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Stereoselective reduction of menthone by molecularly imprinted polymers

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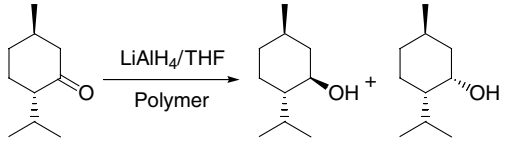
Abstract—Polymeric chiral reductants selective for the reduction of (−)-menthone **1** to the diastereomeric products (−)-menthol **2** and (+)-neomenthol **3** were prepared by a covalent molecular imprinting using **2** as the template. The $LiAlH_4$ derivatized imprinted polymers altered the natural outcome of the reduction reaction ($LiAlH_4$) from 2:1 [(−)-menthol:(+)-neomenthol] to 1:1. The reaction mechanism is discussed in terms of reaction site structure. The molecularly imprinted polymers demonstrated enantioselective recognition for **2** (0.15 μmol enantioselective sites/g polymer) in batch binding experiments.

1. Introduction

Achieving the stereochemical integrity demonstrated in many biological reactions is a major goal for synthetic organic chemistry.[1] In order to achieve this aim, a vast number of different strategies has been investigated, for example, chiral Lewis acids,[2] cavitands,[3] catalytic antibodies,[4] cyclodextrins[5] and, more recently, molecularly imprinted polymers (MIPs).[6]

Molecular imprinting[7] involves the formation of cavities in synthetic polymer matrices that are of complementary functional and structural character to a predetermined template. Appropriately functionalized monomers form complexes, either covalent or noncovalent, with the template, which are subsequently fixed into a rigid network polymer by polymerization in the presence of an excess of an inert crosslinking monomer. Removal of the template reveals recognition sites that are selective for the original template structure. MIPs have been utilized in an ever increasing number of application areas, for example, biosensor recognition elements, solid phase extraction matrices, chromatographic stationary phases and for the mediation of synthetic reactions,[8] both as catalysts[9] and as moderators.[10]

The objective of the present study was to examine the possibility for MIP-directed synthesis using a template of limited size and containing minimal functionality. Menthol has previously been used as a template for noncovalent molecular imprinted polymers[11,12] and silica based gels.[13] The single hydroxyl present in the template suggested the use of a covalent imprinting strategy, which should facilitate the positioning of reactive functionality. The reaction system selected for investigation was the reduction of the terpenoid (−)-menthone **1** by $LiAlH_4$ (Scheme 1). This reaction yields two diastereomeric products, (−)-menthol **2** and (+)-neomenthol **3**. Reductions of **1** using (−)-menthone reductase,[14] cyclodextrins,[15] $LiAlH_4$ or sodium on alumina[16] favour the production of **2** over **3**.



Scheme 1. Reduction of (−)-menthone **1** by $LiAlH_4$ yielding (−)-menthol **2** and (+)-neomenthol **3**.

2. Results and discussion

A series of polymers selective for the (−)-enantiomer of menthol were prepared using acrylate derivatized template, (−)-dimenthylfumarate **4** or (−)-menthylacrylate **5**, in copolymers of styrene **6** and divinylbenzene **7**

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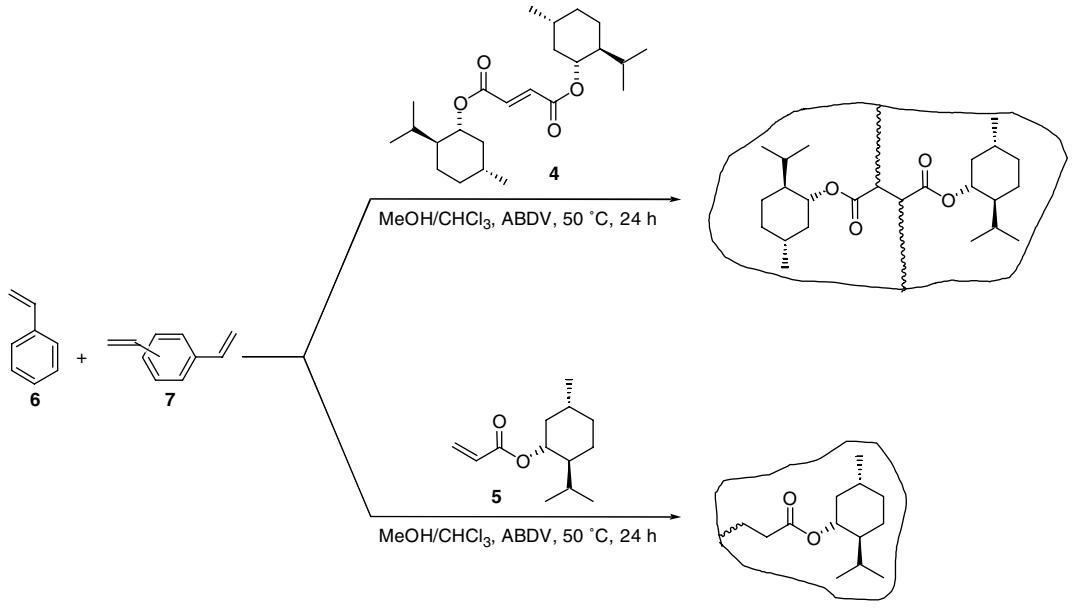
(Scheme 2). A nonimprinted, reference polymer, REF, was prepared using fumaric acid.

In each case, thermally induced polymerization was followed by reductive cleavage of the template by extended exposure to an excess of $LiAlH_4$ to furnish the corresponding primary alcohols (Scheme 3). FT-IR analysis of the resultant polymers showed a decrease of at least 75% of the carbonyl absorption at 1726 cm^{-1} and 1750 cm^{-1}, respectively, (representative spectra are shown in Fig. 1). Extended reaction times did not improve the efficiency of the reduction reaction, thus it was concluded that residual carbonyl absorption is due to the presence of unreacted ester functionalities that were inaccessible to the reagent. After work-up, the hydroxyl moieties were reacted with $LiAlH_4$ (treatment with 1 equiv, based upon theoretical number of sites) to yield polymers with AlH_3-functionalities in the sites created by removal of the template.

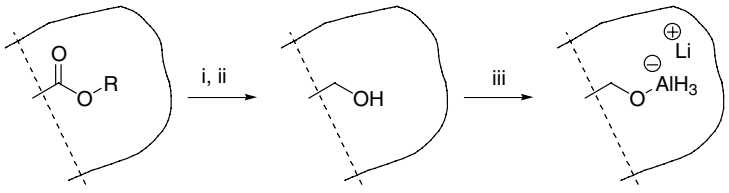
The reduction of (−)-menthone was investigated using three different reactions: (1) reduction with $LiAlH_4$; (2) reduction in the presence of the $LiAlH_4$-activated reference polymer and (3) reduction with each of the $LiAlH_4$-activated imprinted polymers. After standard workup and extensive washing, the reaction products were analyzed by GC–MS.

The reduction of (−)-menthone **1** in THF solution with $LiAlH_4$ was quantitative (GC–MS) and furnished the diastereomeric products **2** and **3** in a ratio of 2:1 (Fig. 2, Table 1). The reference polymer, REF, afforded an identical product distribution, once again in quantitative yield. This indicates that the polymer matrix itself does not influence the stereochemical outcome of the reaction.

However, the use of MIP1, based on (−)-dimenthylfumarate **4**, induced a significant change in the outcome of the reaction, whereby the product ratio was shifted from approximately 2:1 to 1:1, in favour of the naturally unfavoured product, (+)-neomenthol **3**. In the case of MIP2, which was synthesized using 2 equiv of (−)-menthylacrylate **5** instead of **4**, a similar influence on reaction outcome was observed, though the effect was not as pronounced. MIP2 has the same composition as MIP1 with respect to the number of (−)-menthyl moieties, though a slightly lower degree of cross-linking. In the case of MIP3, the polymer was prepared using an equimolar amount of **5** as compared to MIP1, that is half the concentration of (−)-menthyl moieties though with the same degree of cross-linking. Reactions with MIP3 produced the same product profile as seen with the solution and REF reactions. Collectively, these results indicate that the number (stoichiometry) of template structures, and their disposition in the resultant



Scheme 2.



Scheme 3. Reagents and conditions: (i) grinding, sieving/particle sizing; (ii) $LiAlH_4$ (excess), THF, reflux 48 h; (iii) 1 equiv $LiAlH_4$, THF, reflux 24 h.

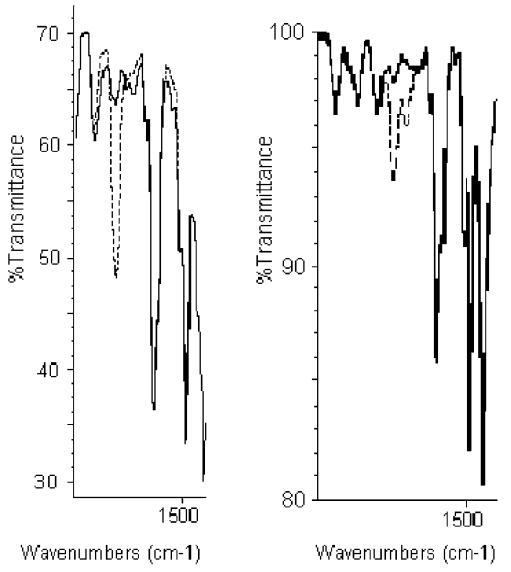


Figure 1. Infrared spectra of MIP1 (left) and REF (right), before template removal (dotted line) and after (black line).

Figure 2. GC–MS traces from the reduction of (−)-menthone **1** to yield (−)-menthol **2** and (+)-neomenthol **3** using $LiAlH_4$, REF and MIP1.

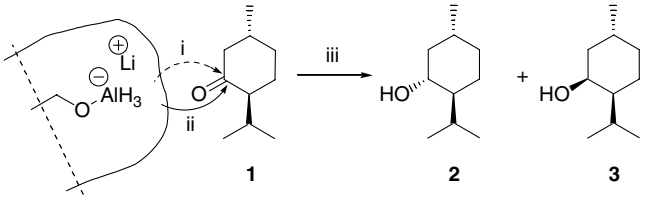
Table 1. (−)-Menthone reduction ratios

Reductant[a]	(+)-Neomenthol (%)	(−)-Menthol (%)
$LiAlH_4$	32 ± 1	68 ± 1
REF	34 ± 3	66 ± 3
MIP1[b]	45 ± 3	55 ± 3
MIP2	39 ± 3	61 ± 3
MIP3[c]	321 ± 3	69 ± 3

[a] Minimum of three experiments with triplicate GC–MS analysis of each.
[b] MIP1: 3 mmol (−)-dimenthylfumarate; MIP2: 6 mmol (−)-menthylacrylate and MIP3: 3 mmol (−)-menthyl acrylate.
[c] 76% reduction of (−)-menthone.

polymer, both influence the stereochemical outcome of the reaction. Moreover, the results imply that some gain is achieved by coordinating the localization of template sites, that is through having sites selective for (−)-menthol in close proximity to one another.

The observed change in the stereochemical outcome of the reaction in the presence of the imprinted polymer MIP1 is concluded to arise from the presence of stereoselective reactive sites in the polymer. A proposed pathway for the reduction of **1** is presented in Scheme 4. The initial orientation of the ester in the binding cavity determines the position of the resultant hydroxyl in the template-selective cavity. Subsequent derivatization with $LiAlH_4$, places the reactive functionality so that hydride delivery to the Si-face of the ketone **1** is favoured, which leads to the production of **3**.

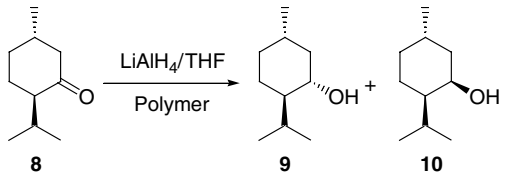


Scheme 4. Reagents and conditions: (i) Re-facial hydride delivery; (ii) Si-facial hydride delivery; (iii) 1 equiv of **1**, THF, reflux 24 h.

To provide additional insight into the selectivity of the reactive site, comparable reaction studies were performed using (+)-menthone **8**, to yield (+)-menthol **9** and (−)-neomenthol **10** (Scheme 5). In this case, MIP1 induced a similar product distribution to that obtained in the solution reaction, 32% **9** and 68% **10** This observation highlights the stereochemical integrity of the reactive sites, that is the stereoselectivity of the polymer induced reactivity.

In order to gauge the inherent stereoselectivities of the resultant polymers, a series of batch binding studies was performed using the nonactivated forms of MIP1 and REF, that is with residual primary hydroxyl functionalities. Studies over the range 0.5 μg/mL–5 mg/mL of the menthol isomers (heptane, 298 K), showed that optimal enantioselectivity was observable at 5 μg/mL. At this concentration a clear distinction between the binding of the (−)- and (+)-enantiomers of menthol was evident (Fig. 3, Table 2). The nonspecific binding to the polymer matrix is reflected in the binding to REF. The recognition of **2** by MIP1 is superior to that of its enantiomer and its diastereoisomers.

The enantioselective binding is concluded to arise from the presence of template selective sites. The theoretical



Scheme 5. Reduction of (+)-menthone **8** by $LiAlH_4$ yielding (+)-menthol **9** and (−)-neomenthol **10**.

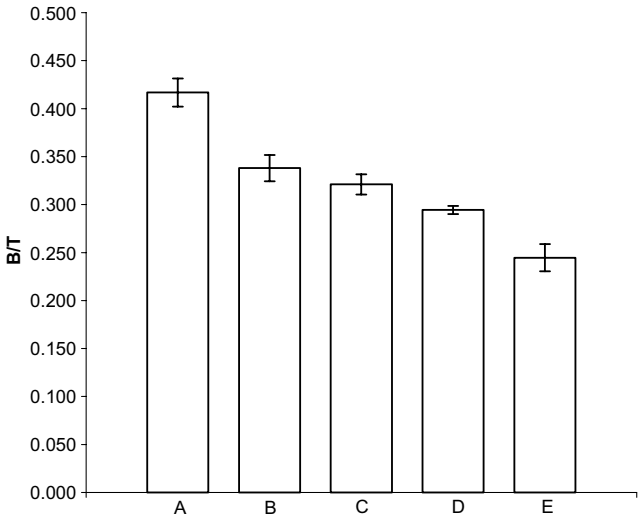


Figure 3. Binding of menthol stereoisomers (5 μg/mL) to MIP1. A: (−)-menthol **2**; B: (+)-menthol **9**; C: (+)-neomenthol **3**; D: (−)-neomenthol **10**; E: average of isomer binding to REF. B/T is the ratio of bound over total added analyte. The results are based on quadruplicate experiments with triplicate analyses of each. Error bars represent RSD for the calculated binding.

Table 2. Menthol isomer binding in heptane

MIP1[a]	B/T[b]	RSD[c]
(−)-Menthol	0.417	0.015
(+)-Menthol	0.338	0.014
(+)-Neomenthol	0.321	0.011
(−)-Neomenthol	0.294	0.004
REF[d]	0.245	0.014

[a] Minimum of four experiments with triplicate GC–MS analysis of each.
[b] B/T is the ratio of bound over total added analyte.
[c] RSD is the relative standard deviation.
[d] Average binding of the menthol isomers.

number of sites selective for **2** in MIP1 is 158 μmol/g polymer (dry weight).[17] The enantioselectivity demonstrated in the batch binding experiments using MIP1 translates to 0.15 μmol/g polymer of sites with selectivity exclusively for **2**. This data provides additional evidence in support of the presence of sites selective for the template.

SEM analysis of MIP1 and REF showed that they have similar morphologies prior to template cleavage (Fig. 4) though, in the case of MIP1, template cleavage results in a somewhat rougher structure. BET-surface analysis (Table 3) indicates a relatively larger gas accessible surface area in the case of the MIP1 after template cleavage, though relatively little change to REF. This may, to some extent, reflect an increase in surface area arising from the presence of polymer cavities/sites left by the template.

Table 3. BET surface area analyses[a] prior and after template removal

Prior		After	
Polymers	BET (m^2/g)	Polymers	BET (m^2/g)
REF	145.4	REF	140.2
MIP1	127.3	MIP1	158.7

[a] Performed on a Micrometrics ASAP 2400, samples were degassed at 100 °C for 24 h before analysis.

3. Conclusions

In summary, polymers selective for (−)-menthol have been synthesized and, after derivatization with $LiAlH_4$, used to alter the stereochemical outcome of the reduction of menthone **1** to (−)-menthol **2** and (+)-neomenthol **3** in favour of the naturally unfavoured product. Moreover, this study demonstrates that this covalent molecular imprinting strategy is amenable to relatively small, poorly functionalized organic structures, and that chiral

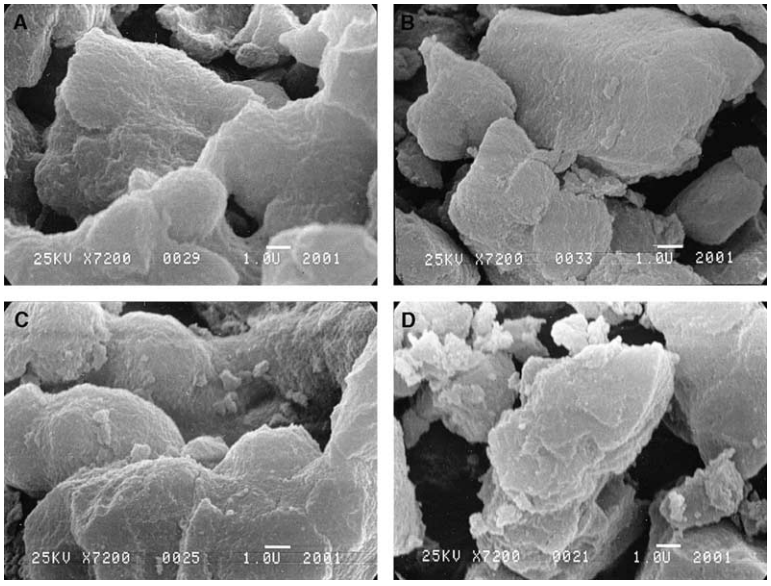


Figure 4. SEM pictures of REF (top), and MIP1 (bottom) before template removal (A and C) and after (B and D) at 7200× magnification.

reductants based on such small organic compounds can be prepared. This underscores the potential for using the molecularly imprinted technique to produce tailor-made reagents for stereoselective organic synthesis.

4. Experimental section

4.1. General methods

IR spectra were recorded on an Avatar 320 FT-IR spectrometer. Solid samples were analyzed using the diffuse reflectance mode employing KBr as dispersant. ^{1}H and ^{13}C NMR spectra were acquired at 250 and 62.5 MHz, respectively, on a Bruker AC-250 MHz instrument. Elemental analyses were performed by Mikrokemi AB (Uppsala, Sweden). Optical rotation was measured on a Perkin–Elmer 141 polarimeter.

BET-surface analyses were performed on a Micromeritics ASAP 2400 instrument. Samples were degassed at 100 °C for 24 h before analysis. SEM studies were carried out using a JEOL JSM-35C Scanning electron microscope. A Polaron Equipment Ltd. SEM Coating unit (E5100 Series II, Cool sputter coater) was used for sample coating.

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, 200 °C; column pressure, 50 psi; carrier gas flow, 1.0 mL/min; split ratio, 72; column oven temperature program, 90 °C (2 min) to 180 °C, at 10 °C/min.

Materials: all chemicals and reagents were purchased from either Aldrich (Germany) or Fluka (Germany) and were used as received unless otherwise stated. Solvents used were purchased from Aldrich and were of HPLC grade. Monomers containing inhibitors were distilled under vacuum prior to use. Anhydrous THF was prepared by distillation from $LiAlH_4$ under an inert atmosphere (N_2).[18] Anhydrous methanol was prepared by distillation from Mg and I_2 under N_2.[18]

4.2. (−)-Menthyl acrylate 5

Compound **5** was prepared by a modification of the original procedure described by Lee–Ruff et al.[19] To a stirred cooled solution (0 °C, ice) containing (−)-menthol (3.12 g, 20 mmol) and pyridine (1.9 mL) in heptane (20 mL) under an atmosphere of under N_2 was added acryloyl chloride (2.18 g, 24 mmol) over a period of 30 min. Stirring was continued for 30 min., whereupon the solution was allowed to warm to rt and stirring continued overnight. The reaction mixture was washed with saturated $NaHCO_3$ (aq) (6 × 10 mL), water (6 × 10 mL) and brine (6 × 10 mL). The combined aqueous phase was extracted with heptane (3 × 20 mL) and combined with the initial organic phase, dried over Na_2SO_4, filtrated and reduced in vacuo (rotary evaporator) to furnish a yellow oil (3.51 g). Flash column chromatography (silica gel, heptane/ethyl acetate; 9:1) afforded a colourless oil (2.90 g, 69%). $[\alpha]_D^{20} = -83.6$ (c 0.97, CH_2Cl_2), lit. $[\alpha]_D^{25} = -85.4$.[19] IR(neat) 1721, 1195 cm^{-1}; ^{1}H NMR: δ 0.75 (d, 3H), 0.9 (dd, 6H), 1.0 (m, 2H), 1.4 (m, 2H), 1.65 (m, 3H), 1.85 (m, 1H), 2.0 (m, 1H), 4.75 (m, 1H), 5.8 (d, J = 10 Hz, 1H), 6.0 (dd, J = 17, 10 Hz, 1H), 6.4 (d, J = 17 Hz, 1H); ^{13}C NMR: δ 16.4, 20.7, 22.0, 23.5, 26.3, 31.4, 34.2, 40.9, 47.1, 74.3, 129.0, 130.1, 165.8. MS (EI, 70 eV) m/z 210 (M+). Spectral properties were in agreement with those previously reported.[19]

4.3. MIP1 (nonreduced form)

A solution of styrene (120 mmol), divinylbenzene (120 mmol) and (−)-dimenthylfumarate (3 mmol) in anhydrous methanol (7.5 mL) and chloroform (20.1 mL) was stirred at rt. ABDV (azobis-[2,4-dimethylvaleronitrile]) (300 mg) was added and the mixture was sonicated under vacuum. The resultant solution was purged with dry N_2 at 0 °C for 5 min. Polymerization was performed at 50 °C for 24 h. The solvents were removed and the polymer was ground and sieved to yield particles in the size range 50–60 μm. The polymer particles were filtered under vacuum and dried before being stored in a desiccator under vacuum until use. IR(KBr) 1726 cm^{-1}.

4.4. REF (nonreduced form)

A reference polymer was prepared using the procedure described for MIP1, with the substitution of fumaric acid (3 mmol) for (−)-dimenthylfumarate. IR(KBr) 1750 cm^{-1}.

4.5. MIP2 and MIP3 (nonreduced forms)

The synthesis of MIP2 and MIP3 was performed as described above for MIP1, though with the substitution of (−)-dimenthylfumarate **2** (3 mmol) with (−)-menthyl acrylate **5** (MIP2, 6.0 mmol; MIP3, 3.0 mmol).

4.6. Polymer reductions

In a typical polymer reduction, $LiAlH_4$ (15 equiv, 522 mg) was carefully added to a slurry of the polymer (MIP1, 4.50 g) in dry THF (70 mL) under N_2. The solution was heated at reflux for 48 h. The reaction mixture was carefully poured into water (800 mol) and the polymer filtered off and washed successively with HCl (1 M, 100 mL), water (100 mL), ethanol (95%, 100 mL) and diethyl ether (100 mL). The polymer was then dried under vacuum overnight. FT-IR analyses were used to determine the extent of ester reduction (carbonyl absorption bands 1726 cm^{-1}) and acid reduction (carbonyl absorption bands 1750 cm^{-1}). MIP1: Anal. found: C, 90.3; H, 8.1. REF: Anal. found: C, 88.7; H, 8.0.

4.7. Polymer activation

$LiAlH_4$ (1 equiv per theoretical hydroxyl, 40 mg) was added to a slurry of polymer (0.500 g) in dry THF (30 mL) and the mixture was heated at reflux under N_2 for 24 h prior to use in subsequent reaction studies.

4.8. Reaction assays—representative example of (−)-menthone reduction using polymers

(−)-Menthone **2** (1 equiv per theoretical hydroxyl, 17.6 μL) was added to the activated polymer slurry (0.500 g/30 mL) and the mixture was heated at reflux for 24 h. The reaction mixture was poured into water (50 mL) and HCl(aq) (1 M, 5 mL) was added. The reaction mixture was extracted with CH_2Cl_2 (3×20 mL), the combined organic phases were washed with water (30 mL) and brine (30 mL). The polymer was collected by vacuum filtration and washed extensively with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered and concentrated to yield a mixture of the diastereomeric products (yield 85%). GC–MS analysis revealed >99% consumption of starting material in all cases. Assays were performed in triplicate on each of at least four separate batches of polymer. Solutions of commercial (−)-menthone, (+)-neomenthol, (−)-neomenthol, (+)-menthol and (−)-menthol (1 mg/mL) were used as standards.

4.9. Representative binding experiments

The menthol isomer (0.5 mL, 10 μg/mL) was added to 0.5 mL of polymer slurry (33.3 mg/mL, 2.2 mL) and incubated at rt in glass vials for 19 h. Centrifugation at 10,000 rcf for 6 min was followed by removal of 700 μL of the supernatant for GC–MS analysis. All experiments were performed in quadruplicate.

Acknowledgements

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