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Search for antibody catalysts for the ene reaction

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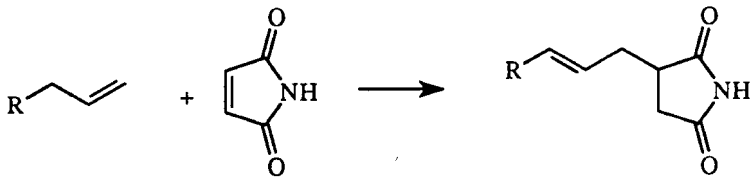
Abstract The design, computational studies and synthesis of transition state analogs for an ene reaction are described. The most promising analogs were used to elicit antibodies that should catalyze an ene reaction between maleimide and 1-decene. Preliminary results indicate that successful catalytic antibodies for the ene reaction can be produced in this manner. In parallel, an antibody library is being developed for the same purpose.

Introduction

In less than ten years, catalytic antibodies[1] (abzymes, or catabs) have become a very actively developing area of organic synthetic methodology. Compared[2] to enzymatically catalyzed reactions, abzymes can offer exciting advantages such as the ability to catalyze "unnatural" reactions, or the fact that a desired specificity can in principle be tailored into a catalytic antibody. On the other hand, the production of abzymes may be time-consuming, laborious, and costly; comparatively large quantities of abzymes are often needed for a catalytic reaction; and so far, no reactions beyond the scale of a gram or two[3] have been realized.

Abzymes have recently been developed for and used in spectacular difficult, or near-impossible, synthetic transformations[4] such as a cyclization[5] that proceeds contrary to the Baldwin rules, or a *syn* elimination in



(1)

an acyclic substrate to give a *cis* olefin.[6] One of the simplest thermal reorganization processes, the all-carbon *ene reaction*[7] (eq 1) discovered by Kurt Alder[8] is another appropriate target for studies on catalysis by abzymes owing to the harsh conditions and low yields usually associated with uncatalyzed alkene ene reactions. Reaction temperatures, typically 200-250 °C, are higher than those needed for the corresponding (and closely related) Diels-Alder reactions. This of course reflects the higher activation energies associated with the ene reactions, arising from the fact that the two electrons of the allylic σ bond replace the two π electrons of the Diels-Alder diene. Although catalysis of both reactions by Lewis acids is now common practice, the yields in the all-carbon ene reactions still remain rather modest in many cases, and reactions involving monosubstituted enes are particularly difficult.

The "classical" way of obtaining catalytic antibodies is based on the design and synthesis of stable molecules (*haptens*) that resemble the transition state (t.s.) of a reaction of interest. The haptens are then used to elicit antibodies by the immune system of a living organism. Since there is now an imprint of the t.s. at an active site of the antibody, the latter should be able to accommodate, and stabilize, the t.s., thus lowering its energy of formation. The reaction will then proceed faster, and the overall process will turn in catalytic cycles if the reaction product does not adhere to the antibody active site.

Reaction mechanism and transition state

To apply these principles to the ene reaction, the reaction mechanism and the t.s. were first examined.[9] Propene was used as a representative 1-alkene and maleimide or maleic anhydride as the enophile. The MP2/6-31G*//HF/6-31G* level of accuracy was highly satisfactory for the *ab initio* reaction modeling, the inclusion of electron correlation correction reducing the activation energy to a great extent. Although the semiempirical AM1 method does not provide accurate reaction energetics, the t.s. geometries were found to be in good agreement with the corresponding HF/6-31G* structures. The difference between the *endo* and *exo* activation energies is 2.1 kcal/mol, favoring the former. Adopting absolute entropies from the vibrational energy calculations to the HF/6-31G* optimized transition structures, the *exo* - *endo* difference in entropy amounted to 0.27 kcal/mol. This is too small to alter the preference for the *endo* reaction route. The synchronicity of the reaction was studied by analyzing the HF/6-31G* optimized transition structures. It appears that the C-C bond formation slightly precedes the proton migration step, the *endo* route being the more asynchronous one. Nevertheless the reaction is unquestionably a concerted one. To compare our results against available experimental data, the reaction between propene and maleic anhydride was studied[9] at the UMP2/6-31G*//RHF/3-21G level. The calculated activation energies for the reactions via the *endo* and *exo* routes were 20.4 and 22.4 kcal/mol, respectively, and agree quite well with the experimental activation energy (21.5 kcal/mol).[10]

Hapten design, optimization and synthesis

Next, 36 plausible t.s. analogous structures were visualized (Fig. 1), with a view of mimicking both the steric and electrostatic characteristics of the t.s.. For optimization by AM1 and t.s. comparison by the CoMFA/SYBYL software,[11] the R side chain (see below) was truncated to methyl. In **1**, one of the compounds giving a good fit, the ring C=C conforms to the t.s. where considerable double bond character is suggested at the corresponding site (i.e., the allyl C2-C3 bond). The final hapten will have to carry a handle (a COOH group joined via a spacer) for attachment to the carrier protein. Thus, the two carboxylic

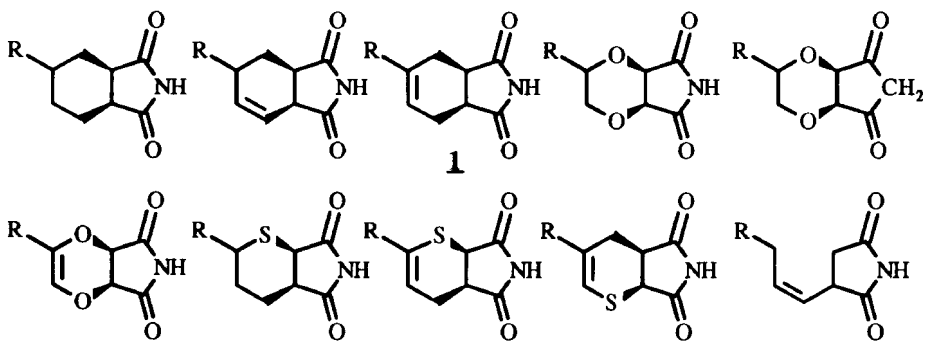
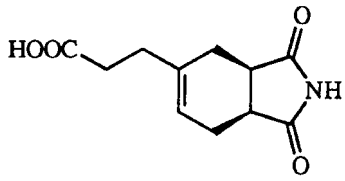


Fig. 1. Some suggested transition state analogs of the ene reaction.



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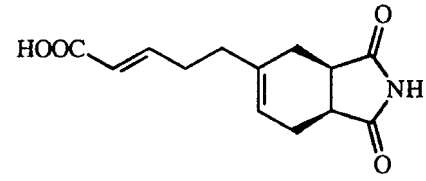

3

acids (**2**, **3**) were readily synthesized by Diels-Alder techniques; in **3**, the acyclic C=C serves to rigidify the side chain, keeping the bicyclic ring at a reasonable distance from the carrier. An superimposition of the X-ray and the AM1 optimized structures of **3** showed a very good overlap. In these haptens, the enophile component is maleimide rather than maleic anhydride owing to the expected lability of the latter under biological conditions, and the problems foreseen in attempting to couple an anhydridic carboxylic acid hapten with a protein.

Raising the antibody

The two haptens **2** and **3** were attached to keyhole limpet hemocyanin (KLH), and the conjugates were used for immunization of mice. Spleen cells were taken and fused with myeloma cells and cloned. Immunoglobulin G type monoclonal antibodies resulted from the hapten **3** only, **2** giving rise to the much less desirable IgM antibodies. This is probably a result of insufficient spacer length between the point of attachment to KLH and the recognition site of the hapten. Both types were nevertheless purified and screened for binding activity using bovine serum albumin -linked hapten in an affinity column.

For the construction of an antibody gene library, spleen cells from immunized mice were again used. The antibody genes have been isolated, and the heavy and light chain genes have been cloned. The construction of the phage antibody library is underway and we expect to commence measurements of the catalytic activity of purified library antibodies shortly.

Catalytic activity

Purified monoclonal IgM antibodies were used in small scale test reactions between 1-decene and maleimide in a two-phase system (4 days). At ambient temperatures, there is no demonstrable background reaction, as the thermal reaction only proceeds at temperatures above 180 °C. Thus, any positive results are likely to be due to catalytic antibody activity. Using GC detection, we have observed in a test reaction the formation of a product that corresponds to authentic 2-decen-1-ylsuccinimide. We will now try to optimize the monoclonal antibody catalyzed reaction by using the IgG class catalysts and by conducting the reaction under microemulsion conditions.

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