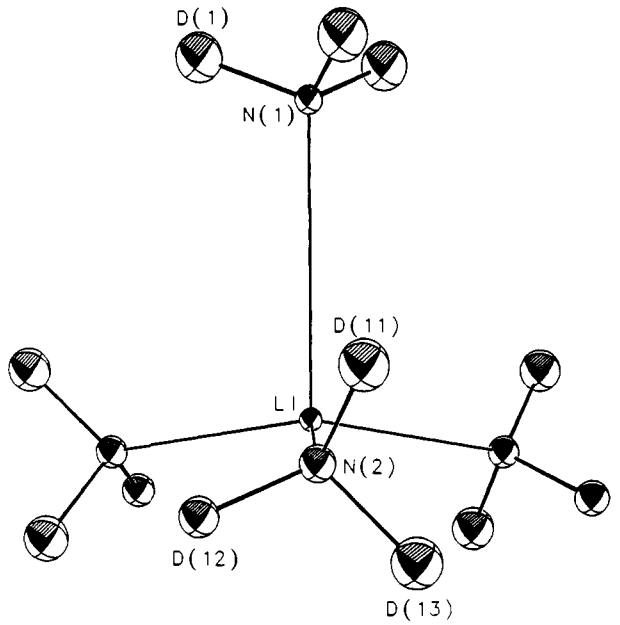

Figure 1. ORTEP drawing of $Li(ND_3)_4$. Selected bond distances and angles are as follows: Li-N(1) = 2.488 (16) Å; Li-N(2) = 1.984 (4) Å; N(1)-D(1) = 0.970 (5) Å; N(2)-D(11) = 0.985 (5) Å; N(2)-D(12) = 0.976 (4) Å; N(2)-D(13) = 1.018 (6) Å; N(1)-Li-N(2) = 98.4 (5)°; N(2)-Li-N(2) = 117.9 (2)°; D(1)-N(1)-D(1) = 108.3 (4)°; D(11)-N-(2)-D(12) = 109.7 (5)°; D(11)-N(2)-D(13) = 97.4 (6)°; D(12)-N-(2)-D(13) = 108.0 (5)°.

Å did not. Next, the rigid-body constraints were replaced with fractional-coordinate soft constraints while all N-D bonds were held at 1.01 (3) Å. This led to a solution having all but one D atom in chemically reasonable locations. Inclusion of data at higher *d* spacings from the 90° bank and evaluation of subsequent difference-Fourier maps led to a proper placement of the remaining D atom. A complete refinement of the structure, including absorption and neutron primary extinction corrections without the application of constraints, was then possible. Isotropic thermal parameters were refined, which led to a weighted residual (R_{wp}) of 4.66% for a combined refinement using both 148° and 90° banks of data.

The three-dimensional drawing shown in Figure 1 shows that all ammonia molecules of an individual $^7Li(ND_3)_4$ complex are coordinated to lithium. Therefore, there is not hydrogen bonding between these complexes in phase II. A most interesting aspect of this refinement is that one N atom bonds to the Li atom at a much longer distance (2.488 Å) than the other three (1.984 Å), so that the $Li(ND_3)_4$ complex has a distorted pyramidal shape. Therefore, this compound is perhaps better described by the formula $^7Li(ND_3)_3{\cdot}ND_3$. The more weakly bound ND_3 can provide some insight into the nature of the phase I-phase II transition that occurs at 82 K in $Li(NH_3)_4$. Upon warming, the weakly bound NH_3 molecules may reorient or become dissociated from the tetraamine unit, which could drive an order-disorder phase transition, as has been suggested for the phase I-phase II transition.[8] This structure determination for phase II permits band-structure and ab initio calculations to be undertaken and sets the stage for a similar refinement of the low-temperature antiferromagnetic phase III of $^7Li(ND_3)_4$. A full account of the structural refinement of phase II of $^7Li(ND_3)_4$ will be published elsewhere.[12]

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Supplementary Material Available: Final difference profile plots for the 148° and 90° detector banks, a table of intermolecular and intramolecular nonbonded distances, and TOF NPD peak profiles (23 pages); a listing of structure factors (5 pages). Ordering information is given on any current masthead page.

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Antibody Catalysis of a Diels-Alder Reaction†

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Recent studies have focused on the mammalian immune system as a source of highly specific, tailored catalysts. With transition-state analogues as haptens, it has been possible to elicit antibodies that promote a variety of chemical transformations, including ester and amide hydrolysis,[1,2] photochemical processes,[3] a sigmatropic rearrangement,[4] and a β-elimination.[5] We report here application of this strategy to the catalysis of a bimolecular [2 + 4] cycloaddition.

The Diels-Alder reaction is one of the most important and versatile transformations available to organic chemists for the construction of complex natural products, therapeutic agents, and synthetic materials of all kinds. It involves concerted addition of a conjugated diene to an olefin to give a cyclohexene derivative. The bimolecular process has a large entropic barrier, with activation entropies typically in the range -30 to -40 cal K^{-1} mol^{-1}.[6] Theoretical considerations suggest that it should be possible to pay for this substantial loss in translational and rotational entropy, and greatly accelerate the rate of reaction, by binding the two reactants together in an antibody combining site.[7]

The transition state of a Diels-Alder cycloaddition is highly ordered, resembling product more closely than starting material.[8]

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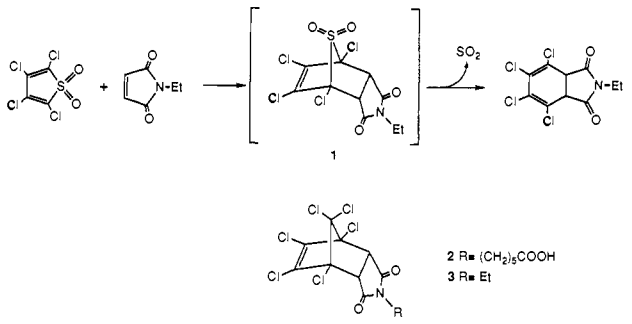

Figure 1.

However, the reaction product is not an appropriate hapten for generating catalytic antibodies, since severe product inhibition would be expected to prevent efficient turnover of the catalyst. An alternate strategy is shown in Figure 1. Tetrachlorothiophene dioxide (TCTD) reacts with *N*-ethylmaleimide (NEM) to give an unstable, bicyclic intermediate **1** that subsequently extrudes SO_2, to give a dihydrophthalimide as product.[9] We reasoned that a stable analogue of the bicyclic adduct could elicit an antibody combining site with the proper shape for promoting the target reaction. As the final product does not closely resemble the transition state of the reaction, product inhibition would be minimized, allowing multiple turnovers of the catalyst.

To test this notion, we prepared five high-affinity monoclonal antibodies against hapten **2**,[10,11] a stable analogue of bicyclic adduct **1**. Because TCTD reacts with lysine residues on the surface of immunoglobulins, it was necessary to reduce the nucleophilicity of the amino groups by exhaustive reductive methylation with formaldehyde and sodium cyanoborohydride.[12] TCTD was shown to be stable in the presence of the methylated antibodies which also retained high affinity for the hapten as judged by ELISA.[13] Chemical modification of immunoglobulins in this way is likely to be of general value, as it will allow reactive molecules like epoxides, Michael acceptors, or other alkylating agents to be employed as substrates for catalytic antibodies.

The methylated antibodies were assayed at 25 °C for their ability to promote the Diels-Alder reaction between TCTD and NEM in aqueous buffer (20 mM MES, 100 mM NaCl, pH 6.0) containing 10% acetonitrile. Cycloadditions were followed by monitoring the disappearance of TCTD at 330 nm and by analytical reverse-phase HPLC.[14] The products of the reaction, dihydro-*N*-ethyltetrachlorophthalimide and the fully oxidized *N*-ethyltetrachlorophthalimide, were isolated and characterized. Sulfur dioxide was detected independently by bleaching of malachite green at 617 nm. In the preliminary assays, one antibody (secreted by hybridoma 1E9) significantly accelerated the rate of reaction over the uncatalyzed process and was characterized further.

Antibody 1E9 promotes the target reaction with multiple (>50) turnovers, and several lines of evidence demonstrate that the observed catalysis is not artifactual. The process is first order with respect to immunoglobulin concentration, and its substrate specificity matches expectations based on hapten structure. Thus, *N*-ethylmaleimide is a good substrate for the catalyst, but maleimide is not. Also, the catalyzed reaction is strongly inhibited by compound **3**,[10] a close analogue of the hapten used for immunization. Preincubation of the antibody (5 μM) with an equimolar amount of **3** stops the catalyzed reaction completely, even at concentrations of maleimide 10^3-fold greater than the concentration of inhibitor. Finally, methylated antibodies other than 1E9 fail to catalyze the cycloaddition.

When initial rates with the antibody were measured as a function of NEM concentration, the concentration of TCTD being held constant, saturation kinetics were observed. At 0.61 mM TCTD, for example, the apparent values of k_{cat} and $(K_m)_{NEM}$ were 4.3 ± 0.3 min^{-1} and 21 ± 4 mM, respectively. Low solubility of TCTD prevented determination of its K_m value and, hence, the true k_{cat} for the reaction. Nevertheless, comparison of $(k_{cat})_{app}$ obtained at 0.61 mM TCTD with the second-order rate constant for the uncatalyzed cycloaddition (0.040 ± 0.007 min^{-1}) yields an apparent effective molarity of at least 110 M/binding site. Because $(k_{cat})_{app}$ is linearly dependent on TCTD concentration in this range, the true effective molarity must be substantially higher than this value.

These experiments provide the first example of an antibody-catalyzed Diels-Alder cycloaddition and demonstrate the feasibility of using antibody technology to promote important nonphysiological reactions. Particularly noteworthy aspects of this study include the following: (1) the design of the hapten so as to minimize product inhibition, and (2) the use of chemically modified antibodies to permit study of reactive substrate molecules. We are currently extending these concepts to other [2 + 4] cycloadditions. Tailored "Diels-Alderase" antibodies will be particularly valuable as tools for studying proximity effects in catalysis and for effecting regio- and stereoselective transformations in organic synthesis.

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(11) The *N*-hydroxysuccinimide ester of hapten **2** was coupled to keyhole limpet hemocyanin, and the resulting hapten-protein conjugates were used to generate an immune response in 129 GIX$^+$ mice. Standard protocols were used to fuse mouse spleen cells with SP2/0$^+$ myeloma cells.[4a] IgG antibodies specific for the hapten were isolated and purified as previously described.[4a] Proteins were judged >95% pure by sodium dodecylsulfate polyacrylamide gel electrophoresis with Coomasie blue staining. Antibody concentration was determined from A_{280} ($\epsilon^{0.1\%}$ = 1.40 mL mg^{-1} cm^{-1}) and a molecular weight of 160 000.
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Photoinduced Destabilization of Bilayer Vesicles

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The polymerization of lipid bilayer vesicles (liposomes) is an effective method to enhance the long-term colloidal and chemical stability of the aqueous suspensions.[1] A host of methods to polymerize vesicles have been described since the first reports in the early 1980s.[2] It was soon recognized that the polymerization of two-component vesicles, where only one component was polymerizable, resulted in phase separation of the lipids into polymeric and monomeric domains.[3-5] The polymerizable lipids form

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