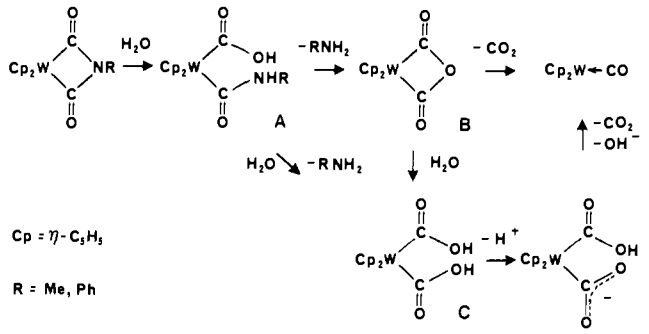
Scheme I. Possible Mechanisms for the Solvolysis of the Metallocycloimides **2** and **3** to the Carbonyl Complex **1**



supernatant was decanted off and the precipitate washed with pentane and vacuum dried to give 0.331 g (0.72 mmol ≡ 87%) of analytically pure $[W(\eta\text{-}C_5H_5)_2[C(O)N(Ph)C(O)]]$ (**3**).[13]

The most reasonable mechanism for formation of **2** and **3** involves initial η^1-C coordination of a Lewis acidic isocyanate to the basic metal center (eq 1). In the zwitterionic intermediate

(1)

the heteroallene is nucleophilic at the β position and the carbonyl is electrophilic at the α position, and subsequent reaction gives a four-membered metalloheterocycle. Ring closure through nitrogen probably reflects the greater strength of C=O bonds as compared with C=N bonds: the isomeric metalloisoimide contains one C=O and one C=N bond in place of the two C=O bonds in **2** and **3**.[14]

Although metallocycloimides might be formed from many carbonyl substrates, we have discovered no other reports of metalloheterocycle formation from the reaction of isocyanates, nor any other heteroallene, with neutral carbonyl complexes. It is known, however, that carbonyls of the group 6 and 8 metals react with isocyanates to form isocyanide complexes,[15] and this reaction has been used as the first step in the catalytic conversion of isocyanates to carbodiimides and CO_2.[16] Both reactions probably proceed through metallocycloisoimides related to **2** and **3**, and metallocycloisoimides and metallocycloimides may be more accessible than previous reports imply.

Hydrolysis of the metallocycloimides is more facile than hydrolysis of cyclic organic imides[17] and does not require basic or acidic catalysis. A THF solution of the *N*-phenyl complex **3** reacts with a large excess of water to regenerate **1** in 1 day. The *N*-methyl complex **2** is less water sensitive than **3** (presumably because the N lone pair is in conjugation with the phenyl ring[17]), and **2** was inert to excess water in acetone at ambient temperatures for 1 day. It was, however, converted cleanly to **1** when the temperature was raised to 55 °C for 20 h. Hydrolysis of cyclic organic imides typically gives amidic acids,[17] and tungsten analogues of such species (A) are reasonable intermediate in the hydrolysis of **2** and **3** (Scheme I). The details of the conversion of A to **1** are unclear at this point, but some possibilities are shown.[18]

Attempts to extend the reaction of **1** with heteroallenes to the preparation of the metalloanhydride B have been unsuccessful. In toluene **1** was inert to 1.7 atm of CO_2 (65 equiv) up to 85 °C, and **1** recovered from a toluene solution after 3 h at 65 °C under 6.5 equiv of $^{13}CO_2$ at a pressure of ca. 0.14 atm did not contain ^{13}CO (MS and IR). We conclude that the exceptional stability of CO_2 precludes metalloanhydride formation by a sequence analogous to that in eq 1.

Acknowledgment. We thank the Office of Naval Research for financial support. We thank Dr. Donald Wink and Dr. Jim Fox for assistance with the X-ray diffraction study.

Registry No. 1, 39333-44-3; **2**, 106865-69-4; **3**, 106880-55-1; MeNCO, 624-83-9; PhNCO, 103-71-9.

Supplementary Material Available: Figures showing atomic numbering scheme and packing within the unit cell; tables of atomic positional and thermal parameters, intramolecular bond lengths and angles, least squares planes, interplanar angles, and angles about tungsten (5 pages); tables of structure factors (11 pages). Ordering information is given on any current masthead page.

(13) ^{1}H NMR (acetone-d_6, 300.13 MHz) δ 7.42-7.36, 7.26-7, 7.10-7.03 (m, 5, C_6H_5), 5.10 (s, 10, $2C_5H_5$); $^{13}C\{^1H\}$ NMR (CD_2Cl_2, 0 °C, 75.47 MHz) δ 176.6 (s, satellites J_{W-C} = 91.6 Hz, C=O), 134.5 (s, C_6H_5), 128.6 (s, C_6H_5), 126.0 (s, C_6H_5), 123.7 (s, C_6H_5), 84.4 (s, C_5H_5). Anal. Calcd for $C_{18}H_{15}NO_2W$: C, 46.87; H, 3.28; N, 3.04. Found (Galbraith Laboratories, TN): C, 47.10; H, 3.30; N, 3.15.

(14) Cyclic organic imides are also more stable than the corresponding isoimides. See, e.g.; Poshkus, A. C.; Herweh, I. E. *J. Org. Chem.* **1965**, *30*, 2466.

(15) Manuel, T. A. *Inorg. Chem.* **1964**, *3*, 1703.

(16) Sayigh, A. A. R.; Tucker, B.; Ulrich, H. *Tetrahedron Lett.* **1967**, *18*, 1731.

(17) (a) Hargreaves, M. K.; Pritchard, J. G.; Dave, H. R. *Chem. Rev.* **1970**, *70*, 439. (b) Challis, B. C.; Challis, J. A. In *Comprehensive Organic Chemistry*; Barton, D., Ollis, W. D., Eds.; Pergamon: Oxford, 1979; Vol. 2, Part 9.9.

(18) The formation of aniline and CO_2 after partial hydrolysis of **3** was confirmed by ^{1}H NMR (aniline) and GC (CO_2) analysis of a sample in acetone-d_6.

Catalytic Antibodies

Jeffrey Jacobs and Peter G. Schultz*

Department of Chemistry, University of California Berkeley, California 94720

Renee Sugasawara and Michael Powell

IGEN Incorporated, Rockville, Maryland

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Antibodies bind biological macromolecules as well as small synthetic molecules with enzymelike affinities and specificities.[1] Moreover, because antibodies can be generated selectively against almost any target molecules of interest, antibodies have become one of the most important classes of receptors in medicine and biology today.[2] The development of strategies for introducing catalytic activity into the combining sites of antibodies might therefore afford a general route to catalysts with *tailored specificities*. We[3] and others[4] recently reported that antibodies that bind phosphate and phosphonate tetrahedral transition-state analogues catalyzed the selective hydrolysis of carbonates or esters. We now report the rational generation of a catalytic antibody that selectively hydrolyzes a predefined substrate. The antibody-catalyzed hydrolytic reaction displays saturation kinetics and substrate specificity and is competitively inhibited by the corre-

* Author to whom correspondence should be addressed.

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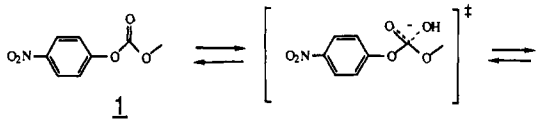
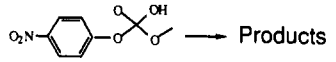
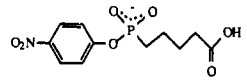
sponding phosphate or phosphonate transition-state analogue.

Monoclonal antibodies were specifically elicited to the tetrahedral nitrophenyl phosphonate transition-state analogue **2** for the aqueous hydrolysis of the corresponding carbonate **1**.



1





2

Preferential binding and stabilization of the transition state arising from attack of water on antibody-complexed substrate **1** should result in a decrease of the free energy of activation for the reaction. Several factors were considered in the experimental design: (1) phosphonates have been reported to act as transition-state inhibitors for proteolytic enzymes;[5] (2) considerable mechanistic information is available on the nonenzymatic hydrolysis of carbonates and esters;[6] (3) carbonates undergo a substantial structural and electronic change on the reaction pathway for hydrolysis, allowing differential binding and stabilization of the transition state; (4) antibody–substrate interactions should exist independent of the transition-state geometry;[7] and (5) a nucleophilic residue is not required for hydrolysis, only water (or hydroxide ion) accessibility to the Ig-complexed carbonate moiety. Characterization of this simplified system should provide insight into both the role transition-state stabilization plays in catalysis and those factors critical to the generation of additional catalytic antibodies.

4-Nitrophenyl phosphonate **2** was synthesized in five steps from triethyl phosphite and methyl 5-bromopentanoate.[8] Phosphonate **2** was then coupled to the carrier proteins bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH) in dilute aqueous HCl, pH 5.0, by using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide, followed by exhaustive dialysis against aqueous 10 mM phosphate, 150 mM NaCl buffer, pH 7.4.[9] Quantitation of the hapten/carrier ratio by hydrolysis of the nitrophenyl phosphonate conjugate typically afforded ratios in the range 15:1. The orientation and length of the five-carbon spacer was chosen to maximize the probability that the antibody would bind the hapten with the phosphonate moiety near the antibody surface and accessible to water.[9,10]

Balb/c mice were immunized with the BSA–phosphonate conjugate emulsified in complete Freund's adjuvant.[11] A fusion was carried out by standard methods using Sp2/0 myeloma as the fusion partner.[12-14] IgG was purified from ascites fluid by affinity chromatography on protein A coupled Sepharose 4B and dialyzed exhaustively against reaction buffer.[15] Antibodies were judged to be homogeneous by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis with Coomassie blue staining.[16]

The rates of hydrolysis of carbonate **1** in the presence (k_{obsd}) and absence (k_{un}) of 4.4 μM antibody were determined as a function of substrate concentration.[17] Carbonate hydrolysis was followed in 10 mM Tris·HCl, pH 8.5 at 30 °C, by following the increase in absorbance at 400 nm due to nitrophenolate ion release. One IgG was found to catalyze the hydrolysis of carbonate **1** with kinetics consistent with the Michaelis–Menten rate expression, (1) The value of k_{cat} and the Michaelis constant, K_m, were found

$$v = \frac{k_{cat}[Ig][\mathbf{1}]}{K_m + [\mathbf{1}]}$$

$$[Ig] + [\mathbf{1}] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Ig\cdot\mathbf{1}] \xrightarrow{k_{cat}} [Ig] + \text{products} \qquad (1)$$

to be 1.4 ± 0.2 min^{-1} and 660 ± 120 μM, respectively. The substrate is therefore reversibly bound by antibody to form a Michaelis complex, followed by intracomplex catalysis (with rate constant, k_{cat}) and release of product. As expected, the catalytic activity of the antibody can be destroyed by heat denaturation at 100 °C. In addition, a control antibody specific for staphylococcal enterotoxin B, which has no appreciable binding affinity for phosphonate **2**, did not catalyze the hydrolysis of carbonate **1**.

The antibody-catalyzed hydrolysis of carbonate **1** was inhibited by the addition of the anionic tetrahedral-transition-state analogue, methyl 4-nitrophenyl phosphate. The dissociation constant K_i for the formation of the antibody–methyl 4-nitrophenyl phosphate complex was determined by measuring the rate of hydrolysis of 215 μM **1** in the presence of 4.4 μM Ig at varying inhibitor concentrations. A Dixon plot[18] afforded a K_i of 3.3×10^{-6} ± 0.8 M at 30 °C in 10 mM Tris·HCl, pH 8.5. Again these results demonstrate that catalysis is occurring in the antibody combining site. Moreover, the differential binding energy of antibody to the transition-state analogue and substrate is consistent with lowering of the free energy of activation for hydrolysis.

The specificity of the antibody-catalyzed hydrolytic reaction was also characterized. Antibody did not catalyze the hydrolysis of 1 mM methyl 2-nitrophenyl carbonate to any appreciable extent at 30 °C in 10 mM Tris·HCl, pH 8.4. The Michaelis constants for the antibody-catalyzed hydrolysis of methyl 3-methyl-4-nitrophenyl carbonate were found to be k_{cat} = 4 min^{-1} and K_m = 7 mM under these conditions. These results are consistent with the highly selective recognition of ligands by antibodies.

The pH dependence of the hydrolysis of **1** was examined in the presence of 4.4 μM antibody and 200 μM **1** between pHs 7.5 and 9.25 in 10 mM Tris·HCl at 30 °C. The k_{cat} of the antibody-catalyzed reaction exhibited a first-order dependence on hydroxide ion concentration whereas K_m was relatively unaffected in this pH range: k_{cat} = 0.2 min^{-1}, K_m = 630 μM (pH 7.5); k_{cat} = 0.5 min^{-1}, K_m = 625 μM (pH 7.9); k_{cat} = 1.4 min^{-1}, K_m = 660 μM (pH 8.5); k_{cat} = 5.9 min^{-1}, K_m = 1.3 mM (pH 9.25). Hydroxide ion may be directly attacking either the polarized carbonyl of the Ig-complexed substrate or an intermediate covalent methyl carbonate antibody adduct. Since the substrate can also be cleaved in the absence of Ig by direct nucleophilic attack of hydroxide ion, we can compare this rate, $v_{uncat} = k_{uncat}[\mathbf{1}][OH^-]$, with the

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(8) 4-Nitrophenyl phosphonate **2** was synthesized by an Arbuzov reaction between triethyl phosphite and methyl 5-bromopentanoate at 156 °C, followed by distillation of product under reduced pressure. Acidic hydrolysis (12.5 M aqueous HCl, 100 °C), followed by removal of solvent and reaction with thionyl chloride, generated the triacid chloride. Subsequent condensation with 4 equiv of nitrophenol afforded the trinitrophenyl ester. The ester was then hydrolyzed (0.2 M aqueous NaOH, 100 °C) and chromatographed by reverse-phase high-pressure liquid chromatography (Bondapak C_{18}, 5–30% CH_3CN gradient in 0.1 M aqueous triethylammonium bicarbonate buffer, pH 7.4) to afford phosphonate **2**.

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(11) Hurn, B.; Chantler, S. *Methods Enzymol.* **1980**, *70*, 104.

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(13) Six of 74 hybridomas were stable producers of antibodies against the KLH–phosphonate conjugate and did not react with BSA in an enzyme-linked immunosorbent assay (ELISA).

(14) Engvall, E.; Perlmann, J. *J. Immunol.* **1972**, *109*, 129.

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(17) Protein molarity was determined by absorbance at 280 nm by using $E_{1\,cm}^{0.1\%}$ = 1.37 and a molecular weight of 150 000 for IgG. Reactions were initiated by adding 10 μL of a stock substrate solution (THF) to the antibody in 0.5 mL of reaction buffer. Kinetic constants were determined by the method of initial rates.

(18) Dixon, G. *Biochem. J.* **1953**, *55*, 170.

rate of hydrolysis in the antibody–substrate complex, $v_{complex} = k_{complex}[complex][OH^-]$.[19] The ratio of $k_{complex}/k_{uncat}$, which reflects the acceleration of hydrolysis by the antibody, is 810. We are carrying out additional experiments to unravel the mechanism of the antibody-catalyzed hydrolysis and are screening 20 additional IgGs for catalytic activity.[20]

We have described the rational generation of a catalytic antibody with predefined specificity. Much work remains to be done to define those elements necessary for the generation of catalytic antibodies with high-turnover numbers and specificity. At the same time we are pursuing additional strategies for introducing catalytic activity into antibodies, including chemical and genetic modification of antibodies. In conclusion, the diversity of receptors that can be generated by the immune system offers tremendous opportunities for investigations of the chemical mechanism of molecular recognition and catalysis.

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(19) The value of $k_{uncat}[OH^-]$ was determined to be 1.75×10^{-3} min^{-1} at 30 °C in 10 mM Tris·HCl, pH 8.5, by extrapolation of the rate of the uncatalyzed reaction to zero buffer concentration.

(20) Generation of monoclonal antibodies against the KLH–phosphonate **2** adduct afforded 20 IgG's, which were inhibitable in a competition ELISA assay. One such IgG catalyzes the hydrolysis of carbonate **1** with $k_{cat} = 29$ min^{-1} and $K_M = 350$ μM, a rate acceleration of 16 000 above background.

Unusual Chemoselectivity Using Difunctional Allylic Alkylating Agents

Barry M. Trost,* Gerald B. Tometzki, and Ming-Hong Hung

Department of Chemistry, University of Wisconsin Madison, Wisconsin 53706

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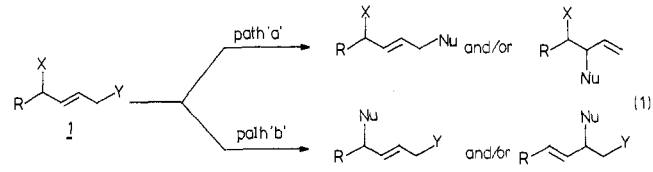
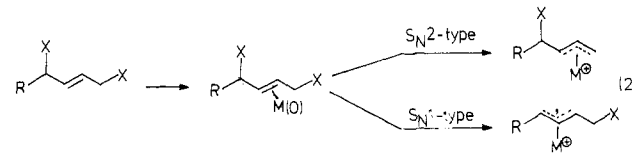
Chemoselective alkylations using difunctional alkylating agents like **1** require differentiating the two leaving groups[1]—a task that may be very cumbersome. A more appealing solution to such a problem would be to have identical leaving groups which may be differentiated simply by modification of reaction conditions to favor path "a" or path "b" (eq 1). An auxillary problem is the question

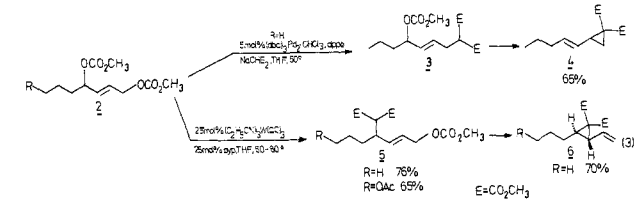


of regioselectivity in such alkylations. Thus, from a single substrate such as **1**, four different constitutional isomers in addition to the stereoisomer and geometrical isomers possible (for a total of eight isomers) may arise. In this paper, we report the remarkable ability to fully control the reactivity of such substrates by appropriate catalyst choice.

In examining the question of metal-catalyzed alkylations, the effect of both olefin substitution and the degree of substitution of the carbon bearing the leaving group must be evaluated. However, for substrates like **1**, only the latter concerns us since ionization of either leaving group occurs from the same olefin–metal(0) complex (see eq 2). The sensitivity of palladium-catalyzed reactions to steric effects[2] led us to propose its ability to ionize would resemble an S_N2-type displacement and, thereby, favor cleavage of the leaving group at the primary carbon. On the other hand, tungsten catalysts showed a greater dependence on electronic effects[3] which should more closely resemble an S_N1-type displacement and, thereby, favor cleavage of the leaving group at the more substituted carbon.

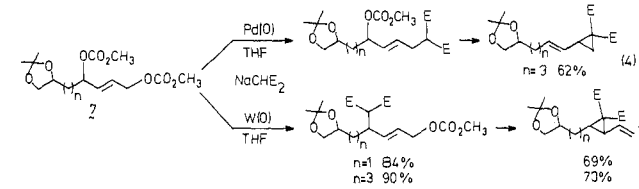


In the event, treatment of the dicarbonate **2** with dimethyl sodiomalonate in the presence of a Pd(0) catalyst at 50 °C led to a mixture of **3** and **4**, the latter arising from further reaction of the former. Allowing the reaction to proceed to completion



produced the vinylcyclopropane **4**[4] as the sole product.[5] Clearly, initial ionization occurred cleanly at the primary carbon. On the other hand, a tungsten catalyst generated by mixing 25 mol % of $(C_2H_5CN)_3W(CO)_3$,[6] 25 mol % of bpy, and 75 mol % of additional propionitrile with the nucleophile[7] and then adding the dicarbonate **2** led cleanly to only **5**.[4,8] While cyclopropane **6**[4] may arise by further treatment with a tungsten catalyst, a smoother conversion occurred using typical Pd(0) conditions.

A similar complementarity occurred with the dicarbonate **7** ($n = 3$) as summarized in eq 4. The W(0)-catalyzed reactions



showed a strong dependence on inductive effects. The electron-withdrawing effect of placing the acetonide directly on the secondary carbon bearing the leaving group (i.e., **7**, $n = 0$) led only

(1) For excellent illustrations of this point related to transition metals, see: Backvall, J. E.; Nystrom, J. E.; Nordberg, R. E. *J. Am. Chem. Soc.* **1985**, *107*, 3676. Ferroud, D.; Genet, J. P.; Kiolle, R. *Tetrahedron Lett.* **1986**, *27*, 23. Tsuji, J.; Shimizu, I.; Minami, I.; Ohashi, Y.; Sugiura, T.; Takahashi, K. *J. Org. Chem.* **1985**, *50*, 1523. Colobert, F.; Genet, J.-P. *Tetrahedron Lett.* **1985**, *26*, 2779. Genet, J. P.; Ferroud, D. *Tetrahedron Lett.* **1984**, *25*, 3579. Tanigawa, Y.; Nishimura, K.; Kawasaki, A.; Murahashi, S. *Tetrahedron Lett.* **1982**, *23*, 5549.

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(4) All new compounds have been fully characterized spectrally and elemental compositions determined by combustion analysis and/or high-resolution mass spectroscopy.

(5) During the course of our studies, the formation of the parent vinylcyclopropane from the dicarbonate of 2-butene-1,4-diol has been reported. However, the question of chemoselectivity of substituted systems has not been addressed. See: Burgess, K. *Tetrahedron Lett.* **1985**, *26*, 3049. Shimizu, I.; Ohashi, Y.; Tsuji, J. *Tetrahedron Lett.* **1985**, *26*, 3835. Also see: Tsuda, T.; Okada, M.; Nishi, S.; Saegusa, T. *J. Org. Chem.* **1986**, *51*, 421.

(6) Cf.: Kubas, G. J. *Inorg. Chem.* **1983**, *22*, 692. We prefer to prepare the catalyst by exchange from $(CH_3CN)_3W(CO)_3$ and propionitrile, analogous to preparation of the benzonitrile analogue. See: Werner, H.; Deckelmann, K.; Schonenberger, U. *Helv. Chim. Acta* **1970**, *53*, 2002.

(7) It is critical to react the tungsten complex with the nucleophile prior to adding the electrophile for optimum results. The exact structure of the active catalyst, presumably an anionic species, will be the focus of future work.

(8) The effect of an adjacent oxygen substituent on the regioselectivity of allylic alkylations has been previously noted for palladium-catalyzed reactions. See ref 1 and: Trost, B. M.; Molander, G. *J. Am. Chem. Soc.* **1981**, *103*, 5969. Tsuji, J.; Kataoka, H.; Kobayashi, Y. *Tetrahedron Lett.* **1981**, *22*, 2575.

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