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Journal of Molecular Catalysis A: Chemical 217 (2004) 31–36
JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL
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An innovative approach to the design of plastic antibodies: molecular imprinting via a non-polar transition state analogue

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Received 7 October 2003; received in revised form 23 March 2004; accepted 24 March 2004

Abstract

An acrylic resin imprinted with a transition state analogue which possesses a dominant non-polar structure was synthesized. As a model for this study the Diels–Alder reaction was chosen since, it is well-known and possesses a stable and synthetically achievable transition state analogue. It is shown that the plastic antibody synthesized in this study catalyzes the Diels–Alder reaction with an efficiency comparable to that of a recently developed monoclonal catalytic antibody. In fact the Lineweaver–Burk V_{max} observed for the two catalysts appears to be of the same order of magnitude. We argue that plastic antibodies can be successfully used to catalyze reactions with a transition state of low polarity. Moreover, they appear to represent a more flexible catalyst when varied reaction conditions and solvent environments are needed.

Keywords: Plastic antibody; Transition state; Imprinted polymer; Acrylic resin; Transition state analogue

1. Introduction

Plastic antibodies, otherwise called imprinted polymers, constitute attractive materials that have been investigated for protein recognition, biosensors development, enantiomer separation and molecular catalysis [1,2].

In the area of molecular catalysis, imprinted polymers seem to be particularly attractive; cavities designed to bind to the transition state of a given reaction should function as a selective catalyst for that reaction. This general strategy is strictly related to the preparation of monoclonal catalytic antibodies [3], which have been demonstrated to be a successful catalyst for organic reactions.

In earlier experiments, where a stable model of the transition state structure (i.e. a transition state analogue (TSA)) was employed as the imprinting substance, the resulting polymers displayed only limited catalytic activity [4]. In some cases, careful optimization of the conditions afforded improved catalysis, most notably in dehydrofluorination reactions [5,6] and hydrolysis reactions [7,8]. It has been shown that, in order to complex the template, a large excess of binding/catalytic sites must be employed. This seems to be a consequence of the fact that TSA binds to the polymer using weak non-covalent interactions. Thus, improved catalytic performance results from imprinting procedures involving non-covalent bonds with sufficiently high association constants that afford complexes having a 1:1 ratio of template to binding site [9]. In general, this type of association is due to highly directed multiple bonds between the polymer matrix and the template [10,11], such as hydrogen bonds or polar interactions. The low efficiency of weak interactions was thought to constitute the reason why there are no examples of molecular imprinted material which catalyzes successfully a variety of organic reactions.

In this study, we attempted to design and synthesize a new polymeric catalyst for a reaction whose transition state is substantially non-polar. In order to demonstrate the feasibility of this project a well-known system which possesses a non-polar transition state was chosen. For this purpose, the Diels–Alder reaction seems to be particularly suitable for the application of the plastic antibodies technology. In fact, this cyclo-addition reaction proceeds through a well-characterized non-polar transition state, whose stable analogue can be easily synthesized.

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doi:10.1016/j.molcata.2004.03.044

2. Experimental

2.1. General procedures

All products and solvents were purchased from Sigma–Aldrich–Fluka and used without any further purification.

The NMR spectra were recorded with a Bruker AC 200 at 100.13 MHz for the ^{1}H and 50.33 MHz for the ^{13}C nucleus. Chemical shifts are reported in ppm utilizing TMS as an internal reference; coupling constants are expressed in Hz.

The IR measurements were recorded with an FT-IR Perkin-Elmer 1600 series spectrometer on KBr pellets.

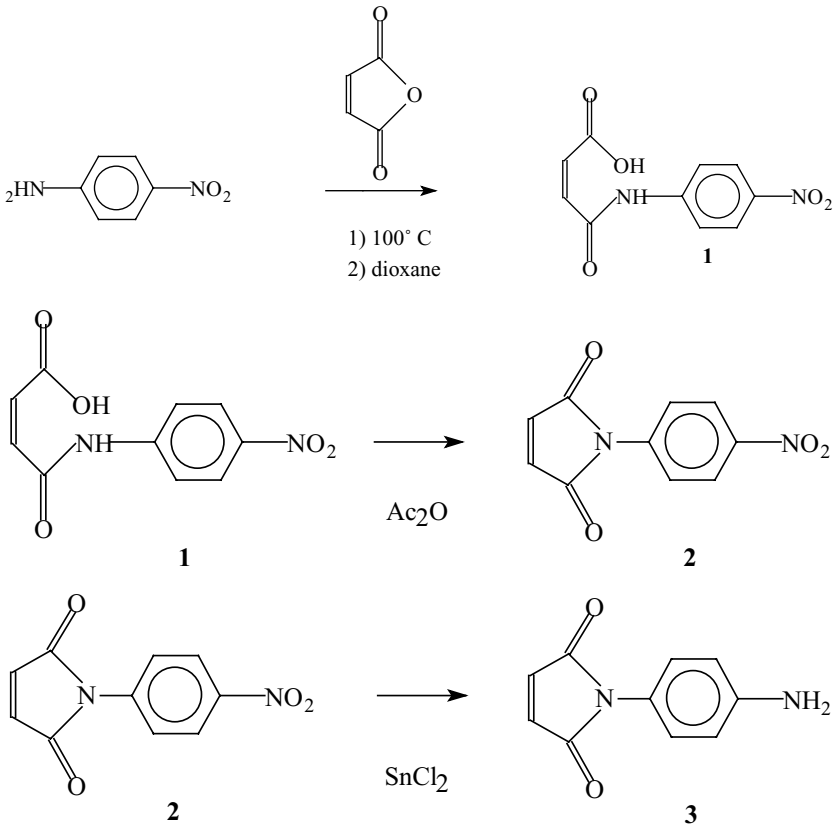
Kinetic measurements were performed by UV-Vis spectroscopy using a UV Hewlett-Packard 8453 instrument (Schemes 1 and 2).

2.2. Preparation of 4-(4-nitro-phenyl)-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (5)

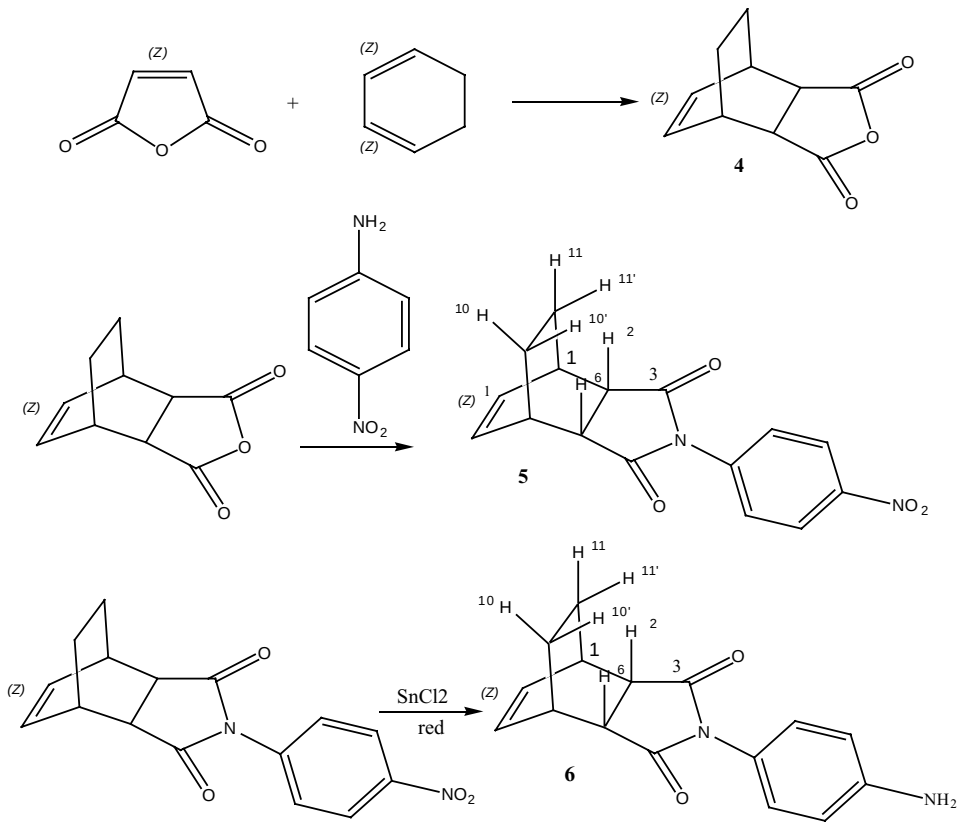
A 1.0 g (5.6 mmol) of (**4**) and 1.55 g *p*-nitroaniline (1.12 mmol) were refluxed in 15 mL of acetic acid for 2 h. The reaction mixture was then cooled and filtered. The product was washed with diethyl ether and crystallized from benzene to give compound **5** (yield 95%) as yellowish crystals, mp 264–266 °C; anal. calcd. (found) for $C_{16}H_{14}N_2O_4$: C, 64.4 (64.5); H, 4.7 (4.6); N, 9.4 (9.5); ^{1}H NMR ($CDCl_3$) δ: 7.46–7.50 and 8.28–8.33 (AA′BB′, 4H, J = 8.2, Ar), 6.30 (dd, 2H, J = 5.4 and 3.0, $H_{8,9}$), 3.29 (br m, 2H, $H_{1,7}$), 3.06 (t, 2H, J = 1.4, $H_{2,6}$), 1.57–1.72 (m, 2H, $H_{10',11'}$) and 1.42–1.53 (m, 2H, $H_{10,11}$); ^{13}C NMR ($CDCl_3$) δ: 23.58 (CH_2), 32.04 (CH), 44.29 (CH), 123.85 (CH), 127.22 (CH), 132.51 (CH), 137.51 (Cq), 146.82 (Cq) and 177.23 (CO).

2.3. Preparation of 4-(4-aminophenyl)-4-azatricyclo-[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (6)

A 1.00 g (3.35 mmol) of compound **5** was added to a slurry of 3.78 g (16.7 mmol) of $SnCl_2{\cdot}2H_2O$ in 6.7 mL of absolute ethanol and the mixture was heated to 70 °C. After 30 min the mixture was cooled to 25 °C and then cautiously quenched into 25 mL 5% Na_2CO_3 solution. The mixture was then extracted with three 15 mL portions of ethyl acetate; the organic extracts were combined, dried (Na_2SO_4) and concentrated by rotary evaporation. The residue was crystallized from benzene to give compound **6** (yield 80%) as white crystals, mp 277–279 °C; anal. calcd. (found) for $C_{16}H_{16}N_2O_2$: C, 71.6 (71.5); H, 6.0 (5.9); N, 10.4 (10.5). ^{1}H NMR ($CDCl_3$) δ: 6.65–6.72 and 6.89–6.96 (AA′BB′, 4H, J = 8.5, Ar), 6.27 (dd, 2H, J = 4.4 and 3.1, $H_{8,9}$), 3.60 (exch. brs, 2H, NH_2), 3.24 (br m, 2H, $H_{1,7}$), 2.97 (t, 2H, J = 1.3, $H_{2,6}$), 1.55–1.68 (m, 2H, $H_{10',11'}$) and 1.38–1.53 (m, 2H, $H_{10,11}$); ^{13}C NMR (DMSO-d_6) δ: 23.19 (CH_2), 31.50 (CH), 43.42 (CH), 113.34 (CH), 120.18 (Cq), 127.34 (CH), 132.16 (CH), 146.65 (Cq) and 178.36 (CO).



Scheme 1. Synthesis of the dienophile **3**. Compounds **1–3** were prepared as reported in [12].



Scheme 2. Synthesis of the template TSA. Compound **4** was prepared as reported in [13].

Endo configuration of the new derivatives **5** and **6** was assigned on the basis of NOE experiments (enhancement of the signals of $H_{10',11'}$ protons by irradiation of $H_{2,6}$).

2.4. Synthesis of the acrylic resin in suspension polymerization

The template (**6**) (50 mg) was dissolved in 910 mg of degassed toluene at 80 °C. A 800 mg of EDMA (ethylene glycol dimethacrylate) and 140 mg of MMA (methyl methacrylate) were added to the toluene solution under nitrogen. The dispersion medium consisted of 7 mL of a 1 wt.% (70 mg) poly(*N*-vinylpyrrolidone) (PVP) and 2 wt.% (140 mg) poly(vinylalcohol) (average M_w 124–186 000) in degassed water. The toluene solution was added under nitrogen and stirring to the dispersion medium and then AIBN (α-α′-azoisobutyro nitrile) as initiator was added. Then, after adjusting the required stirrer revolution (300–500 rpm) the suspension was polymerized at 80 °C for 9 h. Upon cooling, the polymeric beds were washed with hot water and decanted repeatedly with distilled water and methanol. In order to be sure that the template removal was complete the resin was washed with DMSO (dimethyl sulphoxide) and shrink with ethanol until, in the DMSO fraction, the UV band at 270 nm (λ_{max} absorbance of the template) completely disappeared.

The same procedure was performed to synthesize the non-imprinted resin as blank using the equivalent in weight of MMA instead of the template.

2.5. Kinetics

The kinetics of the Diels–Alder reaction were determined by monitoring the change in UV absorbance at 251 nm (the high rate of the catalyzed reaction made the accurate measurements of initial rate impossible via HPLC). Reaction velocities were determined measuring the initial rates at less than 3% reaction completed. All reactions were carried out at 25 °C in dioxane where both reagents are soluble (cyclohexadiene as diene and 1-(4-aminophenyl)-3-pyrroline-2,5-dione as dienophile). As the product and the dienophile have identical extinction coefficients at 251 nm ($\varepsilon_{251} = 14\,000$ in dioxane) the loss in absorbance reflects the reaction of the diene ($\varepsilon_{251} = 4000$ in dioxane) and therefore represents the apparent rate of the catalyzed reaction.

In order to demonstrate that the imprinted resin catalyzes the Diels–Alder reaction the reaction was performed:

(i) in the absence of the resin;
(ii) in the presence of the imprinted resin;
(iii) in the presence of the non-imprinted resin.

The initial rate (measured in the same reaction conditions), in the presence of the non-imprinted resin gave the same value as that with no resin.

Reaction velocities of the reaction in the presence of the imprinted resin were then determined at three different fixed diene concentrations between 125 and 27 μM while varying the concentration of the dienophile from 22 to 62 μM (range of concentrations in which the sum of the absorbance is acceptable for the Lambert–Beer law). Velocities were determined from the slope of the linear portion of the absorbance change. Each velocity represents the average of two runs from the same stock solution and each range of concentrations was run in duplicate. Withdrawals from the reaction flask were done at fixed time, and the absorbance values measured after filtering out the resin (considering that the rate of the reaction at these concentration in the absence of the resin as negligible). A Lineweaver–Burk plot $1/V$ versus 1/[dienophile] was constructed and is shown in Fig. 1.

The K_m value for the dienophile, K_{dp} was figured out at 10 μM from the average of the x-intercepts, as well as the V_{max} value for the dienophile, V_{maxdp} which was found 2 μM min^{-1} from the average of the y-intercepts. A second plot, shown in Fig. 2, can be generated holding the dienophile concentrations (from 58 to 29 μM) and varying the concentrations of the diene (from 190 to 27 μM), which provides the K_m of the diene (K_{di}) as 20 μM and V_{maxdi} as 3 μM min^{-1}. Due to the absorbance of the substrates at 251 nm at higher concentrations it was not feasible to measure the rate of the reaction under saturating conditions. In order to obtain the true V_{max}, the V_{max} values obtained via the Lineweaver–Burk plots are demonstrated against the values of the corresponding concentration (as shown in Fig. 3).

Indeed, in this figure, the y-intercept is equal to $1/V_{max}$ and the x-intercept is equal to $-1/K_m$. The data from both the Lineweaver–Burk plot agrees well and provides a V_{max} of 10 μM min^{-1}.

The K_m values for the diene and the dienophile obtained from this plot are 9 and 1 μM, respectively.

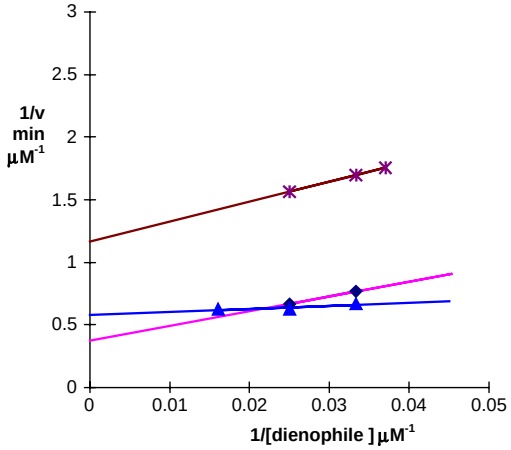


Fig. 1. Lineweaver–Burk plot $1/V$ vs. 1/[dienophile]: (*) 27 μM fixed concentration of diene, varying dienophile concentration (22–62 μM); (▲) 57 μM fixed concentration of diene, varying dienophile concentration (22–62 μM); (◆) 125 μM fixed concentration of diene, varying dienophile concentration (22–62 μM).

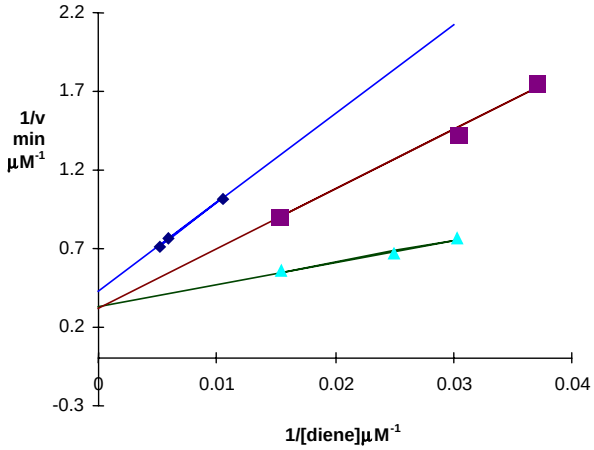


Fig. 2. Lineweaver–Burk plot $1/V$ vs. 1/[diene]: (◆) 29 μM fixed concentration of dienophile, varying diene concentration (27–190 μM); (■) 37 μM fixed concentration of diene, varying dienophile concentration (27–190 μM); (▲) 58 μM fixed concentration of diene, varying dienophile concentration (27–190 μM).

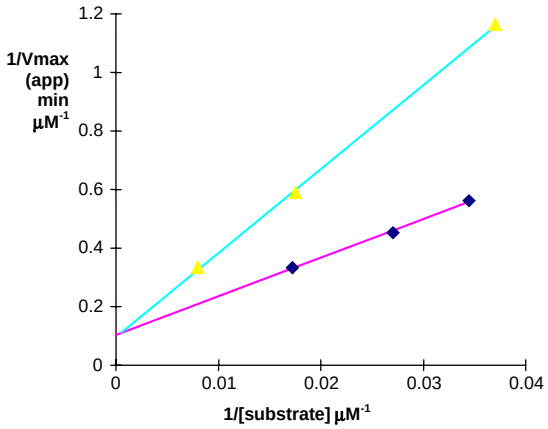
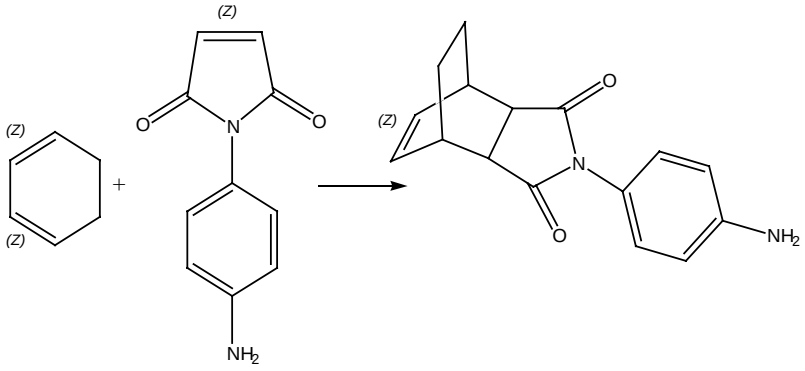


Fig. 3. Lineweaver–Burk plot $1/V_{max}$ vs. 1/[substrate] to obtain true V_{max} value: (▲) re-plotted data of V_{maxdi} vs. dienophile fixed concentrations (29–58 μM); (◆) re-plotted data of V_{maxdp} vs. diene fixed concentrations (27–125 μM).

3. Results and discussion

There exist over 1500 known enzymes which carry out a wide range of chemical reactions with remarkable specificity and rates. It is surprising that there are only a few documented examples of enzyme-catalyzed pericyclic reactions [14,15] (i.e. the chorismate mutase [16] and natural Diels–Alderases [17,18]); yet these are among the most powerful and commonly used reactions in synthetic organic chemistry. The most important of these is the already mentioned Diels–Alder reaction of a diene with a dienophile,



Scheme 3. Cyclohexadiene was used as diene.

which provides a straightforward and highly specific route to synthesize cyclohexene derivatives. In the context of the present work, this well known reaction is used to determine the efficiency of an imprinted plastic antibody in catalyzing reactions with non-polar transition state.

In contrast to the majority of enzyme-catalyzed reactions, Diels–Alder cyclo-additions proceed through a concerted and synchronous transition state [19] involving the simultaneous formation of 2σ carbon–carbon bonds within a cyclic array of interacting orbitals. The only reported approach to the preparation of a specific Diels–Alder catalyst (except for Lewis acid catalyzed reactions) employed generation of monoclonal antibodies against TSA [12] that mimics the Diels–Alder transition structure. This methodology afforded a catalyst yielding a Lineweaver–Burk V_{max} of 81 μM min^{-1}.

In this report, we demonstrate that it is possible to prepare an imprinted acrylic resin with analogue performances, by using the same TSA backbone structure used in the production of monoclonal antibodies. In this case, it is possible to prove that the imprinting on a non-polar TSA successfully leads to production of the targeted catalyst.

The TSA chosen was 4-(4-aminophenyl)-4-azatricyclo-[5.2.2.0^{2,6}]undec-8-ene-3,5-dione. This molecule is the TSA of a Diels–Alder reaction of 1-(4-aminophenyl)-3-pyrroline-2,5-dione (see Section 2) as dienophile (Scheme 3).

A reasonable mechanistic model of the successful imprinting implies that, during the resin polymerization the *p*-amino group of the TSA would initially shape the structure of the forming catalytic cavity by a directional hydrogen bond interaction between the oxygen of the acrylic matrix. Subsequently, the structure of the catalytic cavity will be fully formed on the TSA template through non-polar van der Walls' interactions.

Polar and non-polar interactions are also expected to drive the catalysis: in fact, in the presence of the dienophile and the diene, the cavity will initially interact with the polar dienophile (through the *p*-amino group) via hydrogen bonding. Then, the diene will join the complex and react with the dienophile. Non-polar interactions are expected to orient the diene in a way that mimics the TSA structure.

In order to demonstrate that the imprinted resin catalyzes the Diels–Alder reaction, the reaction was performed:

(i) in the absence of the resin;
(ii) in the presence of the imprinted resin;
(iii) in the presence of the non-imprinted resin.

The results show that the initial rate in the presence of the non-imprinted resin gave the same value as that with no resin, in the same reaction conditions; therefore, a non-specific involvement of the resin in the catalysis can be excluded. The reaction in the presence of the imprinted resin were monitored kinetically by plotting velocities data via the Lineweaver–Burk method (see "kinetics" in Section 2). The results are interesting considering that the observed value of V_{max} (10 μM min^{-1}) (Fig. 3) is only eight times lower than the V_{max} reported for the monoclonal antibody-catalyzed Diels–Alder reaction [12]. In that case, the catalyst, e.g. a monoclonal antibody, is a protein. Therefore, its activity is strictly correlated to the structure and consequently to physiological pH and temperature. In addition, the chemical environment has in general, to be aqueous. Consequently, also the designed transition state analogue has to be water soluble. For this reason, the molecule to be synthesized for the imprinting must be tailored with an hydrophilic part which allows water solubility. Plastic antibodies, made out of cross-linked polymers (in our case acrylate), can be synthesized and then suspended in any solvent; so they are appropriate for the imprinting with any transition state analogue and for the application in any kind of reaction environment. Moreover, due to their pH and temperature stability they can work in any reaction conditions.

4. Conclusions

Until recently plastic antibodies have been mainly used to produce materials which catalyzes hydrolysis reactions [7,8].

In this work, we have successfully applied this technology to the development of a catalyst for Diels–Alder pericyclic reactions.

In analogous study [12], the use of monoclonal catalytic antibody is limited by the fact that in order to preserve the delicate structure of the protein the reaction has to be performed in water. For this reason, the diene and the dienophile had to be chemically modified by introducing polar groups to increase solubility. In contrast, an imprinted polymer can, in principle, operate in all organic solvents and reaction conditions (provided that these conditions are compatible with the solubility and chemical properties of the reactants) and may represent a more flexible technology for the production of Diels–Alder catalysts.

Moreover, it is commonly accepted that the interaction between the template and the catalytic cavity in the imprinted polymer has to be highly directional and polar. This study shows that the plastic antibody approach can also be used to prepare catalysts, for reactions driven by transition states of low polarity. In fact, while we envisioned an imprinting mechanism controlled by a combination of initial polar hydrogen-bonding and subsequent non-polar interactions, the "active site" of the template for the catalysis, i.e. the bicycle[2.2.2]oct-2-ene moiety of the TSA, is completely non-polar. For this reason, plastic antibodies are expected to be applicable to other classes of pericyclic reactions whose pathway presents a non-polar transition state.

Acknowledgements

This study was funded by "Piano di Ateneo per la Ricerca" (PAR) 2002 of the University of Siena.

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