, but produces alcohol **5b** in an enantiomeric ratio of 70:30. The observed enantiomeric excesses presumably represent lower limits on the antibody's true selectivity, given that the substrate is a 4:1 mixture of cis and trans isomers. The uncatalyzed background reaction and some racemization during workup may have further eroded the measured enantioselectivities.

The diastereoselectivity of the antibodies was investigated with compound **3c**, which contains two chiral centers. UV and HPLC assays show that all isomers react at roughly comparable rates in the absence of catalyst but only one pair of enantiomers is accepted by SZ-*trans*-28F8 and SZ-*cis*-39C11 (Figure 1B). At low substrate concentrations, the antibodies further discriminate between the two possible enantiomers of the reactive diastereomer, processing one more than an order of magnitude faster than its antipode. At high substrate

(30) Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90−93.

Table 1. Kinetic Parameters for the Antibody-Catalyzed Evans−Mislow Rearrangement of Allylic Sulfoxides 3[a]

catalyst	substrate	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}\,min^{-1}$)	k_{cat}/k_{uncat}	$(k_{cat}/K_m)/k_{uncat}$ (M^{-1})
SZ-*cis*-39C11	**3a** (R^1 = H, R^3 = H)	0.0076	20	380	160	7.9×10^6
	3b (R^1 = H, R^3 = Me)	0.14	28	5100	820	3.0×10^7
	3c (R^1 = Me, R^3 = H)	0.027	10	2700	47	4.7×10^6
SZ-*trans*-28F8	**3a** (R^1 = H, R^3 = H)	0.0040	<1	>4000	80	$>8.3 \times 10^7$
	3b (R^1 = H, R^3 = Me)	ND	ND	ND		
	3c (R^1 = Me, R^3 = H)	0.090	3.3	27 000	160	4.7×10^7
BSA	**3a** (R^1 = H, R^3 = H)		>700	8.0		1.7×10^5
	3b (R^1 = H, R^3 = Me)	0.022	470	47	130	2.8×10^5
	3c (R^1 = Me, R^3 = H)	0.10	230	430	170	7.4×10^5

[a] The reactions were assayed at 20 °C in 100mM Tris−HCl, 100 mM NaCl (pH 8) in the presence of DTT. ND: no activity detected.

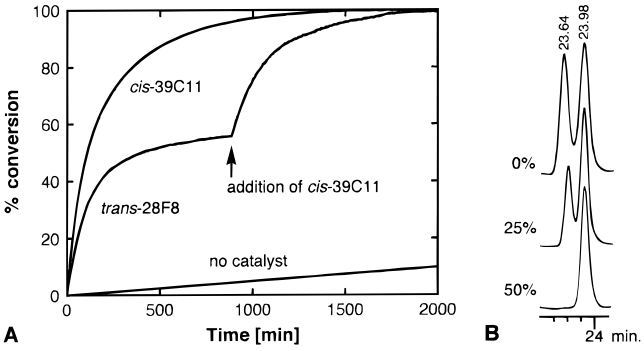


Figure 1. (A) Total time course of the antibody-catalyzed and uncatalyzed rearrangement of allylic sulfoxide **3a**. The reactions were performed at 20 °C in 100 mM Tris−HCl and 100 mM NaCl (pH 8) in the presence of 0.5 mM DTT ([**3a**] = 60 μM; [SZ-*trans*-28F8] = 5 μM; [SZ-*cis*-39C11] = 5 μM) and monitored at 410 nm. After 800 min, SZ-*cis*-39C11 was added to the SZ-*trans*-28F8 reaction to convert the remaining substrate to product. (B) HPLC traces monitoring the SZ-*cis*-39C11-catalyzed conversion of **3c** ([**3c**] = 14 μM; [SZ-*trans*-28F8] = 10 μM). The diastereomers of **3c** were separated on Rainin Microsorb-MV 86-203 (C-18, 4.6 mm i.d.) eluted with acetonitrile/water (0.05% TFA) (20/80 ratio for 10 min, followed by a linear gradient over 5 min to a 50/50 ratio that was then maintained for 45 min). The two diastereomers eluted with retention times of 23.6 and 24.0 min, respectively. Top, t = 0 min; middle, t = 15 min (25% conversion of substrate); bottom, t = 110 min (50% conversion of substrate). Acetophenone was used as an internal standard.

concentrations both enantiomers are converted to product, suggesting a higher K_m for the less reactive species and thus poorer binding as the basis for discrimination. The nonreacting diastereomer is a competitive inhibitor, lowering the catalytic efficiency of the antibodies when present. Accordingly, binding alone appears to be insufficient for catalysis. This conclusion is further strengthened by the observation that the large majority of *anti*-**6** antibodies lack any detectable catalytic activity.

BSA Catalysis. As seen for other medium-sensitive transformations,[10,31] the Evans-Mislow rearrangement is also catalyzed by BSA, albeit with lower efficiencies (for **3a** and **3b**) and substantially higher K_m values than with the antibodies. These results suggest weaker and less specific binding of substrate. Consistent with this interpretation, BSA does not differentiate between the enantiomers of **3a** or **3b** and product **5b** is generated in racemic form. BSA also converts both diastereomers of **3c** to the corresponding allylic alcohols.

Discussion

Mechanism. A reversible pericyclic mechanism has been proposed for the interconversion of allylic sulfoxides and sulfenate esters.[11,27,28] The entropies of activation for the forward and reverse reactions are in the range −0.7 to −10 eu,[28] consistent with a conformationally restricted five-membered cyclic transition state. Electron withdrawing substituents greatly accelerate the rearrangement of allylic aryl sulfoxides, as do nonpolar solvents.[28] In contrast, the reverse process starting from the sulfenate ester is relatively insensitive to solvent change. These results, together with recent calculations,[32] suggest that the transition state more closely resembles the less polar sulfenate ester in its structure and solvation properties than the polar sulfoxide.

Consistent with these conclusions, catalysis of the [2,3]-sigmatropic rearrangement of allylic sulfoxides **3** by antibodies SZ-*trans*-28F8 and SZ-*cis*-39C11 and BSA can be largely ascribed to a simple medium effect. Partitioning of properly configured substrates from aqueous solution into the relatively apolar environment of the antibody or BSA active sites would shift the internal equilibrium in favor of the sulfenate ester **4**, which can then be trapped by DTT either within the binding pocket or upon dissociation into free solution. The rate accelerations we observe are comparable to those seen previously for the medium-sensitive selenoxide eliminations also catalyzed by these proteins.[10] The latter reaction is, however, considerably more sensitive to solvent change than the rearrangement of allylic sulfoxides as judged by correlations of reaction rate with the solvent polarity parameter E_T. For example, the selenoxide elimination is accelerated by a factor of 40 000 in going from aqueous buffer (E_T = 65) to cyclohexane (E_T = 30),[10] whereas the rate of the allylic sulfoxide-sulfenate rearrangement is enhanced only by a factor of about 100.[28,33]

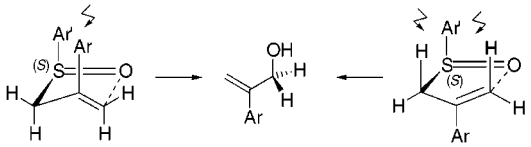
The importance of a medium effect is supported by the similarity in the rate accelerations (k_{cat}/k_{un}) achieved by the antibodies and BSA for most of the reactions examined (Table 1). The conversion of **3b** to **4b** by SZ-*cis*-39C11 appears to be an exception to this general statement, as the rate enhancement in the presence of this antibody is nearly an order of magnitude faster than the comparable reaction with BSA. The γ-methyl substituent in **3b**, which lacks a counterpart in the hapten, presumably makes serendipitous but favorable interactions

(31) (a) Kikuchi, K.; Thorn, S. N.; Hilvert, D. *J. Am. Chem. Soc.* **1996**, *118*, 8184−8185. (b) Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *Nature* **1996**, *383*, 60−63.

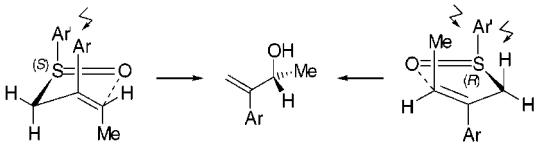
(32) Jones-Hertzog, D. K.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1995**, *117*, 9077−9078.

(33) Preliminary investigations of the uncatalyzed rearrangement of compound **3b** in a limited range of solvents shows that it and the substituted allylic phenyl sulfoxides studied by Tang and Mislow[28] are comparably sensitive to solvent change.

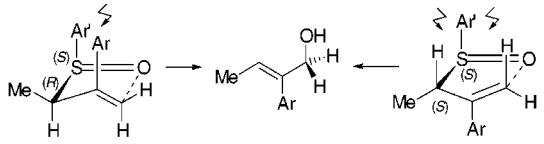
Scheme 6



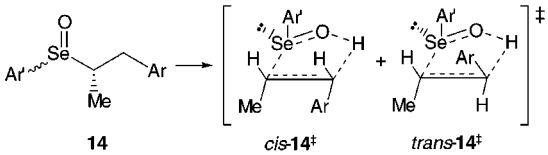
Scheme 7



Scheme 8



Scheme 9



within the SZ-*cis*-39C11 binding pocket that contribute to selective transition state stabilization. The active site of SZ-*trans*-28F8 is less tolerant of substitution at the γ-position of substrate since the rearrangement of **3b** is not detectably catalyzed by this antibody.

Selectivity. The low K_m values and the fact that compound **13** is not a substrate for SZ-*trans*-28F8 or SZ-*cis*-39C11 indicate that the aryl rings contribute significantly to productive binding. They are also clearly important for imparting selectivity and catalytic proficiency to the antibody-catalyzed reactions. Although the rate accelerations for the antibody and BSA-catalyzed reactions (k_{cat}/k_{uncat}) are comparable, the catalytic proficiencies of the former are 50−500-fold larger. This difference is manifest most dramatically in the contrasting stereoselectivities of the antibody and BSA-catalyzed conversions. All the antibody reactions, with the exception of the rearrangement of **3a** promoted by SZ-*cis*-39C11, are highly diastereo- and/or enantioselective. BSA, on the other hand, discriminates poorly, if at all, between the stereoisomers of substrates **3a**−**c**. Interestingly, SZ-*trans*-28F8 is considerably more selective than might have been expected based on its ability to bind both enantiomers of *trans*-**8** and its lack of selectivity toward chiral selenoxides;[10] selectivity in the case of SZ-*cis*-39C11 appears to depend critically on the presence of an α- or γ-substituent.

A complete understanding of the origins of the observed selectivities, and how they correlate to hapten recognition, will have to await detailed structural studies on these catalysts. In principle, the [2,3]-sigmatropic rearrangement can occur either via an endo or an exo transition state as shown in Scheme 6 for substrate **3a**. *Endo* transition states are generally favored due to their completely staggered conformations,[12,13,27] but the presence of the β-aryl moiety and the α- or γ-methyl groups as in **3b** and **3c** complicates matters due to competing 1,2- and 1,3-pseudodiaxial interactions.

Although SZ-*cis*-39C11 does not discriminate between the enantiomers of **3a**, the successful transfer of chirality from sulfur to carbon in the rearrangement of sulfoxide **3b** shows that this antibody can distinguish differently shaped transition states. As depicted in Scheme 7, only modest stereoselectivity is expected in the uncatalyzed reaction of **3b** because both substrate enantiomers can yield the same product enantiomer via competing exo and endo transition states, both of which have unfavorable 1,3-pseudodiaxial interactions between the aryl group on the sulfoxide and either the β-aryl or the γ-methyl substituent. In addition to differentiating between the two substrate enantiomers, SZ-*cis*-39C11 apparently stablizes one of these transition states selectively. In contrast, the BSA-catalyzed reaction is nonselective, suggesting a rather loose, nonspecific interaction with substrate. At the other extreme, SZ-*trans*-28F8 does not catalyze the rearrangement of **3b** at all, presumably because steric clashes between the γ-methyl group and the protein pocket prevent the flexible substrate from adopting a conformation suitable for reaction.

For **3c**, we have found that the product of the catalyzed and uncatalyzed reactions is exclusively trans-allylic alcohol **5c**. Since the different **3c** diastereomers react at similar rates in the uncatalyzed reaction (as judged by HPLC), this product must be formed via both endo and exo transition states of similar energy. Scheme 8 illustrates this with two respresentative diastereomers (note that the *exo*-(*S*,*R*) and *endo*-(*S*,*S*) conformers of **3c** would give rise to the cis-allylic alcohol). We assume that the α-methyl substituent will adopt a pseudoequatorial orientation in the accessible endo and exo transition states to avoid a very unfavorable 1,2-diaxial interaction with the aryl group on the sulfoxide. In the antibody catalyzed reaction, only one pair of enantiomers (the same for both antibodies) is recognized and converted to product, again indicating effective discrimination between competing transition states. The fact that the antibodies catalyze the rearrangement of both enantiomers of this isomer, albeit at significantly different rates, allows the prediction to be made that the preferred substrate has the (*S*,*R*) or (*R*,*S*) configuration. The two enantiomeric endo transition states of this diastereomer are more similar to one another, because of the pseudo plane of symmetry bisecting the molecule, than the two enantiomeric exo transition states of the other diastereomer (Scheme 8). If this hypothesis can be verified, it would suggest that an endo transition state may also be preferred in the antibody-catalyzed conversions of **3a** and **3b**.

Although the selenoxide elimination (Scheme 1) and the allylic sulfoxide−sulfenate rearrangement (Scheme 2) share several common features, the corresponding transition states are subtly different in their steric demands as a consequence of the differing hybridization state of the carbon β to the selenoxide/sulfoxide (sp^3 in the selenoxides, sp^2 in the sulfoxides). For selenoxide **14** (Scheme 9), which is analogous to sulfoxide **3c**, minimization of eclipsing interactions between the α-methyl and β-aryl substituents favors formation of the *trans*-olefin product. In the presence of SZ-*cis*-39C11, this intrinsic

preference can be partially overcome to yield a 45:55 mixture of *cis*- and *trans*-olefins, as programmed by the *cis*-hapten.[10] Because only 50% of the substrate is converted to product, this antibody appears to be extremely sensitive to the stereochemical configuration at C-α (the selenoxide moiety epimerizes rapidly under the experimental conditions) in line with the results obtained here for the allylic sulfoxide rearrangement of substrate **3c**. The fact that some *trans* product is also formed suggests that alternative productive binding modes for substrate are accessible, however. Like antibody DB3, which recognizes a series of conformationally distinct steroids with high affinity, SZ-*cis*-39C11 may have a bifurcated binding pocket. In contrast, SZ-*trans*-28F8 catalyzes the elimination of both epimers of **14** and yields only trans product.[10] The greater selectivity observed for the allylic sulfoxide-sulfenate rearrangement suggests that sulfoxides and selenoxides may bind in quite different ways to the SZ-*trans*-28F8 antibody.

Comparison with Other Antibodies. The X-ray structures of antibodies 1F7[5] and AZ-28,[7] which catalyze the Claisen rearrangement of chorismate and the oxy-Cope rearrangement of a 2,5-diaryl-3-hydroxyhexadiene, respectively, have been solved. These structures show how hydrogen bonding, electrostatic, and van der Waals interactions can be exploited within an antibody-combining site to promote sigmatropic rearrangements.

Antibody AZ-28 was elicited with a 2,5-diarylcyclohexanol derivative, and as we observe for the antibodies reported here, the aryl groups of the ligand appear to be key epitopes for immune recognition and catalysis.[4,7] The hapten binds to the immunoglobulin in a deep cylindrical pocket. Its 5-phenyl substituent sits at the bottom of the pocket tightly packed by the side chains of several aromatic and hydrophobic residues. The cyclohexyl ring adopts the expected chair conformation with the hydroxyl and aryl moieties in equatorial orientations. The hydroxyl group is hydrogen bonded to the NH backbone and the imidazole side chain of HisH96. This same imidazole also packs against the 2-phenyl substituent, which is located near the entrance of the cavity, through π-stacking interactions.

Although hydrogen bonding to the hydroxyl group of the substrate undoubtedly helps to position the flexible substrate within the AZ-28 binding pocket and may contribute to the 5000-fold rate acceleration in the ensuing oxy-Cope rearrangement, the extensive van der Waals contacts with the C-2 and C-5 aryl substituents play a critical role in aligning the $[4\pi + 2\sigma]$ orbitals of the hexadiene for reaction.[7] Because the C-2 and C-5 atoms of the substrate are sp^2 rather than sp^3 hybridized as in the hapten, the ground-state conformation of the substrate is unlikely to be optimal for reaction, however. In fact, the germline precursor of AZ-28 binds the transition state analogue 40-times less tightly but catalyzes the reaction with 30-fold greater efficiency than the mature antibody. These effects can be largely accounted for by somatic mutation of active site residue SerL34 in the germline antibody to an asparagine in AZ-28.[7] This residue influences the conformation of the CDR H3 loop, which in turn orients the 2-phenyl substituent of the ligand.

By analogy with AZ-28, we expect that the conformations adopted by the flexible selenoxides **1** and sulfoxides **3** at the active sites of SZ-*trans*-28F8 and SZ-*cis*-39C11 will be determined primarily by extensive van der Waals interactions with the aryl groups of the ligand. While such interactions must be responsible for the observed selectivities, they may limit catalytic efficiency by locking the flexible substrates into a suboptimal ground state conformation (as is apparently the case for AZ-28). This may simply reflect imperfect mimicry of the transition states for the corresponding [2,3]-sigmatropic rearrangements by the haptens. Interestingly, unanticipated but favorable interactions between the γ-methyl group in compound **3b**, which lacks a counterpart in hapten *cis*-**6**, and the SZ-*cis*-39C11 active site apparently compensate for deficiencies in hapten design to some extent and catalytic effects in excess of the simple medium effect are achieved.

Theoretical investigations of the allylic sulfoxide-sulfenate rearrangement have noted the accelerating effects of a hydrogen bond to the oxygen of the polarized S−O bond in the transition state.[32] The proline ring of haptens *cis*-**6** and *trans*-**6** lacks a suitable counterpart to this bond, so it is unlikely that the active sites of SZ-*trans*-28F8 and SZ-*cis*-39C11 possess an analogue to HisL96 in AZ-28 capable of hydrogen bonding with the sulfoxide oxygen. It will be interesting to see whether, with proper instruction, such an interaction might be deliberately exploited within the antibody combining site to further augment catalytic efficiency.

Perspective. Catalysis of the Evans−Mislow rearrangement extends the repertoire of antibody-catalyzed reactions to a new pericyclic reaction for which natural enzymes are unknown. The experiments presented here show how conformational constraint imposed by an antibody combining site can be exploited in combination with a simple medium effect to achieve significant rate accelerations and selectivities. Although these effects exceed those attained by serum albumins, significant improvements in hapten design and screening will be necessary to identify catalysts with truly enzyme-like properties.

Acknowledgment. We thank Ning Jiang for preparation and purification of the antibodies and Dr. G. Schlingloff for help in determining enantiomeric excesses. This work was supported by the National Institutes of Health (GM38273 to D.H.), Novartis Pharma, and a fellowship of the scientific board of NATO by the DAAD (to A.F.).

JO991299A