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Molecularly imprinted polymer catalysis of a Diels-Alder reaction

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ABSTRACT

A series of synthetic polymers were designed and synthesized for enhancing the rate of the Diels-Alder cycloaddition reaction of 1,3-butadiene carbamic acid benzyl ester (**1**) and *N*,*N*-dimethyl acrylamide (**2**), to yield the corresponding *endo*- (**3**) and *exo*- (**4**) reaction products. Putative transition state analogues (TSAs) for the *endo*- (**5**) and *exo*- (**6**) reaction pathways were used as templates for the synthesis of molecularly imprinted methacrylic acid (MAA)–divinylbenzene (DVB) copolymers. The polymer system utilized was selected based upon a series of ^{1}H NMR studies of complex formation between template and a functional monomer analogue (K_d (app) ≈ 70 mM, d_8-toluene, 293 K). Batch binding studies revealed that the imprinted polymers were selective for the TSA corresponding to the template used in the polymer synthesis. Studies on the influence of the polymers on the catalysis of the reaction of **1** and **2** demonstrated a 20-fold enhancement of the rate of the reaction relative to the solution reaction. A surprising temperature dependence of the reaction of **1** and **2** in the presence of the polymers was observed, which provides support for the role of template-functional monomer complexes in the catalysis of the Diels-Alder reaction.

1. Introduction

The Diels-Alder reaction is one of the most important tools in organic synthesis for the creation of C—C bonds and is widely used in the preparation of physiologically active compounds [1]. Many different approaches have been investigated either with the aim of catalysing this [4 + 2] cycloaddition reaction, or in order to influence its regio- or stereo-selectivity [2]. In one such approach antibodies were raised against stable transition state analogues (TSAs) and were shown to catalyse the corresponding Diels-Alder reaction and to exert stereoselectively over product formation [3]. Despite the significance of this reaction in biology (and for the organic chemist), no corresponding biological catalyst for this reaction has as yet been conclusively confirmed, though a number of biological extracts have demonstrated activities that have inferred the presence of catalysts [4], however alternative reaction mechanisms or partitioning effects may underlie these observations [5].

Molecularly imprinted polymers (MIPs), in which sites complementary in both shape and functionality to a target structure are created by polymerization in the presence of a molecular template [6], are often compared to antibodies or enzymes due to their affinities, selectivities or catalytic activities. Molecular imprinting technology provides the means to create selective recognition sites for a diverse array of chemical entities and even for molecules where it has proven difficult to raise antibodies [7]. In addition, the chemical and physical stabilities of MIPs, such as compatibility with organic solvents and tolerance to a wide range of pH and temperature [8], provide advantages over biological receptors with regard to deployment in non-biological environments. These qualities have led to their use as affinity matrices in many application areas including solid phase extraction (SPE) [9], sensors [10], and capillary electrophoresis (CE) [11].

A promising though challenging area for the application of MIP technology is in organic synthesis [12], and in particular catalysis (for reviews see Refs. [6,13]). While a growing number of enzyme mimics have been designed and synthesized for a number of different reaction types, e.g. ester hydrolysis [14], transamination [15], β-elimination [16], only a few reports deal with C—C bond formation, e.g. aldol [17], Suzuki [18], and Diels-Alder reactions [19]. In nearly all cases, a transition state analogue has been employed as a template for MIP synthesis, which is analogous to the approach adopted for the raising of catalytic antibodies [20]. In this paper the

Abbreviations: 4-VP, 4-vinylpyridine; ACHN, azobis(cyclohexanecarbonitrile); ACN, acetonitrile; BHT, 3,5-di-*tert*-butyl-4-hydroxy-toluene; DVB, divinylbenzene; MAA, methacrylic acid; MIP, molecularly imprinted polymer; TSA, transition state analogue.

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design of TSAs for the production of stereo-selective MIP-catalysts for the Diels-Alder reaction is described, along with the synthesis of the polymers and investigations of their recognition capabilities and catalytic activities.

2. Experimental

2.1. General

Reagents were purchased from Aldrich (Germany) and used as received or purified as described below. Divinylbenzene (80% tech; DVB) was purified from inhibitors by extraction with sodium hydroxide solution (3× 0.1 M), dried over $MgSO_4$ and stored at 4 °C. Before use, the cross-linker was filtered through activated Al_2O_3. Methacrylic acid (MAA), crotonaldehyde, ethyl chloroformate and benzyl alcohol were distilled prior to use. Azobis(cyclohexanecarbonitrile) (ACHN) was re-crystallised from methanol. All solvents were of HPLC grade and, if specified, dried by standard procedures before use [21].

Unless otherwise stated ^{1}H and ^{13}C NMR spectra were acquired at 250 and 62.5 MHz, respectively, on a Bruker AC-250 instrument. Chemical shifts are reported in ppm relative to TMS. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer in the diffuse reflectance (ATR) mode with samples dispersed in KBr or neat. BET-surface analyses were performed on a Micromeritics ASAP 2400 instrument. Samples were degassed at 100 °C for 24 h before analysis. Elemental analyses were performed by Mikrokemi AB (Uppsala, Sweden) and high resolution mass spectrometry was carried out by the Mass-Spectrometry Unit (Lund University, Sweden). GC–MS analyses were performed using a CP-Chirasil-Dex CB 25 m capillary column on a Shimadzu GC-17A instrument equipped with Shimadzu QP-5000 MS detector. GC-parameters: injection volume, 1 μL; carrier gas, He; injector temperature, 250 °C; interface temperature, 210 °C; column pressure, 47 psi; carrier gas flow, 1.2 mL/min; split ratio, 65; column oven temperature program, 40 °C (initial temperature held 3 min) to 120 °C, at 20 °C/min (2 min hold), then to 190 °C at 10 °C/min (2 min hold). HPLC analysis was preformed on a HP1050 or a HP1090 using a Beckman silica column (250 mm × 4.6 mm i.d.). Polymer suspensions were filtered through 2 μm PTFE membrane filter cartridges prior to HPLC analysis.

2.2. Synthesis of 1,3-butadiene carbamic acid benzyl ester (**1**) [22,23]

1,3-Butadienonic acid (2.57 g, 0.0262 mol) in dry acetone (30 mL) was added to a dry, nitrogen purged three neck flask equipped with stirring bar, thermometer and pressure-equalising dropping funnel. *N*,*N*-Diisopropylethylamine (3.60 g, 0.0278 mol) in dry acetone (15 mL) was added over a period of 30 min at room temperature, after which the mixture was cooled to −5 to −10 °C. Ethyl chloroformate (3.02 g, 0.0278 mol) in dry acetone (15 mL) was added dropwise over a period of 30 min while ensuring that the temperature remained between −5 and −10 °C. After stirring at low temperature for an additional 30 min, a cooled solution of NaN_3 (3.62 g, 0.0557 mol) in water (20 mL) was added over a period of 15 min. The mixture was stirred for a further 10 min after which it was poured into ice-water (250 mL) and extracted with toluene (5× 75 mL). The combined organic phases were dried over $MgSO_4$ and the solvent reduced to approximately 40 mL (*Caution*: do not evaporate solvent to dryness as azides can be explosive!).

The azide solution was added dropwise over a period of 30 min to a boiling mixture of benzyl alcohol (2.50 g, 0.0232 mol) and 3,5-di-*tert*-butyl-4-hydroxy-toluene (BHT, 20 mg) in toluene (40 mL), and the mixture was stirred at reflux for 60 min. The solvent was removed in vacuo and the residue repeatedly recrystallized from ethanol followed by column chromatography (ethyl acetate:heptane 2:3, v:v) to furnish the product as white crystals (1.38 g, 26%); m.p. 73 °C (Lit. 74–75 °C) [23]. IR(KBr): 3301, 1700, 1658, 1611, and 1518 cm^{-1}. ^{1}H NMR ($CDCl_3$): 7.36 (s, 5H), 6.80–6.70 (m, 1H), 6.49–6.46 (m, 1H), 6.35–6.20 (m, 1H), 5.73–5.64 (m, 1H), 5.16 (s, 2H), 5.07–4.89 (dd, 2H). ^{13}C NMR ($CDCl_3$): 153.24 (C=O), 135.72 (CH), 134.34 (CH), 128.60 (CH), 128.43 (CH), 128.28 (CH), 126.93 (CH), 113.54 (CH_2), 112.17 (CH), 67.41 (CH_2).

2.3. Synthesis of endo- (**3**) and exo- (**4**) Diels-Alder products [27]

1,3-Butadiene carbamic acid benzyl ester **1** (2.13 g, 10.5 mmol), *N*,*N*-dimethyl acrylamide **2** (5.20 g, 5.25 mmol) and BHT (20 mg) in toluene (25 mL) were added to a 35 mL pressure tube. After purging the contents with nitrogen, the tube was sealed and placed in an oil bath at 140 °C for 48 h. After recovery of the tube contents, the solvent was removed and the crude purified by column chromatography (heptane:ethyl acetate 3:7, v:v), giving the *endo*-product (**3**) as white crystals (0.94 g, 30%, m.p. 71.2–71.7 °C). IR(KBr). 3242, 1712, 1619, and 1542 cm^{-1}. ^{1}H NMR ($CDCl_3$): 7.33 (s, 5H), 5.87–5.68 (m, 2H), 5.31–5.28 (d, 1H, J = 9.44 Hz), 5.06 (s, 2H), 4.52 (s [broad] 1H), 3.09–3.00 (m, 4H), 2.85 (s, 3H), 2.17–1.77 (m, 5H,). ^{13}C NMR ($CDCl_3$): 172.20 (C=O), 155.92 (C=O), 136.64 (C), 129.78 (CH), 128.37 (CH), 127.82 (CH), 126.87 (CH), 66.44 (CH_2), 45.77 (CH), 40.78 (CH), 37.10 (CH_3), 35.45 (CH_3), 23.74 (CH_2), 21.67 (CH_2). Anal. calcd for $C_{17}H_{22}N_2O_3$: C: 67.53; H: 7.33; N: 9.26 Found: C: 67.35; H: 7.3; N: 9.15. HRMS: M+1 = 303.1711 (theoretical 302.163 + 1.0078). The *exo*-product (**4c**) was obtained as white crystals after the appropriate fractions were re-purified with ethyl acetate (100%) and re-crystallized from ethanol (0.72 g, 23%, m.p. 123.5–124.2 °C). IR(KBr): 3242, 1712, 1619, and 1542 cm^{-1}. ^{1}H NMR ($CDCl_3$): 7.33 (s, 5H), 5.83–5.64 (m, 2H), 5.11–5.00 (m, 3H), 4.35 (s [broad] 1H), 3.19–3.11 (m, 1H), 2.95–2.96 (d, 6H), 2.12 (s, 2H), 1.84–1.72 (m, 3H). ^{13}C NMR ($CDCl_3$): 173.75 (C=O), 155.35 (C=O), 136.63 (C), 128.56 (CH), 128.44 (CH), 128.20 (CH), 128.00 (CH), 66.40 (CH_2), 50.34 (CH), 41.24 (CH), 37.13 (CH_3), 35.66 (CH_3), 24.96 (CH_2), 24.13 (CH_2). Anal. calcd for $C_{17}H_{22}N_2O_3$: C: 67.53; H: 7.33; N: 9.26 Found: C: 67.5; H: 7.4; N: 9.25. HRMS: M+1 = 303.1712 (theoretical 302.163 + 1.0078).

2.4. Synthesis of endo- (**5**) and exo- (**6**) transition state analogues

The same procedure as described for **3** and **4** was employed, but starting with cyclohexa-1,3-dienyl-carbamic acid benzyl ester **12** (2.41 g, 0.0105 mol). The products were separated by column chromatography (heptane:ethyl acetate, 3:7, v:v) giving *endo*-TSA (**5**) (1.21 g, 35%) and *exo*-TSA (**6**) (0.38 g, 11%) as white crystals. **5**: m.p. 114–115 °C. IR(KBr): 3321, 1707, 1631, and 1525 cm^{-1}. ^{1}H NMR ($CDCl_3$): 7.34–7.27 (m, 5H), 6.20–6.14 (m, 2H), 5.49 (s, 1H), 5.11–5.08 (d, 1H, J = 12.45 Hz), 4.99–4.97 (d, 1H, J = 12.44 Hz), 3.66–3.62 (q, 1H), 2.91 (s, 3H), 2.79 (s, 3H), 2.59–2.57 (m, 1H), 2.56–2.51 (m, 1H), 2.06–2.01 (m, 1H), 1.64–1.59 (m, 1H), 1.46–1.37 (m, 2H), 1.31–1.26 (m, 1H). ^{13}C NMR ($CDCl_3$): 174.18 (C), 155.16 (C), 136.88 (C), 135.86 (CH), 130.25 (CH), 128.35 (CH), 127.35 (CH), 127.81 (CH), 65.92 (CH_2), 56.18 (C), 43.13 (CH), 36.85 (CH_3), 35.35 (CH_3), 33.82 (CH_2), 29.80 (CH_2), 29.71 (CH), 24.59 (CH_2). Anal. calcd for $C_{19}H_{24}N_2O_3$: C: 69.49; H: 7.37; N: 8.53 Found: C: 69.4; H: 7.35; N: 8.5. HRMS: M+1 = 329.1871 (theoretical 328.1787 + 1.0078). UV(ACN): λ_{max} 199 nm, Log ε 4.31. **6**: m.p. 92–93.5 °C. IR(KBr): 3305, 1717, 1630, and 1530 cm^{-1}. ^{1}H NMR ($CDCl_3$): 7.35–7.28 (m, 5H), 6.60–6.59 (d, 1H, J = 8.52 Hz), 6.21–6.17 (t, 1H, J = 6.55 Hz, J = 8.47 Hz), 5.15–5.13 (d, 1H, J = 12.35 Hz), 5.09 (s, 1H), 5.02–4.99 (d, 1H, J = 12.33 Hz), 3.35–3.32 (q, 1H), 2.84 (s, 3H), 2.80 (s, 3H), 2.71–2.6 (m, 1H), 2.60–2.58 (m, 1H), 1.80–1.74 (m, 1H), 1.73–1.66 (m, 1H), 1.60–1.56 (m, 1H), 1.41–1.34 (m, 1H), 1.19–1.13 (m, 1H). ^{13}C NMR ($CDCl_3$): 174.54 (C), 155.17 (C), 137.58 (CH), 136.79 (C), 131.37 (CH), 128.40 (CH), 128.08 (CH), 127.97 (CH), 66.15 (CH_2), 56.08 (C), 39.92 (CH), 37.31 (CH_3), 35.35 (CH_3), 35.64 (CH_2), 31.09 (CH_2),

29.95 (CH), 27.08 (CH_2), 25.48 (CH_2). Anal. calcd for $C_{19}H_{24}N_2O_3$: C: 69.49; H: 7.37; N: 8.53 Found: C: 69.4; H: 7.35; N: 8.45. HRMS: M+1 = 329.1865 (theoretical 328.1787 + 1.0078). UV(ACN): λ_{max} 199 nm, Log ε 4.29.

2.5. Synthesis of 1-diethylamino-1,3 butadiene (**8**) [24,25]

A dry three neck round bottom flask equipped with pressure-equalising dropping funnel, thermometer and N_2-inlet was charged with diethylamine (32 mL, 0.309 mol) and K_2CO_3 (6 g, 0.043 mol). The suspension was cooled to between −5 and −10 °C and freshly distilled crotonaldehyde (**7**) (10 g, 0.142 mol) in dry toluene (15 mL) was added dropwise over a period of 30 min while keeping the temperature between −5 and −10 °C. After stirring at low temperature for an additional hour, the mixture was allowed to reach room temperature, and stirred over night in the dark. Unreacted K_2CO_3 was removed by filtration and the volatiles were removed in vacuo. The resulting solution was used for the next step without further purification. IR(neat): 2967, 1643, 1452, and 1375 cm^{-1}. ^1H NMR ($CDCl_3$): 6.34–6.19 (m, 2H), 5.07–4.98 (dd, 1H, *J* = 13.41 Hz), 4.74–4.67 (d, 1H, *J* = 16.63 Hz), 4.40–4.43 (d, 1H, *J* = 10.18 Hz), 3.09 (dd, 4H), 1.11–1.06 (t, 6H). ^{13}C NMR ($CDCl_3$): 141.46 (CH), 137.42 (CH), 103.32 (CH_2), 98.00 (CH), 44.96 (CH_2), 13.00 (CH_3). GC–MS: 125 (100%; M+), 110 (97%).

2.6. Synthesis of ethyl 2-diethylamino-3-cyclohexene carboxylate (**9**) [24,25]

1-Diethylamino-1,3-butadiene **8** (34.8 g, 0.278 mol) and dry toluene (60 mL) were added to a round bottom flask followed by ethyl acrylate (30.3 g, 0.303 mol). The mixture was stirred under nitrogen in the dark for 5 days, diluted with diethyl ether (100 mL) and extracted with aqueous HCl (2 M, 3× 50 mL). The combined aqueous phases were washed with diethyl ether (2× 100 mL) and the pH was raised to pH 9–10 with NaOH (6 M). The product was then extracted with diethyl ether (3× 100 mL), the combined ether phases dried over $MgSO_4$, and the volatiles removed in vacuo. The product was purified by column chromatography (heptane:ethyl actetate 1:1, v:v). The product was obtained as a pale yellow oil (27.6 g, 44%). IR(neat): 2969, 1733, and 1685 cm^{-1}. ^1H NMR ($CDCl_3$): 5.93–5.62 (m, 2H), 4.25–4.01 (m, 2H), 3.70–3.57 (m, 1H), 2.71–2.37 (m, 5H), 2.23–1.72 (m, 4H), 1.29–1.22 (dt, 3H), 1.01–0.91 (dt, 6H). ^{13}C NMR ($CDCl_3$): 175.89 (C=O), 174.10 (C=O), 129.61 (CH), 128.55 (CH), 127.61 (CH), 125.95 (CH), 59.95 (CH_2), 59.72 (CH_2), 58.66 (CH_2), 55.54 (CH_2), 46.05 (CH_2), 45.66 (CH_2), 44.87 (CH), 44.31 (CH), 26.05 (CH_2), 24.70 (CH_2), 24.14 (CH_2), 20.05 (CH_2), 14.70 (CH_3), 14.20 (CH_3), 13.79 (CH_3). GC–MS: 225 (12%; M+), 125 (86%), 110 (100%).

2.7. Synthesis of 1-carboethoxy-1,3-cyclohexadiene (**10**) [25]

A solution of ethyl-2-diethylamino-3-cyclohexene carboxylate **9** (30.4 g, 0.135 mol) in acetic acid (150 mL) was heated at reflux overnight. The mixture was quenched with ice water (300 mL) and extracted with diethyl ether (5× 100 mL). The combined organic phases were extracted with water (2× 100 mL), saturated $NaHCO_3$ (2× 100 mL) and brine (2× 100 mL). After drying over $MgSO_4$ the solvent was removed and the product purified with column chromatography (ethyl acetate:heptane 1:9, v:v). The product was obtained as a pale yellow oil (4.31 g, 21%). IR(neat): 2979, 1702, 1574, 1263, and 1220 cm^{-1}. ^1H NMR ($CDCl_3$): 7.01–6.97 (d, 1H, *J* = 4.82), 6.17–6.02 (m, 2H), 4.25–4.16 (m, 2H), 2.50–2.40 (m, 2H), 2.30–2.20 (m, 2H), 1.36–1.27 (m, 3H). ^{13}C NMR ($CDCl_3$): 167.42 (C=O), 133.27 (CH), 132.9 (CH), 127.35 (C), 123.88 (CH), 60.25 (CH_2), 22.79 (CH_2), 20.67 (CH_2), 14.23 (CH_3). GC–MS: 152 (18%, M+), 79 (100%).

2.8. Synthesis of 1-carboxylic acid-1,3-cyclohexadiene (**11**) [25]

1-Carboethoxy-1,3-cyclohexadiene **10** (6.27 g, 0.0412 mol) was dissolved in THF (50 mL) and aqueous KOH (3 M, 50 mL) was added. The mixture was stirred at 40 °C overnight, cooled on ice and washed with diethyl ether (3× 50 mL). The aqueous phase was acidified with HCl (6 M) to pH 1–2 and extracted with diethyl ether (3× 50 mL). The combined organic phases were dried over $MgSO_4$ and the solvent removed to yield the product as white crystals (2.86 g, 56%), m.p. 42.5 °C (Lit [25]: 38–40 °C). IR(KBr): 2940 (br), 1670, 1631, and 1570 cm^{-1}. ^1H NMR ($CDCl_3$): ∼12 (s [broad]; OH), 7.15–7.12 (d, 1H, *J* = 6.49 Hz), 6.25–6.06 (m, 2H), 2.38–2.25 (m, 2H). ^{13}C NMR ($CDCl_3$): 172.83 (C=O), 135.46 (CH), 134.73 (CH), 126.35 (C), 123.91 (CH), 22.81 (CH_2), 20.20 (CH_2). GC–MS: 124 (26%, M+), 79 (100%).

2.9. Synthesis of cyclohexa-1,3-dienyl-carbamic acid benzyl ester (**12**) [22,23]

The same procedure as described for **1** was employed though excluding the final recrystallization step, starting with 1-carboxylic acid-1,3-cyclohexadiene **11** (2.88 g, 0.0262 mol), and the product was obtained as a yellow wax-like solid (3.84 g, 64%). IR(KBr): 3033, 1778, 1702, and 1498 cm^{-1}. ^1H NMR ($CDCl_3$): 7.39–7.37 (m, 5H), 6.18–6.15 (d, 1H, *J* = 5.82 Hz), 5.97–5.91 (m, 2H), 5.13 (s, 2H), 2.27 (s; 1H). ^{13}C NMR ($CDCl_3$): 152.80 (C=O), 136.02 (C), 132.90 (C), 128.55 (CH), 128.26 (CH), 124.46 (CH), 120.43 (CH), 105.55 (CH), 66.83 (CH_2), 26.63 (CH_2), 22.90 (CH_2). HRMS: M = 229.1104 (theoretical 229.1103).

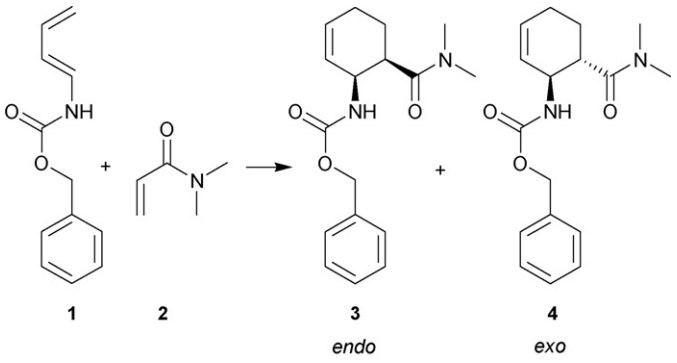
2.10. NMR titrations

A solution of *endo*- (**5**) or *exo*-TSA (**6**) (0.065 M in either $CDCl_3$, d_3-ACN, or d_8-toluene) was titrated with consecutive additions of a solution containing one of the functional monomer analogues (d_4-acetic acid 8.73 M, d_5-pyridine 6.24 M and acetamide 1.02 M) and **5** or **6** (0.065 M) in the same solvent. Apparent dissociation constants K_d(app) were calculated by non-linear regression using the software package Graph Pad Prism 3.02 (Graph Pad Software Inc., San Diego, CA, USA).

Job's method of continuous variation was employed for determining the stoichiometric relationship between **5** and **6**. NMR tubes (11) containing different molar fractions of **5** or **6** and d_4-acetic acid in d_8-toluene or $CDCl_3$ (ranging from pure **5** or **6** to pure acetic acid, in increments of 0.1 and with a constant total concentration of all solutes = 15 mM) were studied.

2.11. Polymer synthesis

A representative protocol for preparing 1 g of MIP is as follows: *endo*-TSA (**5**) (0.0933 g, 0.284 mmol, 5 mol%), MAA (0.0979 g, 1.14 mmol; 20 mol%), DVB (0.592 g, 4.55 mmol, 80 mol%), the porogen (toluene, 2 mL) and the initiator ACHN (1 mol% relative to double bonds) were added to a polymerisation tube fitted with a ground glass joint for connection to a vacuum line. The mixture was degassed by repeated freeze-thaw cycles (three times), after the last cycle left under nitrogen and placed under a UV light at 8 °C for 24 h. The polymers were ground, sieved through a 65 μm sieve and the fines removed by repeated sedimentation from acetone (400 mL, 3 × 25 min). To remove the template and additional fines, Soxhlet extraction was carried out in acetone. More complete template removal from the polymer was achieved by packing the polymer into a stainless steel HPLC column and subjecting it to extensive washing under a variety of conditions, as described elsewhere [26]. The control polymers were prepared in the same manner though in the absence of template.



Scheme 1. Diels-Alder reaction utilized in this study.

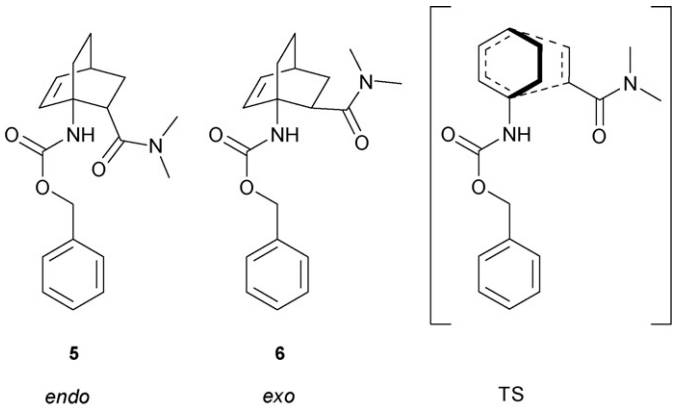


Fig. 1. *Endo*- and *exo*-TSA employed as template for the fabrication of MIPs. The putative transition state (TS) is also shown.

2.12. Batch binding studies

A solution of **5** or **6** in toluene (0.5 mL, 0.1 or 1 mM) was added to MIP or control polymer (5 ± 0.1 mg) in a 2 mL screw top vial. The resultant suspension was shaken overnight. The polymer was filtered and the concentration of the TSA remaining in solution determined by HPLC. Elution was carried out in 90% heptane containing acetic acid (0.1%): isopropanol (10%) at a flow rate of 1 mL/min with UV detection at 220 nm.

2.13. Swelling studies

Polymer (1 mL) was weighed into a 5 mL glass cylinder with stopper, the weight was recorded and the dry density d calculated (Eq. (1)). Toluene (3 mL) was added and the cylinder was sealed with a glass stopper. The increase in polymer volume (swelling, *SW*), was recorded after 12 h. The specific swelling (SW_{sp}) was calculated as follows:

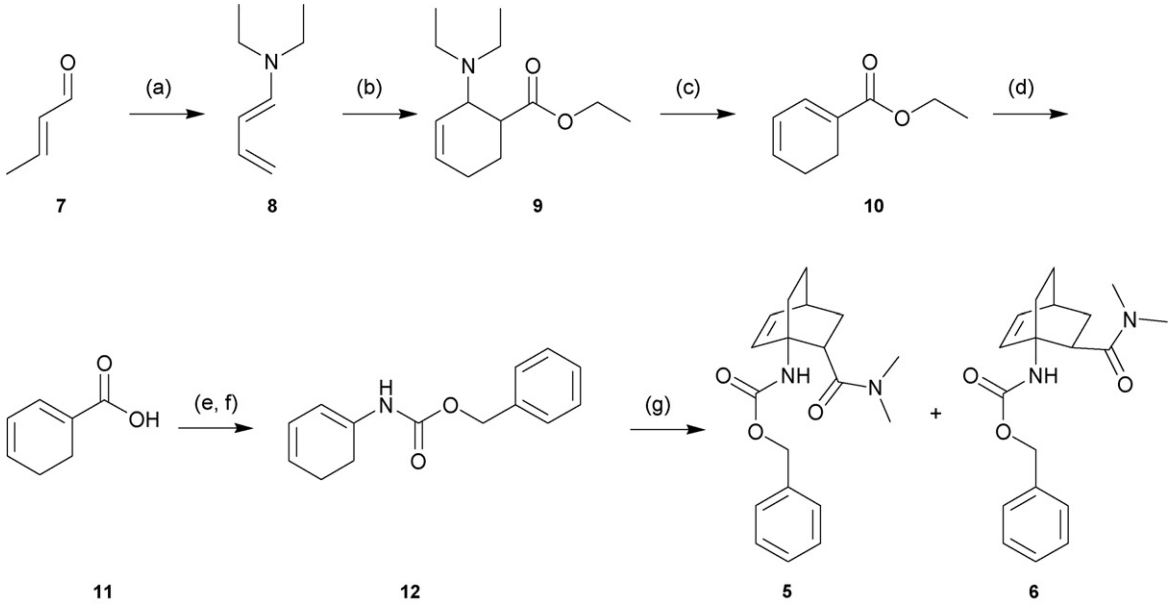
$$SW_{sp} = \frac{SW}{d} \quad (1)$$

2.14. Kinetics studies

A stock solution was prepared containing 1,3-butadiene carbamic acid benzyl ester **1** (20 mM), *N*,*N*-dimethyl acrylamide (20 mM) and BHT (10 mg) in toluene. Portions (3 mL) of this solution were added to pressure tubes containing *endo*- or *exo*-imprinted polymer (100 mg), control polymer (100 mg) or no polymer (solvent reaction). The tubes were purged with nitrogen and placed in an oil bath at the appropriate temperature (120 and 20 °C). Samples were taken at appropriate intervals (during sample acquisition the tubes were put on ice to arrest reaction). Samples (1 mL) were taken and centrifuged for 5 min (10 000 rcf, 20 °C). The supernatant from each (0.5 mL) was filtered as described earlier, into a HPLC vial and HPLC analyses were run in duplicate to determine the amount of product formation. Elution was carried out with heptane containing acetic acid (0.1%, v/v): 15% isopropanol (15%, v/v) at a flow rate of 1 mL/min with UV detection at 210 nm. After the second injection the polymer was re-suspended and the mixture returned to the pressure tube. The tube was purged with nitrogen, sealed and the reaction mixture then returned for incubation at the appropriate temperature. Studies using inhibitor were performed similarly.

3. Results and discussion

Inspired by the work of Gouverneur et al. [27] on the utilization of antibodies for the catalysis of a series of Diels-Alder cycloaddition



Scheme 2. Synthesis of the *endo*- (**5**) and *exo*- (**6**) transition state analogues (TSAs). (a) Dimethylamine, K_2CO_3, toluene −5 °C to r.t.; (b) ethylacrylate, toluene r.t.; (c) acetic acid, reflux; (d) KOH, THF 40 °C; (e) diisopropyl ethylamine, ethylchloroformate, acetone, NaN_3, −5 °C; (f) benzyl alcohol, 3,5-di-*tert*-butyl-4-hydroxy-toluene (BHT), toluene, reflux; (g) *N*,*N*-dimethyl acrylamide (**2**), BHT, toluene reflux.

reactions, molecularly imprinted polymers were designed, synthesized and evaluated as catalysts for the reaction of a 1-substituted diene (**1**) and *N*,*N*-dimethylacrylamide (**2**), which affords a mixture of the *endo*- (**3**) and *exo*- (**4**) cycloaddition products, Scheme 1.

In the present study, the putative transition state structures **5** and **6** were proposed, which correspond to the reactions leading to the *endo*- (**3**) and *exo*- (**4**) products, respectively, based upon those used previously for the raising of catalytic antibodies, Fig. 1 [27].

3.1. Synthesis of endo- and exo-TSA and Diels-Alder products

The diastereoisomeric TSAs **5** and **6** were synthesized using a modification of a seven step reaction pathway commencing with crotonaldehyde (**7**) utilized by Gouverneur et al. (Scheme 2) [21]. The desired products were obtained in reasonable (35 and 11%, respectively) overall yield. The Diels-Alder products **3** and **4** (Scheme 1) were synthesized from **1** and **2**, according to previously described procedures [21], and were used as standards in the establishment of an assay for the study of the kinetics of the chosen reaction.

3.2. Prepolymerization studies

The selective interaction of a template structure and a functional monomer is essential for formation of the complexes necessary for synthesis of polymers capable of ligand-selective recognition. We [28] and others [29,30] have previously demonstrated that spectroscopic studies, in particular NMR titrations, can be used for identifying functional monomer–template–solvent combinations suitable for use in polymer synthesis. ^{1}H NMR titration studies of potential systems were performed to assist in the identification of a suitable polymerization system, namely one capable of recognizing the intended template structures, the TSAs for the *endo*- (**5**) and *exo*- (**6**) outcomes of the Diels-Alder reaction studied, Fig. 2.

Three functional monomers were selected as potential candidates, methacrylic acid, 4-vinylpyridine (4-VP) and acrylamide, which have acidic, basic and neutral characteristics, respectively. In order to simplify the NMR spectra and avoid possible polymerisation, monomer analogues were employed. For MAA and 4-VP the deuterated monomer analogues d_4-acetic acid and d_5-pyridine were used [28] while acetamide was used instead of acrylamide. Titrations were performed in each of three solvents $CDCl_3$, d_3-ACN or d_8-toluene by monitoring changes in the chemical shifts of H^a and NH, which reflect monomer interactions with the carbamate and amide functionalities. Only titrations with d_4-acetic acid resulted in saturation isotherms, Table 1.

Table 1
K_d(app) values obtained from ^{1}H NMR titration of *endo*- and *exo*-TSAs with d_4-acetic acid.

	Endo-TSA (**5**)/mM		*Exo*-TSA (**6**)/mM	
	H^a	NH	H^a	NH
$CDCl_3$	141 ± 12	53 ± 6	257 ± 42	155 ± 21
d_3-ACN	466 ± 12	178 ± 21	n.d.	n.d.
d_8-Toluene	76 ± 3	64 ± 2	74 ± 6	81 ± 7

Duplicate titration experiments with duplicate integrations.

The lowest apparent dissociation constants (K_d(app)) determined were found using d_4-acetic acid in d_8-toluene. No appreciable difference was observed between the strengths of the complexes formed with **5** and **6** in toluene. The weaker interaction observed in the case of titrations performed in ACN reflects the higher dielectric constant of ACN, i.e. weaker electrostatic (hydrogen binding) interactions [31].

Analysis of complexation of the carbamate NH moieties of both **5** and **6** in $CDCl_3$ using Job's method of continuous variation [24] revealed that the monomer–template interaction at the carbamate involves two functional monomers per template, i.e. a 2:1 complex stoichiometry between acetic acid and the NH of **5** and **6** (Fig. 3). The same stoichiometry was observed when the experiment was performed with **6** in d_8-toluene, which is in agreement with a recent study carried out by Baggiani et al. [32] where the interaction of MAA and carbamate moieties was investigated by molecular modelling. However, in the case of **5** in d_8-toluene a 1:1 complex was observed. Based upon preliminary semi-empirical AM1 studies, we suggest that this behaviour could arise as a consequence of π-π-interactions involving a stacking arrangement with a molecule of toluene intercalating between the C–C double bond and the amide moiety. Here the energy minimized structure demonstrated adequate space for insertion of a toluene molecule between these groups. This would reduce the accessibility of a second monomer (analogue). This cooperative binding of a toluene molecule by these two functional groups of the TSA is not possible in the *exo*-form.

3.3. Polymer synthesis and evaluation

On the basis of the NMR studies described above, methacrylic acid–divinylbenzene–copolymers were synthesized using toluene as porogen. The polymers were prepared with the composition: methacrylic acid (MAA, 20 mol%) and divinylbenzene (DVB,

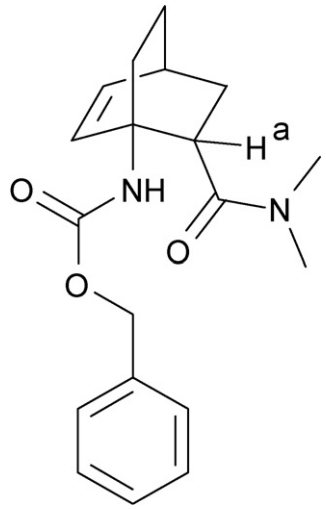


Fig. 2. Structure of *endo*-TSA **5** protons followed during ^{1}H-titration (NH and H^a).

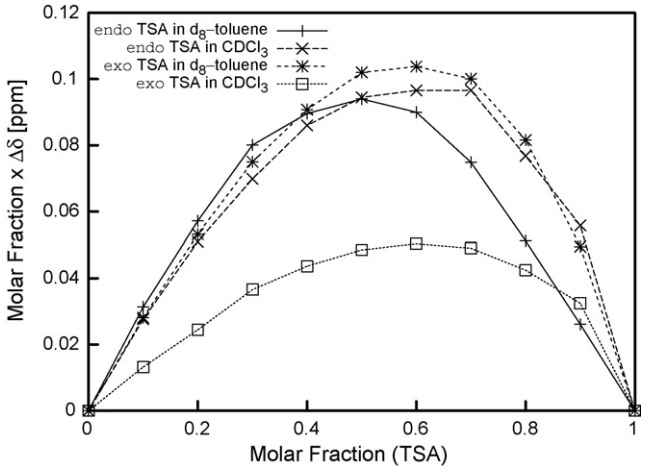


Fig. 3. Job plot of *endo*- and *exo*-TSA on NH with d_4-acetic acid.

Table 2
Physical and elemental analysis of polymers.

Polymer	Specific swelling/ml g^{-1}	Surface area/m^2 g^{-1}	Anal. Calcd./%[a]
P_{ENDO}	5.57	11.5	C: 84.2, H: 7.6, N: 0.4
P_{EXO}	4.14	4.7	C: 84.0, H: 7.6, N: 0.4
P_{REF}	5.75	8.4	C: 82.8, H: 7.6, N:0.4

[a] Theoretical calculations: C: 86.82, H: 7.69, N: 0.37 (based upon complete incorporation of monomers and initiator).

Table 3
Specific binding of the *endo*- or *exo*-TSA (c = 1 or 0.1 mM) in toluene to the different polymers.

Entry	Ligand	Polymer	Specific uptake (μmol/g polymer)	
			1 mM	0.1 mM
1	*endo*-TSA (**5**)	P_{ENDO}	4.20 ± 0.16	1.12 ± 0.14
2	*endo*-TSA (**5**)	P_{EXO}	3.47 ± 0.34	0.66 ± 0.14
3	*exo*-TSA (**6**)	P_{ENDO}	2.99 ± 0.32	0.66 ± 0.05
4	*exo*-TSA (**6**)	P_{EXO}	9.17 ± 0.37	1.08 ± 0.16

Errors reflect standard deviations of mean of duplicate analysis of triplicate experiments. A ligand concentration of 0.1 mM corresponds to 10 μmol g(polymer)$^{-1}$. Binding to P_{REF} was found to be 22.55 ± 0.09 μmol/g and 33.99 ± 0.23 μmol/g at 1 mM and 3.00 ± 0.11 μmol/g and 1.82 ± 0.04 μmol/g at 0.1 mM for **5** and **6**, respectively.
The uptakes are the specific uptakes, i.e. the binding to the control subtracted from the binding to the MIP.

80 mol%), with either *endo*- (**5**) or *exo*- (**6**) TSA (5 mol% equivalents) and azobis(cyclohexanecarbonitrile) (ACHN) as photoinitiator, furnishing the corresponding *endo*-(P_{ENDO}) or *exo*-(P_{EXO}) MIPs. The bulk polymers were subjected to standard work-up and particles of ≤63 μm were obtained and used in subsequent studies. A non-imprinted reference polymer (P_{REF}) was prepared identically, though in the absence of template. The physical characterisation of the materials by swelling studies, BET analysis and elemental analysis, demonstrated comparable characteristics for the three polymers (Table 2). The relatively low surface areas observed are comparable to those for a number of similar systems [15].

Batch binding studies in toluene using **5** and **6** as ligands (at 0.1 or 1 mM) were used to examine the recognition properties of the imprinted and reference polymers, Table 3. Both imprinted polymers demonstrated specific uptakes (specific uptake = binding to P_{ENDO} or P_{EXO} − binding to P_{REF}) at a TSA concentration of 0.1 mM demonstrate selective recognition of the template structure. At a higher concentration (1 mM) the specific uptake is small relative to the total uptake, i.e. the system is dominated by non-specific interactions, though even at this higher concentration some selectivity is observed, again implying the presence of highly selective sites.

3.4. Assay

Studies of the influence of the polymers on the reaction of the diene (**1**) and *N*,*N*-dimethylacrylamide (**2**), were performed in toluene in the presence, or absence, of the three polymers. The formation of the dominant Diels-Alder product, the *endo*- (**3**) product, was used as a basis for monitoring the reaction outcome. Corresponding analyses based upon the less easily detected *exo*-product (**4**) were effectively the same.

Initial studies were performed at room temperature (20 ± 3 °C), Fig. 4. All three of the polymers were observed to induce an increase in reaction rate, up to 20-fold, relative to the solution reaction, as determined by production of the dominant *endo*-product, **3**. Unfortunately, the less favoured *exo*-product (**4**) could not be detected to any significant extent over the time frame studied. It should be noted that the reaction itself was very slow at room temperature (with only 1.5% product formed after >1000 h), a fact which perhaps alone justifies the need for a catalyst for this reaction. Based upon the theoretical number of sites (calculated from the amount of template used in the polymer synthesis), this enhancement corresponds to 5.3 turnovers (mol/mol site) per day. However, taking the more realistic assumption that the number of sites accessible during the assay is equal to the number of template selective sites, as determined in the recognition (uptake) studies (1.12 μmol/g polymer, at 0.1 mM ligand concentration), the turnover corresponds to 22.4 turnovers (mol/mol site) per second. Importantly, although the template imprinted polymers P_{ENDO} and P_{EXO} provided a slightly greater rate enhancement than P_{REF}, no significant difference between the results for P_{ENDO} and P_{EXO} was evident. This indicates that there is no difference in the sites selective for the *endo*- and *exo*-TSAs with respect to binding of the actual transition state under the conditions studied. It should be noted that polymer elemental compositions after use at room temperature were comparable to those of the unused material.

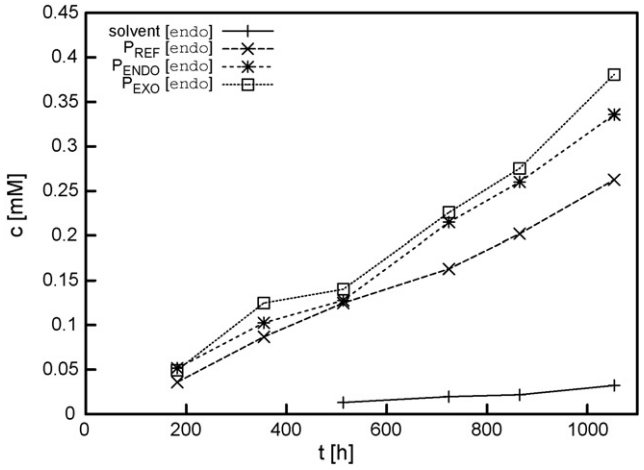


Fig. 4. Formation of **3** in toluene and in the presence of P_{ENDO}, P_{EXO} or P_{REF} at room temperature. (Errors estimated to ± 10%, based upon duplicate analyses and duplicate experiments.)

Markedly different results were obtained when experiments were performed at 120 °C using sealed-tube reaction vessels, Fig. 5. Surprisingly, the solvent reaction proceeded significantly faster than reactions performed in the presence of the various polymers. Furthermore, no significant difference was observed in the influence of the polymers on the reaction rate.

In reactions with P_{REF}, P_{ENDO} and P_{EXO} the product concentration decreased at longer (>50 h) reaction times. This suggests that in the presence of polymer, decomposition or additional reactions occur.

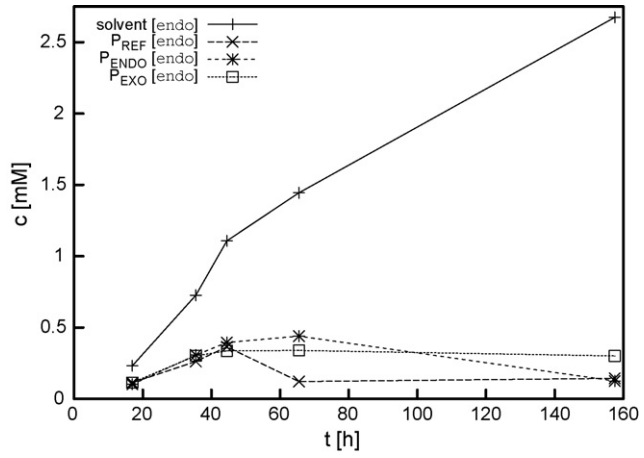


Fig. 5. Time course study of *endo*-D-A product formation in toluene (120 °C), and in the presence of P_{ENDO}, P_{EXO} or P_{REF}. (Errors estimated to ±10%, based upon duplicate analyses and duplicate experiments.)

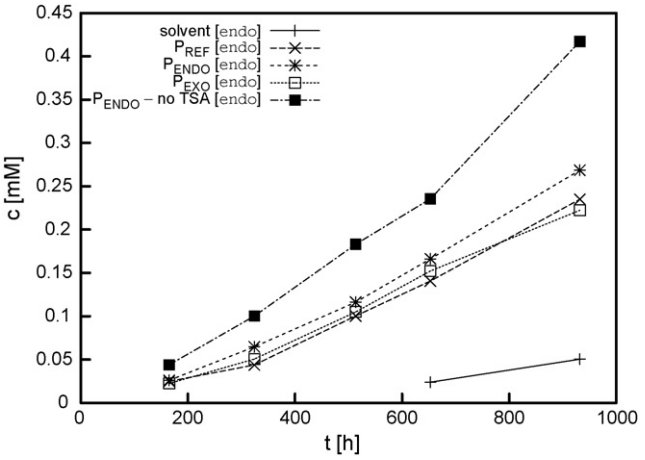


Fig. 6. Formation of **3** in toluene and in the presence of P_{ENDO}, P_{EXO} or P_{REF} and 20 mM *endo*-TSA at room temperature. (Errors estimated to ±10%.)

The latter proposition was supported by elemental analysis data of polymers recovered after use which revealed a higher nitrogen content (0.6–0.8%), suggesting that the diene may have engaged in reactions with residual double bonds present in the polymer matrix. This, in turn, over time reduces the amount of product due to the equilibrium nature of the reaction.

To provide additional evidence for the role of the TSA-selective sites on the observed reaction rate increases, reactions were performed in the presence of the *endo*-TSA (**5**) (20 mM) at room temperature, Fig. 6. While **5** had no effect on the rate of the solution reaction, its presence resulted in a 30% reduction in the performance of both P_{ENDO} and P_{EXO}. Importantly, the presence of **5** had effectively no influence on the role of P_{REF}. Comparable results were obtained using the *exo*-TSA (**6**) (data not shown). This result is significant in that it, again, implies that sites selective for **5** are responsible for the superior activity of P_{ENDO} (and P_{EXO}). This in turn indicates that approximately 30% of the observed product formed under the reaction conditions used arises from reactions taking place within the imprinted sites. The similar activity of P_{EXO} regarding production of the *endo*-product (**3**) suggests that the sites are not able to adequately discriminate between the reaction pathways leading to the two products. We suggest that this may result from the physical closeness of the sites in the two templates at which the monomers interact (carbamate and amide moieties). This physical closeness does not allow for sufficient differences in the corresponding polymers to steer the stereochemical outcome of the reaction. This suggests that more efficient polymers may be obtained by providing additional more well-defined points for interaction.

An important finding, the first of its kind yet reported concerning molecularly imprinted polymer catalysts, is the surprising influence of temperature on the role of the polymers on the outcome of the reaction. The solution reaction was approximately 100 times faster at 120 °C than at 20 °C, while the reaction in presence of polymer showed a fundamentally different behaviour. As the temperature dependence of similar reactions – as can be assumed for the Diels-Alder reaction and the reaction of one of the substrates with the polymer – would be expected to be similar as well, one would expect to find an incorporation of substrate into the polymer even at room temperature. That this could not be observed for the present system suggests that other processes which might be specific for the polymer system are involved.

4. Conclusions

In this preliminary study, a series of polymers capable of enhancing the reaction rate for the Diels-Alders reaction of **1** and **2** to yield **3** and **4** have been designed with the assistance of NMR studies, and synthesised using transition state analogues for the reaction pathways leading to these two products. Template selective recognition was observed. The polymers were found to enhance the rate of the reaction, and the significance of the sites selective for the template was demonstrated by studies performed using the template as an inhibitor. At elevated temperature (120 °C) the polymers not only failed to exert an influence over the reaction outcome, but seemed to inhibit the reaction. At higher temperature the polymer was also found to change in composition under the reactions' progress. This suggests that the polymer actively participates in the reaction instead of catalysing it; the exact processes of which are not known as of now. Further characterisation of this and related systems is ongoing in our laboratory.

Acknowledgements

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