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Formation of Bridge-Methylated Decalins by Antibody-Catalyzed Tandem Cationic Cyclization

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Abstract: We report the antibody catalysis of an electrophilic tandem ring forming process that yields a bicyclic ring system at neutral pH. Three closely related decalin systems that represent rings A and B of the steroid nucleus account for 50% of the overall products. The linear diene substrate has been designed to mimic the first two isoprene units of 2,3-oxidosqualene, where the epoxide oxygen has been substituted by an arylsulfonate as leaving group. The hapten is based on a decahydroquinoline system with an *N*-oxide functionality as the key structure to elicit a combining site architecture capable of promoting leaving group release. The k_{cat} for the formation of sulfonic acid in the catalyzed reaction was determined to be 0.021 min^{-1}. The efficiency of the antibody-catalyzed process is underscored by the fact that the bicyclic products are not formed in the absence of the antibody catalyst under our mild conditions.

Introduction

The conversion of 2,3-oxidosqualene to lanosterol or cycloartenol as part of sterol biosynthesis is one of the most demanding processes controlled by an enzyme (2,3-oxidosqualene-lanosterol cyclase, OSC) because in a single step six new stereocenters and four carbon−carbon bonds are formed.[1] In this regard, we have been interested in eliciting antibodies that catalyze[2] these complex processes. Initially, we have focused on cationic monocyclization reactions where antibodies were shown to control the reaction with remarkable efficiency.[3] In these experiments, antibodies catalyzed highly selective syntheses of cyclohexanol, cyclopentylmethylcarbinol, and, most remarkably, a *cis*-fused cyclopropanocyclopentane.[4] We suggested that these reactions proceeded via a protonated cyclopropane intermediate that was selectively partitioned along distinct reaction coordinates by the antibody catalyst. Subsequently we were able to cyclize terpenoid-like substrates to form the cyclohexene core structures of the α- and γ-irones.[5] We now extend these studies to achieve tandem cationic cyclizations to form bridge-methylated bicyclic systems that resemble rings A and B of the steroid nucleus (Figure 1).

Results and Discussion

Polyene cyclization can be broken up into three events, initiation, propagation, and termination.[6] While each of these processes is distinct, the success of a polyene cyclization reaction still depends on the complex interplay between the method of initiation, the nucleophilicity of the double bonds involved, and

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(3) Li, T.; Lerner, K. D.; Janda, K. D. *Acc. Chem. Res.* **1997**, *30*, 115−121.

(4) Li. T.; Janda, K. D.; Lerner, R. A. *Nature* **1996**, *379*, 326−327.

(5) Hasserodt, J.; Janda, K. D.; Lerner, R. A. *J. Am. Chem. Soc.* **1996**, *118*, 11654−11655.

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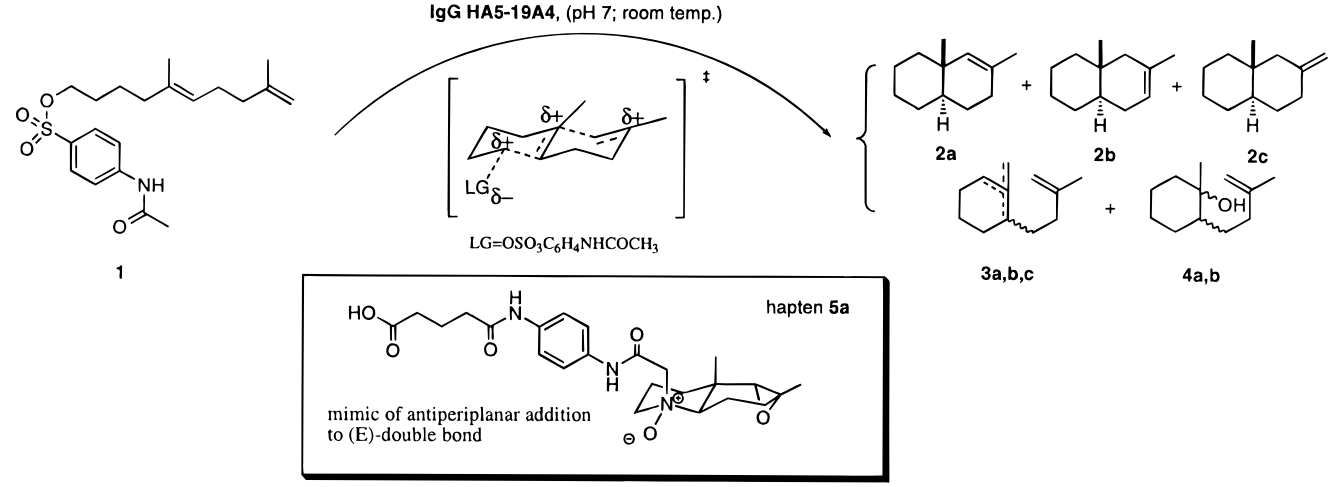


Figure 1. Antibody-catalyzed tandem cationic cyclization.

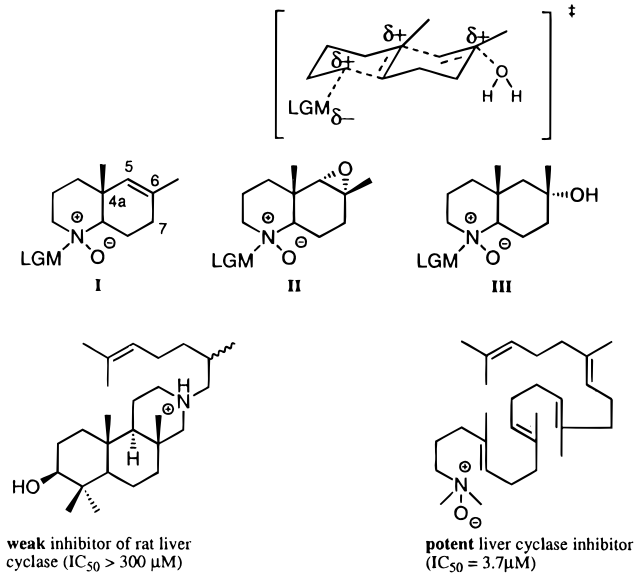


Figure 2. Hapten design and comparison with structures of known inhibitors of natural squalene cyclases (LGM = leaving group mimic).

the mechanism of termination. To properly address these points in our hapten-design considerations, we started with structures I−III (Figure 2). On the basis of inhibition studies of a variety of cyclase enzymes[1a] (Figure 2), we reasoned that a key feature of the initiation event is the stabilization of the first carbocation. For this purpose, we relied on our concept of bait-and-switch catalysis[7] which we have previously shown to be successful in eliciting antibodies that accomplish monocyclization processes.[8] Thus, the *N*-oxide moiety displayed within structures I−III is appropriately positioned to elicit amino acid residues in the antibody combining site for the purpose of initiating the cyclization reaction via solvolysis of the sulfonate functionality found within **1**. *N*-Oxides have also been used in inhibition studies of natural oxidosqualene cyclases according to the example given in Figure 2.[1a]

Unlike cyclization processes in which a single ring is formed, a tandem process also requires control over the propagation event. Given that the activation energy for initiation (formation of the first cationic center) has already been overcome by the antibody, the process of ring cyclization can be envisaged to be essentially a relaxation process. Here, the role of the catalyst is to enforce the appropriate conformation of the substrate **1** (Figure 1) and stabilize any developing cationic centers during the cyclization cascade.[9] The second six-membered ring in the hapten design and its functionality at C5 and C6 were designed to induce an antibody combining site that controls this stage in the reaction. The half-chair conformation of the second ring of structures I−III is expected to induce a binding pocket that causes the otherwise extended conformation of substrate **1** to adopt the pseudo-chair conformation in which the terminal double bond is properly positioned to capture the developing second carbocation as the cascade proceeds.

The termination step generally gives rise to either olefinic (elimination) or hydroxyl (water addition) products and should therefore be represented in the hapten in a comparable fashion (I or III, Figure 2) to elicit hydrophobic or polar amino acid residues in the binding pocket, respectively. Structure II may provide a microenvironment of intermediate hydrophilicity because the epoxide functionality can accept but not donate a hydrogen bond. Geometrically, structure II still resembles I because carbon atoms 4a and 5−7 and the 6-methyl substituent are positioned only slightly out of plane compared to structure I.[10] Structure II has the advantage of having little similarity with the possible products (alcohols or olefins), thereby minimizing the chance of product inhibition. Moreover, structure II might lead to an enhanced immune response by covalent interaction in the antibody binding pocket (epoxide opening by a nucleophile), thereby functioning as a reactive immunogen.[11] Finally, the linker, by which the hapten is coupled to a protein for immunization, is positioned distal to the second ring, thus favoring an antibody binding pocket in which the second olefinic process might be placed at a deeply buried site in the protein. In essence this would help to exclude water and favor termination by an elimination event.

To test these ideas, five specific haptens that represent the *trans*- and *cis*-fused isomers of structures I and II have been utilized for antibody induction. Their synthesis will be presented elsewhere.[12] One of the five candidates, a diastereomeric mixture (1:1) of **5a**,**b** belonging to category II (Figure 2 and

(7) Janda K. D.; Weinhouse M. I.; Danon T.; Pacelli K. A.; Schloeder D. M. *J. Am. Chem. Soc.* **1991**, *113*, 5427−5434.

(8) (a) Li T.; Janda K. D.; Ashley J. A.; Lerner R. A. *Science* **1994**, *264*, 1289−1293. (b) Li, T.; Hilton, S.; Janda, K. D. *J. Am. Chem. Soc.* **1995**, *117*, 3308−3309.

(9) For two different explanations for carbocation stabilization, see: (a) Johnson, W. S.; Telfer, S. J.; Cheng, S.; Schubert, U. *J. Am. Chem. Soc.* **1987**, *109*, 2517−2518. (b) Dougherty, D. A. *Science* **1996**, *271*, 163−168.

(10) See X-ray structure of synthesis intermediate **14a** in: Hasserodt, J.; Janda, K. D. *Tetrahedron* **1997**, in press.

(11) Wirsching, P.; Ashley, J. A.; Lo, C.-H.; Janda, K. D.; Lerner, R. A. *Science* **1995**, *270*, 1775−1782.

(12) For a complete listing of hapten structures and their synthesis, see ref 10.

Scheme 1

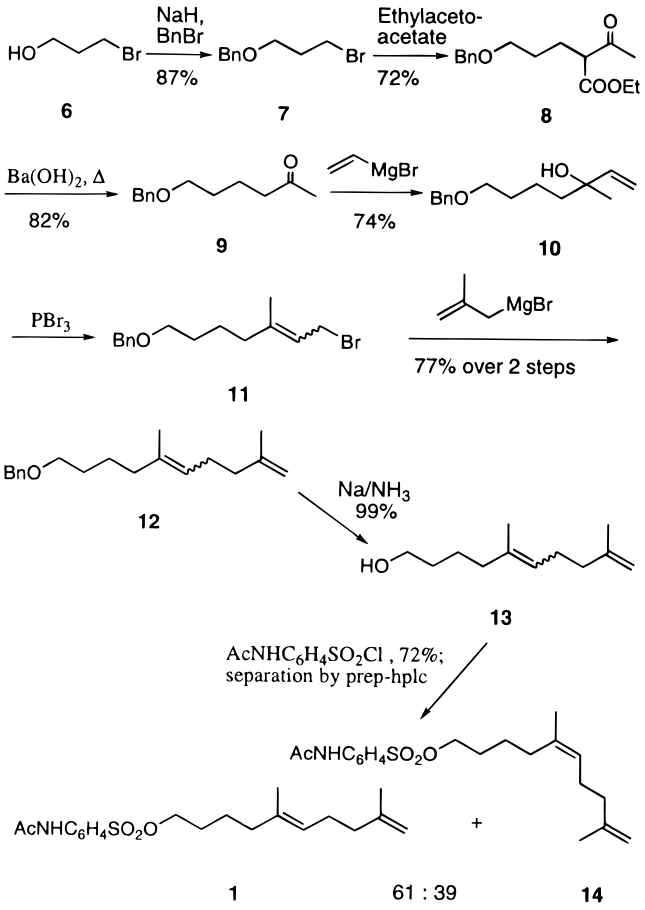
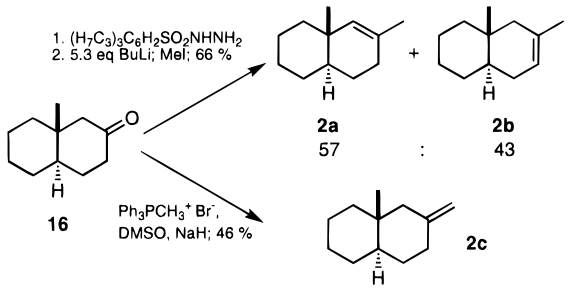


Figure 4), successfully elicited several catalytic antibodies with specificity for substrate **1**. All monoclonal antibodies generated against two haptens belonging to category I did not show any catalytic activity toward solvolysis of substrate **1** but are currently being checked for activity toward related substrate structures. Attempts to synthesize hapten structures of category III, that reflect the outcome of an antiperiplanar addition (as is required by the Stork−Eschenmoser hypothesis) of a water molecule to the terminal double bond, have been unsuccessful.

Diastereomers **5a**,**b**, only differing in the configuration at the tertiary *N*-oxide center, were coupled to the carrier protein KLH (keyhole limpet hemocyanin) and *coimmunized* as racemates.[13] The half-life of hapten **5a** was determined under physiological conditions (37 °C, PBS) to be approximately 100 h, which strongly suggests that the immune system encountered the epoxide structure and not one of the two possible hydrolysis products.[14] Twenty-two monoclonal antibodies were selected from the immune response for their affinity to bind to **5a**,**b**−BSA conjugate (bovine serum albumin) by ELISA methods.[15] When tested for their ability to enhance the solvolysis of substrate **1** to form acetamidobenzenesulfonic acid, three of the antibodies were found to be catalytic. HA5-19A4 was by far the most active and was subsequently studied in more detail.

Substrates **1** and **14** were synthesized (Scheme 1) according to a scheme outlined by Johnson and co-workers[16] for a homologous compound. The key step involves the transformation of the tertiary allylic alcohol **10** to the primary allylic bromide **11**, creating a complete isoprene moiety (C_5) that can be coupled to a second, incomplete isoprene moiety (C_4) by reaction with methallyl magnesium bromide. The lack of a methyl group on this terminal isoprene unit results in simplified product analysis and should have no substantial electronic effects on the finally evolving tertiary carbocation in the cyclization process. The two methyl substituents of the first isoprene unit of **1** were omitted in order to suppress background solvolysis involving a stable tertiary carbocation. The finally obtained *Z*- and *E*-mixture of **13** was separated as its mixture of sulfonates (**1** and **14**) by preparative HPLC to 95% purity in one purification step.

Scheme 2



The antibody catalyst released sulfonic acid from **1** but not from the *Z*-configurated **14**, suggesting that only **1** is a cyclization substrate for this antibody. Release of the leaving group is a necessary but not sufficient requirement for the formation of carbocycles from the hydrocarbon portion of **1**. Consequently, we analyzed the organic phase of our biphasic assay.[17] To resolve and reliably assign the major products of the antibody-catalyzed reaction by gas capillary chromatography, we produced 10 mg of organic products by antibody catalysis using **1** as a substrate. These products were separated by silica gel chromatography into an olefinic fraction (one spot by thin layer chromatography, containing several unresolved species), accounting for 70% of the product mixture and four pure alcohols (30%).[18] As was established by GC analysis of the olefinic fraction, the closely related, regioisomeric decalins **2a**−**c** were the major antibody products as they represent 70% of the olefinic mixture, therefore accounting for 50% of the overall product mixture including hydroxylated products. The identity of each olefin was determined by comparison to authenic samples synthesized by an independent route (Scheme 2): Decalone **16** was obtained through a known procedure[19] and then converted to a 57:43 mixture of **2a**,**b** by a modified Shapiro reaction.[20] This unequal ratio enabled us to assign the two corresponding GC signals of the authentic compounds **2a**,**b** unambiguously (see NMR spectra in Supporting Information). Furthermore, transformation of **16** to **2c** by means of a Wittig

(13) For a detailed procedure, see Supporting Information of ref 5.

(14) See Supporting Information for a plot of the decrease of **5a** over time.

(15) Köhler, G.; Milstein, C. *Nature* **1975**, *256*, 495−497.

(16) Brady, S. F.; Ilton, A. I.; Johnson, W. S. *J. Am. Chem. Soc.* **1968**, *90*, 2882−2889. The authors never pursued that synthesis scheme further because of the difficulty in separating the configurational isomers first formed in the transformation of allylic tertiary alcohols to primary allylic bromides (see: Ruzicka, L.; Firmenich, G. *Helv. Chim. Acta* **1939**, *22*, 392−396). Instead they adapted the stereospecific Julia olefin synthesis to their system (Julia, M.; Julia, S.; Guegan, R. *Bull. Soc. Chim. Fr.* **1960**, 1072−1079). We chose the former pathway to obtain the *Z*-isomer, too.

(17) 83% hexane/2% chloroform/15% phosphate buffer, 50 mM, pH 7.1; shaken in a sealed Teflon tube for defined time units. See also: Ashley, J. A.; Janda, K. D. *J. Org. Chem.* **1992**, *57*, 6691−6693.

(18) For a presentation of the TLC and the three alcohol structures, see also the Supporting Information.

(19) (a) Nagata, W.; Kikkawa, I.; Fujimoto, M. *Chem. Pharm. Bull. (Tokyo)* **1963**, *11*, 226−235. (b) Nagata, W.; Kikkawa, I. *Ibid.* **1963**, *11*, 289−293.

(20) Chamberlin, A. R.; Stemke, J. E.; Bond, F. T. *J. Org. Chem.* **1978**, *43*, 147−154.

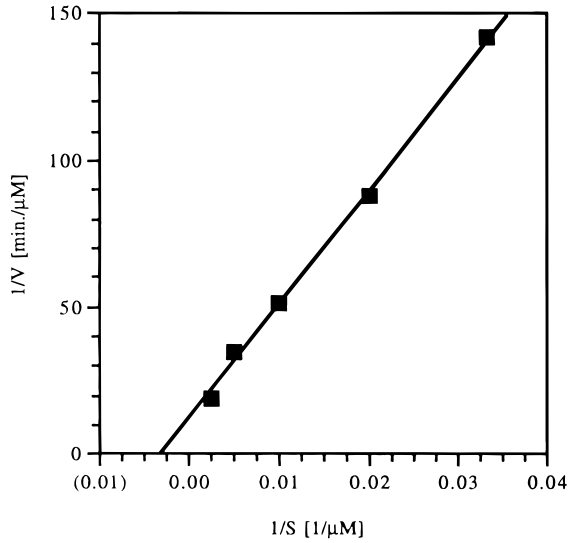


Figure 3. Lineweaver−Burke plot of leaving group release from **1** catalyzed by IgG HA5-19A4 (2 μM). Biphasic conditions: 83% hexane, 15% phosphate buffer (50 mM, pH 7.0), 2% $CHCl_3$, room temperature.

reaction led to the complete elucidation of the bicyclic products formed by antibody HA5-19A4 as well as their ratio.[21]

Three of the four alcohols were identified by ^{1}H NMR as follows: They contain the unaltered terminal double bond but lack the olefinic proton signal of the internal double bond in **1**; two of them are therefore assigned diastereomeric cyclohexyl alcohols **4a**,**b** (Figure 1), and one is tentatively assigned a six-membered ring structure[18] resulting from 1,2-hydrogen shift prior to water addition. The fourth alcohol could not be characterized due to immeasurable low amounts after isolation.

Compounds **2a**,**b**,**c** were formed by the antibody in a ratio of 2:3:1, which is in accord with the expected stabilities of these regioisomers. This suggests that the antibody combining site does not provide tight regiospecific control on the deprotonation process as part of the termination. The formation of monocyclic but not bicyclic alcohols may be explained by the partial accessibility of the combining site to water. The epoxide functionality of **5a** may leave polar space in the binding pocket close to the intermediate carbocation of the cyclization process but not the final carbocation. Under the current reaction conditions (pH 7.0, biphasic, 50 mM phosphate buffer), the regioisomeric olefins **2a**,**b** were formed with an average[22] enantiomeric excess of 53%, determined by means of chiral gas capillary chromatography; **2c** yielded an ee of 80%.

The kinetic characterization of IgG HA5-19A4 was based on sulfonic acid release. A Lineweaver−Burke plot (Figure 3) yielded a k_{cat} of 0.021 min^{-1} and a K_M of 320 μM. The k_{uncat} for solvolysis was determined to 9.2×10^{-6} min^{-1}, giving a rate acceleration for leaving group release of 2.3×10^3. The rate acceleration for formation of the decalin products must be greater than the observed k_{cat}/k_{uncat} for solvolysis since **2a**,**b**,**c** cannot be detected in the uncatalyzed reaction under our assay conditions. Initial inhibition studies were performed on both hapten isomers originally used as a mixture for coimmunization (Figure 4). A dose−response plot[23] shows that hapten **5a** is a

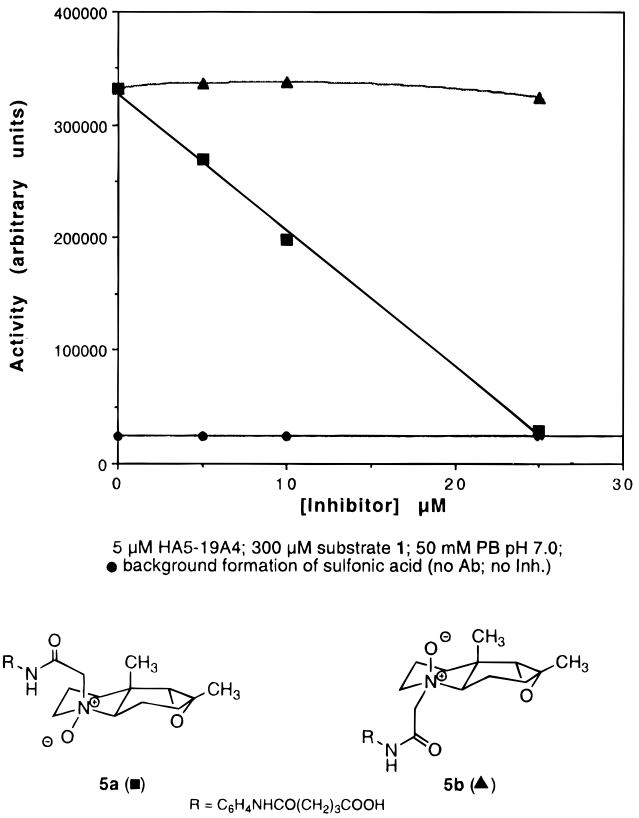
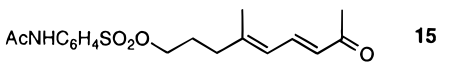


Figure 4. Inhibition study of IgG HA5-19A4 with haptens **5a**,**b**.

potent inhibitor of catalysis (K_i = 1.4 μM, IC_{50} = 8.2 μM) whereas **5b** has no impact on IgG HA5-19A4 catalysis, strongly suggesting that **5a** induced the antibody we studied. The single inversion at the tertiary *N*-oxide center causes the antibody to distinguish between guest and nonguest, thereby demonstrating the remarkable precision with which antibodies achieve complementarity and catalysis. Although the Stork−Eschenmoser concept requires the leaving group to be expelled pseudo-equatorially, our hapten design favors axial departure of the leaving group. It is likely that the $N(O)CH_2CONH$ moiety of the axial leaving group mimic leaves enough conformational flexibility for the system to sufficiently resemble the transition state with pseudo-equatorial extrusion of the sulfonate. Indeed, kinetic analyses from our labs have shown that catalytic antibodies may display complex binding modes including such phenomena as *induced fit*.[24]

Finally, compound **15** was found not to be a substrate for IgG HA5-19A4, even though it carries a number of common structural features with **1**. Instead, **15** functioned as a competi-



15

tive inhibitor (K_i = 24 μM, IC_{50} = 32 μM) when added to the reaction with substrate **1**, proving that the antibody binds to **15** but does not promote its cyclization. This suggests that the antibody does not form an initial carbocation by mere leaving group cleavage but rather argues for anchimeric assistance of the internal double bond in **1**. The trisubstituted double bond in **15** is apparently not as nucleophilic; thus, the higher energy of activation is not compensated by the antibody due to a lack of electronic and steric complementarity to the transition state, involving a six-membered rather than a five-membered ring system.

(21) Johnson, W. S.; van der Gen, A.; Swoboda, J. J. *J. Am. Chem. Soc.* **1967**, *89*, 170−172. The authors prepared a mixture of **2a**,**b**,**c** from **16** by reaction with methyllithium and subsequent elimination.

(22) The major enantiomers of **2a**,**b** as one group as well as their minor enantiomers as the other could not be completely resolved either with trifluoroacetylated γ-cyclodextrin nor with methylated β-cyclodextrin as chiral immobile phase; therefore, an average ee was calculated from the combined peak areas.

(23) Copeland R.; Lomabardo D.; Giannaras J.; Decicco C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1947−1952.

(24) Wirsching, P.; Ashley, J. A.; Benkovic, S. J.; Janda, K. D.; Lerner, R. A. *Science* **1991**, *252*, 680−685.

Conclusion

This paper describes an antibody catalyst accelerating the transformation of a linear polyisoprenoid-like substrate (**1**) to bicyclic olefins. This is to be contrasted to previous studies on acid-catalyzed cyclizations of analoguous systems in organic solvents in which the monocyclic alcohols were observed as the major products.[25] The success of an antibody catalyst can be attributed to the ability of the experimenter to program a binding pocket that can exert control over several features of the transition state on the reaction coordinate, thereby favoring the desired product distribution. In the present case, the major differences between the catalyzed and the uncatalyzed reaction would appear to involve both the ability of the antibody to catalyze initiation and favor the second electrophilic addition. These events may be concerted in that the presence of an electron-rich double bond is required for efficient sulfonate release.

The generation of catalytic antibodies with cyclase activity and investigations into their mechanism can already be expected to contribute answers to the question how proteins can direct electrophilic cyclizations. In addition, such catalysts may ultimately lead to artificial enzymes that process naturally occurring polyisoprenoid substrates to various useful terpenoid and steroid structures. Thus, our focus now turns to the challenge of creating an artificial biocatalyst for steroid formation from oxidosqualene-type substrates.

Experimental Section

General Procedures. The 500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR spectra were recorded on a Bruker AMX-500 instrument. Chemical shifts (δ) are given in parts per million relative to $CHCl_3$ in $CDCl_3$ (7.27 ppm, ^{1}H; 77.00 ppm, ^{13}C). Signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), qnt (quintet), m (multiplet), and ψ (pseudo-coupling pattern). High-resolution mass spectra (HRMS) were recorded at The Scripps Research Institute on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) conditions.

All reactions were monitored by thin-layer chromatography (TLC), using 0.25 mm Merck silicagel glass plates (60F-254), fractions being visualized by UV light or staining with *p*-anisaldehyde or phosphomolybdic acid solutions with subsequent heat application. Column chromatography was carried out with Mallinckrodt SilicAR 60 silicagel (40−63 μm). Reagent grade solvents for chromatography were obtained from Fisher Scientific. Reagents and anhydrous solvents were obtained from Aldrich Chemical Co. and used as is. All reactions were carried out under anhydrous conditions and an atmosphere of argon, unless otherwise noted. Reported yields were determined after purification to homogenous material.

3-(Benzyloxy)propyl Bromide (7). NaH (60% disperson in mineral oil, 6.70 g, 167.5 mmol, 1.29 equiv) is placed in a 1 L one-necked round-bottom flask, equipped with a large stirring bar, washed three times with 15 mL of pentane, dispersed in 550 mL of DMF anhydrous, and kept under nitrogen. Benzyl bromide (23.03 g, 134.7 mmol, 1.03 equiv) is added, and the mixture is cooled to −78 °C under vigorous stirring, followed by the dropwise addition of 3-bromo-1-propanol (18.07 g, 130.0 mmol) over 2 h. The reaction flask is kept in the cooling bath which gradually warms to room temperature (rt) over 4 h, and stirring is continued overnight. Then, 500 mL of water and 450 mL of hexanes are added. After phase separation, the aqueous/DMF phase is reextracted with 100 mL and 50 mL of hexanes and the combined organic phases are washed with 250 mL and 125 mL of brine, dried over $MgSO_4$, and evaporated to give **7** as a light yellow oil containing, according to TLC (R_f = 0.45 hexanes/EtOAc, 7:1) and proton NMR spectrum, 10% benzyl bromide (25.98 g, 102.0 mmol of **7**, 78%). This material is introduced into the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.15 (2H, qnt, J = 6.0 Hz), 3.55 (2H, t, J = 6.6 Hz), 3.62 (2H, t, J = 5.8 Hz), 4.54 (2H, s), 7.31−7.39 (5H, m).

5-(Benzyloxy)-2-(2-oxoethyl)pentanoic Acid, Ethyl Ester (8). Anhydrous ethanol (46 mL) is placed in a 250 mL two-necked round-bottom flask, equipped with reflux condenser, drying tube, magnetic stirring bar and septum, and small pieces of sodium (2.05 g, 89.0 mmol) are dissolved in it. Ethyl acetoacetate (11.58 g, 89.0 mmol) is added in one portion, and the mixture is heated to reflux before **7** (22.50 g, 98.2 mmol, 1.1 equiv) is added gradually via syringe. Refluxing is continued for 8 h followed by filtration from the precipitate, evaporation *in vacuo*, redissolution in ethyl acetate, and filtering through Celite. The crude product is purified using silica gel chromatography (pentanes/EtOAc, 20:1 → 2:1) to yield **8** as a colorless oil (17.74 g, 63.8 mmol, 72%, R_f = 0.67 CH_2Cl_2/EtOAc (9:1), stains yellow-green with anisaldehyde). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.27 (3H, t, J = 7.1 Hz), 1.61 (2H, m), 1.96 (2H, ψ-q, J = 7.6 Hz), 2.22 (3H, s), 3.47 (1H, t, J = 7.5 Hz), 3.49 (2H, td, J = 6.3, 1.5 Hz), 4.19 (2H, qd, J = 7.1, 2.1 Hz), 4.49 (2H, s), 7.28−7.36 (5H, m). ^{13}C NMR ($CDCl_3$): δ 14.0, 25.0, 27.4, 28.8, 59.4, 61.3, 69.6, 72.8, 127.5, 127.6, 128.3, 169.7, 203.1. LRMS (FAB, NBA/NaI): found for $C_{16}H_{22}O_4$ (M + H$^+$) 279.

6-(Benzyloxy)-2-hexanone (9). A mixture of **8** (18.38 g, 66.1 mmol), 50 mL of ethanol (95%), 140 mL of water, and 45 g of $Ba(OH)_2 \cdot 8H_2O$ is heated to reflux for 4 h. Then 350 mL of H_2O, 90 mL of 10% HCl, and 350 mL of Et_2O are added, and the organic phase is washed with brine, dried over $MgSO_4$ to give, upon evaporation, 14.36 g of crude oil, that shows only one stainable spot on TLC (R_f = 0.60 CH_2Cl_2/EtOAc (9:1), grey-brown with anisaldehyde). Distillation with a microdistillation apparatus (120−130 °C, dynamic oil pump vacuum) yields **9** as a colorless oil (11.11 g, 53.86 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.61−1.68 (4H, m), 2.13 (3H, s), 2.46 (2H, t, J = 7.4 Hz), 3.48 (2H, t, J = 6.0 Hz), 4.50 (2H, s), 7.28−7.37 (5H, m). ^{13}C NMR ($CDCl_3$): δ 20.5, 29.1, 29.8, 43.3, 69.9, 72.9, 127.5, 127.6, 128.3, 138.4, 208.9. LRMS (FAB, NBA/NaI): found for $C_{13}H_{18}O_2$ (M + H$^+$) 207.

6-(Benzyloxy)-2-vinyl-2-hexanol (10). A stirred solution of vinylmagnesium bromide (23.27 mL, 1 M in THF, 28.6 mmol) is cooled to 0 °C and **9** (4.80 g, 23.3 mmol), dissolved in 6.4 mL of Et_2O anhydrous, and 3.21 mL of anhydrous THF is added dropwise. Stirring is continued for 2 h at 0 °C and for 3.5 h at rt. The mixture is treated with 5.8 mL of saturated NH_4Cl containing 3 drops of NH_4OH. The organic layer is decanted from the solid precipitate, which is then dissolved in slightly acidic water and extracted with Et_2O. The combined organic phasesare washed with brine and dried over $MgSO_4$, and the solvent is evaporated. Purification is carried out on a silica gel column (pentanes/EtOAc, 5:1 → 2:1) to yield **10** (4.05 g, 17.3 mmol, 74%, R_f = 0.27 hexanes/EtOAc 3:1) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28 (3H, s), 1.40−1.44 (2H, m), 1.53−1.57 (2H, m), 1.60−1.65 (2H, m), 3.48 (2H, t, J = 6.5 Hz), 4.51 (2H, s), 5.05 (1H, dd, J = 10.8, 1.1 Hz), 5.21 (1H, dd, J = 17.4, 1.1 Hz), 5.91 (1H, dd, J = 17.4, 10.8 Hz), 7.29−7.36 (5H, m). ^{13}C NMR ($CDCl_3$): δ 20.6, 27.6, 30.0, 42.0, 70.2, 72.8, 73.2, 111.6, 127.5, 127.6, 128.3, 138.5, 145.1. HRMS (FAB, NBA/NaI): calcd for $C_{15}H_{22}O_2$ (M + Na$^+$) 257.1517, found 257.1528.

Bromide 11. A stirred solution of **10** (2.42 g, 10.3 mmol) in 2.8 mL of hexanes anhydrous at −10 °C, containing 0.26 mL of anhydrous pyridine, is treated with a solution of PBr_3 (1.37 g, 0.48 mL, 5.05 mmol, 1.47 equiv) in 1.4 mL of hexanes. A white precipitate forms immediately. The mixture is warmed to rt over 1.5 h before being cooled to 0 °C, quenched with 0.6 mL of saturated $NaHCO_3$, and extracted with Et_2O. The combined organic phases are washed with brine, dried over $MgSO_4$ to give, upon evaporation, crude **11** as a colorless oil (2.83 g, 9.5 mmol, 92%, R_f = 0.62 hexanes/EtOAc (3:1), stains blue with anisaldehyde), suitable for the next step according to TLC and proton NMR.

(5*E*,*Z*)-5,9-Dimethyldeca-5,9-dienyl Benzyl Ether (12). Magnesium turnings (2.43 g, 100.0 mmol) are suspended in 20 mL of anhydrous THF, and 3-chloro-2-methylpropene (4.94 mL, 4.53 g, 50.0 mmol) in 15 mL of THF is added slowly while stirring at 0 °C. Afterward the mixture is stirred at rt for 1 h, before the supernatant solution is decanted by a syringe and added to a solution of crude **11** (2.83 g, 9.5 mmol) from the previous step in 4 mL of anhydrous THF. After considerable heat evolution has ceased, the mixture is heated to reflux for 0.5 h with a condenser attached to the reaction flask before

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being stirred overnight. The mixture is quenched at 0 °C with saturated NH_4Cl (made basic with a few drops of NH_4OH), extracted with Et_2O, the combined organic phases washed with brine, and dried over $MgSO_4$ to give, upon evaporation, 2.53 g of crude product ($R_f = 0.44$ hexanes/benzene (1:1), stains turquoise with anisaldehyde) as a colorless oil. Silica gel chromatography (hexanes/Et_2O, 20:1 → 3:1) gives **12** (2.15 g, 7.9 mmol, 77% over two steps) as a mixture of *Z*- and *E*-isomers (31:69). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46−1.49 (2H, m), 1.57−1.66 (2H, m), 1.60 (3H, s, *E*-), 1.68 (3H, s, *Z*-), 1.726 (3H, s, *Z*-), 1.734 (3H, s, *E*-), 1.98−2.06 (4H, m), 2.10−2.16 (2H, m), 3.47−3.50 (2H, m), 4.51 (2H, s), 4.69 (1H, s, br), 4.71 (1H, s, br), 5.11−5.15 (1H, m), 7.27−7.31 (1H, m), 7.34−7.36 (4H, m). LRMS (FAB, NBA/NaI): found for $C_{19}H_{28}O$ ($M + Na^+$) 295.

(5*E*,*Z*)-5,9-Dimethyldeca-5,9-dienol (13). A solution of **12** (100 mg, 0.37 mmol) in 1.7 mL of Et_2O anhydrous is added dropwise to a stirred solution of 50 mg of sodium in 5 mL of liquid ammonia and 2 mL of Et_2O anhydrous at −78 °C. The cooling bath is removed and stirring is continued for 0.5 h until 10 mL of saturated NH_4Cl and 10 mL of Et_2O are added cautiously. After evaporation of NH_3, the phases are separated, the aqueous phase is extracted with Et_2O, and the combined organic phases are washed with brine and dried over $MgSO_4$ to give, upon evaporation, **13** as a colorless oil (71 mg, 0.37 mmol, 100%). TLC check shows no side product formation and no starting material left ($R_f = 0.26$ hexanes/EtOAc, 3:1, yellow + blue over one another [*Z*+*E*] with anisaldehyde). LRMS (FAB, NBA/NaI): found for $C_{12}H_{22}O$ ($M + H^+$) 183.

Sulfonates 1 and 14. A solution of **13** (32 mg, 0.18 mmol), in 0.4 mL of pyridine anhydrous is treated with *N*-acetylsulfanilyl chloride (155 mg, 0.66 mmol, 3.8 equiv) and a few crystals of DMAP and stirred for 5 h. The mixture is washed with 5% citric acid and extracted with CH_2Cl_2. The combined organic phases are washed with brine and dried over $MgSO_4$, and the residue is purified, upon evaporation, using silica gel chromatography (hexanes/EtOAc, 15:1 → 1:1) to give a mixture of **1** and **14** as a slightly yellow oil (48 mg, 0.13 mmol, 72%, $R_f =$ 0.28 hexanes/EtOAc (1:1), stains turquoise with anisaldehyde). HRMS (FAB, NBA/NaI): calcd for $C_{20}H_{29}O_4S$ ($M + Na^+$) 402.1715, found 402.1731. Separation of the *Z*- and the *E*-isomers (31:69) is carried out on preparative HPLC [Vydac 201HS1022, 300 Å silica, 10 μm, C_{18}, 250 × 22 mm, isocratic elution of CH_3CN/H_2O (no TFA!) (60:40), flow rate 20 mL/min].

4-(Acetylamino)phenylsulfonic Acid, (5*E*)-5,9-Dimethyldeca-5,9-dienyl Ester (1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (2H, Ψ-qnt, J = 7.8 Hz), 1.55 (3H, s), 1.61 (2H, m), 1.72 (3H, s), 1.93 (2H, t, J = 7.5 Hz), 2.01 (2H, t, J = 7.1 Hz), 2.10 (2H, Ψ-q, J = 7.8 Hz), 2.23 (3H, s), 4.02 (2H, t, J = 6.5 Hz), 4.66 (1H, Ψ-q, br, J = 0.6 Hz), 4.70 (1H, s, br), 5.06 (1H, t, J = 6.9 Hz), 7.68 (1H, s, br), 7.72 (2H, d, J = 8.7 Hz), 7.83 (2H, d, J = 8.7 Hz). ^{13}C NMR ($CDCl_3$): δ 15.7, 22.4, 23.4, 24.7, 26.1, 28.2, 37.7, 38.7, 70.8, 109.8, 119.3, 124.7, 129.2, 130.5, 134.2, 142.8, 145.8, 168.7.

4-(Acetylamino)phenylsulfonic Acid, (5*Z*)-5,9-Dimethyldeca-5,9-dienyl Ester (14). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (2H, m), 1.60−1.66 (2H, m), 1.63 (3H, s), 1.71 (3H, s), 1.99 (4H, Ψ-t, J = 7.9 Hz), 2.04−2.09 (2H, m), 2.24 (3H, s), 4.03 (2H, t, J = 6.4 Hz), 4.66 (1H, Ψ-q, br, J = 0.8 Hz), 4.71 (1H, Ψ-q, br, J = 0.6 Hz), 5.13 (1H, t, br, J = 6.7 Hz), 7.60 (1H, s, br), 7.72 (2H, d, J = 8.7 Hz), 7.84 (2H, d, J = 8.7 Hz). ^{13}C NMR ($CDCl_3$): δ 22.5, 23.2, 23.6, 24.8, 26.0, 28.6, 30.9, 37.9, 70.7, 109.9, 119.2, 125.4, 129.2, 130.6, 134.4, 142.8, 145.7, 168.7.

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Supporting Information Available: A listing of the determinations of K_i and half-life of **5a**, the GC chromatograms of antibody product distribution, the inhibition diagram of **15**, the characterization data of compounds **1**−**2c**, **6**−**16**, and a synthesis scheme of **16** (38 pages). See any current masthead page for ordering and Internet access instructions.

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