to V_2O_5 sheets in the orthorhombic V_2O_5 layer structure,[8] raising the possibility that other *nido*-polyoxoanion cages might be derived from other layered structures such as the α-$VOPO_4$ structure.

Preliminary investigations indicate that the $V_{12}O_{32}^{4-}$ host is quite robust and is likely to form a variety of different inclusion complexes not only with other uncharged guest molecules but also with anions capable of bonding to vanadium(V) centers and cations capable of interacting with anionic oxygen centers. Moreover, molecular analogues of redox intercalation reactions of layered compounds such as $VOPO_4 \cdot 2H_2O$[17] or V_2O_5[18] may be possible since the cyclic voltammogram of compound **1** in CH_3CN shows a reversible wave with $E_{1/2} = -0.52$ V vs F_{c^+}/F_c.

Acknowledgment. We acknowledge the National Science Foundation for support of this work. Chris Frank provided invaluable technical assistance.

Supplementary Material Available: Crystal structure analysis report, Table I (atomic coordinates for non-hydrogen atoms), Table II (anisotropic thermal parameters for non-hydrogen atoms), Table III (atomic coordinates for hydrogen atoms of the cations), Table IV (bond lengths and bond angles for the anion), Table V (bond lengths and bond angles for the cations), Table VI (bond lengths and bond angles for acetonitrile solvent molecules), Figure 2 (parts a and b) (perspective ORTEP plots of $(C_6H_5)_4P^+$ cations), Figure 3 (perspective ORTEP plots of the non-hydrogen atoms of acetonitrile molecules), and Figure 4 (perspective ORTEP plot of the anion) (28 pages); table of observed and calculated structure factors for $[(CH_3CN)V_{12}O_{32}][(C_6H_5)_4P]_4 \cdot 3CH_3CN \cdot 4H_2O$ (20 pages). Ordering information is given on any current masthead page.

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Stereospecific Hydrolysis of Alkyl Esters by Antibodies

Scott J. Pollack, Paul Hsiun, and Peter G. Schultz*

Department of Chemistry, University of California Berkeley, California 94720

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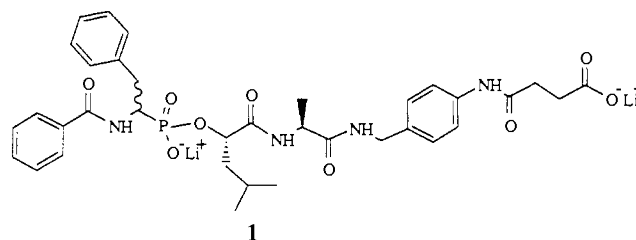
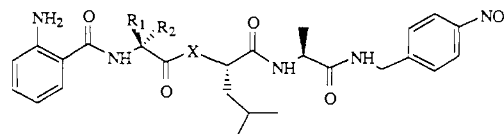
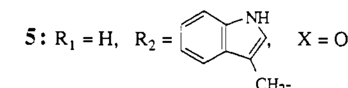
Enzymes are playing an increasingly important role as catalysts in chemistry, due to their ability to catalyze highly selective transformations of complex polyfunctional molecules.[1] However, in many cases enzymes are unavailable for a specific reaction of interest. We[2] and others[3] have recently demonstrated that the high binding specificity of the immune system can be exploited to develop new biological catalysts with tailored specificities. Antibodies have been generated with catalytic groups in the binding site,[4] with cofactor binding sites,[5] semisynthetic antibodies have been generated,[6] and the notions of approximation[7] and transition state stabilization[2,3,8] have been applied to the generation of catalytic antibodies. We now report application of the latter strategy to the generation of antibodies which catalyze the *stereospecific* hydrolysis of *alkyl* esters.[9] Antibodies generated to phosphonate hapten **1** were found to catalyze the hydrolysis of ester **2** with a greater than 200/1 preference for the (*R*)-phenylalanine-containing isomer relative to the (*S*)-phenylalanine-containing isomer.



1

Antibodies were generated against hapten **1**, a tripeptide transition state analogue for the hydrolysis of ester **2** or peptide **3** under basic conditions. Hapten **1** was synthesized as a roughly equimolar mixture of two diastereomers and coupled via its carboxylic acid to the carrier protein keyhole limpet hemocyanin[10] in order to elicit an immune response.[11] Note that the hapten also contains analogues of fluorogenic groups which allow hydrolysis of substrates **2** and **3** to be monitored by observing the fluorescence increase which occurs when the fluorescent 2-aminobenzoyl group is separated from the quenching 4-nitrobenzylamide in the reaction.[12] (This sensitive assay may allow direct screening of ELISA plates for catalytic activity.)



2a: R_1 = H, R_2 = $PhCH_2$-, X = O
2b: R_1 = $PhCH_2$-, R_2 = H, X = O
3a: R_1 = H, R_2 = $PhCH_2$-, X = NH
3b: R_1 = $PhCH_2$-, R_2 = H, X = NH
4: R_1 = H, R_2 = $(CH_3)_2CHCH_2$-, X = O
5: R_1 = H, R_2 =  X = O

Ester hydrolysis was monitored by fluorescence measurement with a Perkin-Elmer LS 5B spectrophotometer using 340 nm for excitation and 415 nm for emission. Reactions were carried out in 0.2 M borate, 0.15 M sodium chloride (BBS), pH 8.0, at 24 °C, in the presence of 3.33 μM antibody.[13] Although none of the 31 IgGs[14] specific for **1** accelerated the hydrolysis of peptide

* Author to whom correspondence should be addressed.
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(13) Reactions were initiated by adding a stock solution (dimethyl sulfoxide) of the ester substrate **2** (5 μL) to the antibody in 0.5 mL of reaction buffer. Fluorescence values for hydrolysis products were calibrated by alkaline hydrolysis (pH 12, by addition of 7 N NaOH [10 μL] to the reaction mixture) followed by adjustment of the pH to assay conditions with 12 N HCl and correcting for dilution. The fluorescence changes upon complete hydrolysis showed a consistent linear dependence on substrate concentration up to 50 μM substrate. Protein molarity was determined from A_{280} ($E_{1cm}^{0.1\%}$ = 1.37) and a molecular weight of 150 000 for IgG.

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Table I. Catalytic Properties of Antibodies

IgG	k_{cat}(**2a**) ($min^{-1} \times 10^3$)	K_m(**2a**) (μM)	K_i(**1**) (μM)	$k_{cat}/[k_{OH^-}[OH^-]]$	k_{2b}/k_{2a}
6E4D5	2.3 ± 0.3	4.4 ± 0.5	0.5 ± 0.1	33	<0.005
3E10D8	7.0 ± 0.7	6.0 ± 0.6	0.8 ± 0.2	100	0.025
3E9F2D10	5.8 ± 0.6	4.5 ± 0.5	1.6 ± 0.4	83	0.018
2H12E4	18.7 ± 2.0	14.8 ± 1.0	2.4 ± 0.6	267	<0.005
2B5B11	9.8 ± 1.0	6.2 ± 0.6	1.4 ± 0.3	140	<0.005

3 to a measurable extent under a variety of conditions, 18 antibodies were found to catalyze the hydrolysis of the corresponding ester **2**.

Interestingly, all of the 18 antibodies found to accelerate the hydrolysis of ester **2** were selective for the D-phenylalanine-containing diastereomer, **2a**, although a mixture of diastereomers was used to elicit the antibodies. One explanation is that haptens containing D amino acids (or analogues thereof) are more immunogenic than those containing L amino acids.[15] Of these 18 catalytic antibodies, five IgGs were selected for further study. All five exhibited saturation kinetics and competitive inhibition by phosphonate **1**, as determined by Eadie–Hofstee and Dixon plots, respectively (Table I).[16] With all the antibodies, multiple (>10) turnovers were observed with no loss of catalytic activity. Because the antibody-catalyzed reaction rate is first-order in hydroxide ion concentration (vide infra), the value of k_{cat} can be compared with $k_{OH^-}[OH^-]$ for the hydrolysis of ester **2a** to give the rate enhancements ($k_{cat}/k_{OH^-}[OH^-]$) shown in Table I.[17] In other antibody-catalyzed reactions, greater rate enhancements can be attributed to a higher degree of differential binding of the antibody to transition state vs ground state and/or the presence of catalytic groups in the antibody combining site. With relatively large transition state analogues such as these tripeptides, the tetrahedral phosphonate probably contributes proportionally less to the overall binding energy of the hapten to the antibody.

The antibody-catalyzed reaction shows a high degree of stereospecificity for the D-phenylalanine-containing ester **2a**. With all five of the antibodies studied, hydrolysis of the L-phenylalanine-containing ester **2b** (30 μM) was catalyzed at <2.5% the rate of **2a** (initial rates) (Table I). With three of the five IgGs, the ratio of **2b**:**2a** hydrolysis rates was less than 0.5%. This value represents the upper limit on the ratio of the **2b**:**2a** rates, since the **2b** rate with these antibodies was essentially indistinguishable from the background hydrolysis rate at pH 8.0. The antibodies show a high degree of substrate specificity. Esters **4** and **5**, containing leucine or tryptophan, respectively, in place of phenylalanine, are not hydrolyzed by any of the antibodies ($k_4/k_{2a} < 0.005$ and $k_5/k_{2a} < 0.005$ at 30 μM substrates, pH 8.0, 24 °C).

The catalytic activity of antibodies 6E4D5, 2H12E4, and 2B5B11 shows a linear dependence on hydroxide ion concentration in the range of pH 7–10.[18] Interestingly, with antibodies 3E10D8 and 3E9F2D10, the pH dependence shows an inflection at pH 9.2. This result suggests the presence of a catalytic amino acid side chain in these two antibodies, such as a tyrosine residue, which could act as a nucleophile, producing a labile tyrosine ester intermediate. Chemical modification of tyrosine residues with tetranitromethane[19] destroyed the catalytic activity of all the antibodies.

We have described the generation of antibodies which catalyze the stereospecific hydrolysis of esters, a potentially useful class of organic reactions for synthetic chemistry. In addition to providing new biological catalysts, these rationally designed antibodies may suggest clues to the evolution and mechanism of enzymes.

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Supplementary Material Available: Synthesis details and characterization (mp, IR, ^{1}H NMR, MS) of compounds **1**–**5** (17 pages). Ordering information is given on any current masthead page.

(14) IgGs were purified from ascites fluid by affinity chromatography using protein A coupled Sepharose 4B[22] followed by Pharmacia Mono Q 10/10 anion exchange chromatography using a linear gradient of 85–150 mM sodium chloride (20 mM Tris, pH 7.8) over 20 min (flow rate of 4.0 mL/min). The antibody-containing fractions were dialyzed exhaustively against assay buffer and judged to be homogeneous by 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis with Coomasie blue staining.[23]

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(17) The rate $v_{uncat} = k_{OH^-}[\mathbf{2a}][OH^-]$ was compared directly to the rate of hydrolysis in the IgG–substrate complex, $v_{complex} = k_{complex}[complex][OH^-]$. The value of the pseudo-first-order rate constant $k_{OH^-}[OH^-]$ was determined to be $(7.0 \pm 0.2) \times 10^{-5}$ min^{-1} at 24 °C, pH 8.0, by extrapolation of the rate of the uncatalyzed reaction to zero buffer concentration.

(18) The rates of hydrolysis were measured at 10 different pH values between pH 7 and 10, in BBS, at 24 °C, with 50 μM **2a** (approximately V_{max} conditions). The slope of log (v_{obs}) vs pH was 0.82 ± 0.05 for all five IgGs except where the rate levelled off at high pH with 3E10D8 and 3E9F2D10 as noted in the text.

(19) The antibodies (20 μM) were treated with 2.0 mM tetranitromethane[24] in BBS, pH 8, for 1 h at 25 °C, followed by dialysis against BBS. In separate experiments, hapten **1** (250 μM) was also present. Greater than 90% of the catalytic activity was retained in the latter cases.

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Infinite Polymeric Frameworks Consisting of Three Dimensionally Linked Rod-like Segments

Bernard F. Hoskins and Richard Robson*

Department of Inorganic Chemistry
University of Melbourne
Parkville, Victoria, 3052, Australia

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We propose that a new and potentially extensive class of solid polymeric materials with unprecedented and possibly useful properties may be afforded by linking together centers with either a tetrahedral or an octahedral array of valencies by rod-like connecting units. For example, if ways could be devised of linking together tetrahedral centers and appropriate molecular rods, an infinite molecule would be generated which might be expected to adopt a structure based on either the cubic diamond lattice or the hexagonal Lonsdaleite lattice in which each C–C bond of the prototype had been replaced by a molecular rod. These frameworks would generate a regular array of cavities, interconnected by windows. When models of 3D networks based on tetrahedral centers are constructed, a very striking feature is the relatively large size of the cavities and windows produced even by rods of only modest length.

Lattices of this general type seemed to us to offer a number of features of sufficient potential interest and utility to warrant attempting the synthesis of some exploratory examples. For instance, they may show interesting molecular sieve and ion exchange properties, they may have unusual mechanical and elec-

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