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Scalemic and racemic imprinting with a chiral crosslinker

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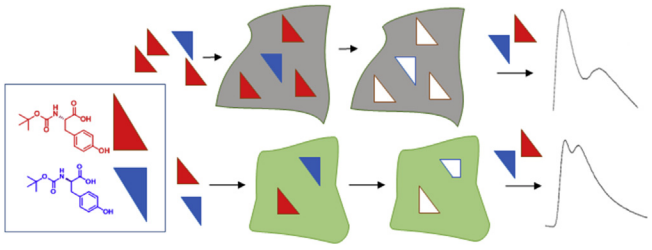
HIGHLIGHTS

- First example of scalemic molecularly imprinted polymers.
- Scalemic imprinting provides partial chromatographic separation using achiral or chiral crosslinker.
- Racemic imprinting provides partial chromatographic separation using chiral crosslinker.
- Batch rebinding of each enantiomer on the racemic imprinted polymer shows different affinity distribution.

GRAPHICAL ABSTRACT



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ABSTRACT

The development of molecularly imprinted chiral stationary phases has traditionally been limited by the need for a chiral pure template. Paradoxically, availability of a chiral pure template largely defeats the purpose of developing a chiral stationary phase. To solve this paradox, imprinting of scalemic and racemic template mixtures was investigated using both chiral (N-α-bismethacryloyl-L-alanine) and achiral (N,O-bisacrylamide ethanolamine) crosslinkers. Imprinting of scalemic mixtures provided polymers capable of partial separation of Boc-tyrosine enantiomers with virtually the same results when using either the chiral or achiral crosslinker. However, the chiral crosslinker was required for chiral differentiation by the racemic imprinted polymers which were evaluated in both batch rebinding and chromatographic modes. Batch rebinding analysis revealed intersecting binding isotherms for the L- and D-Boc-tyrosine, indicating bias for the D or L enantiomer is concentration dependent. Partial chromatographic separation was achieved by the racemic imprinted polymers providing variable D or L bias in equal probability over multiple replicates of polymer synthesis. Correlation of enantiomer bias with the batch rebinding results and optimization of HPLC parameters are discussed.

1. Introduction

Nearly all examples of chiral selectivity by molecularly imprinted polymers require the use of a chiral pure template for the imprinting process [1–5]. However, there are relatively few enantiopure compounds available compared to the universe of chiral compounds which are often found (e.g. natural products) or synthesized (e.g. pharmaceuticals) in racemic or scalemic mixtures [6]. Therefore, it is of tremendous interest to broaden the capabilities of molecular imprinting beyond requiring pure enantiomers as templates, to the use of imprinting racemic or scalemic mixtures. The molecular imprinting process using a scalemic mixture (a mixture of enantiomers at a ratio other than 1:1) and similarly for a racemic mixture is outlined in Scheme 1 where monomers and scalemic template are equilibrated in solution (a), then polymerized to give the imprinted material (b), subsequently washed to leave binding sites (c) with chiral selectivity for one of the template enantiomers (d) [7].

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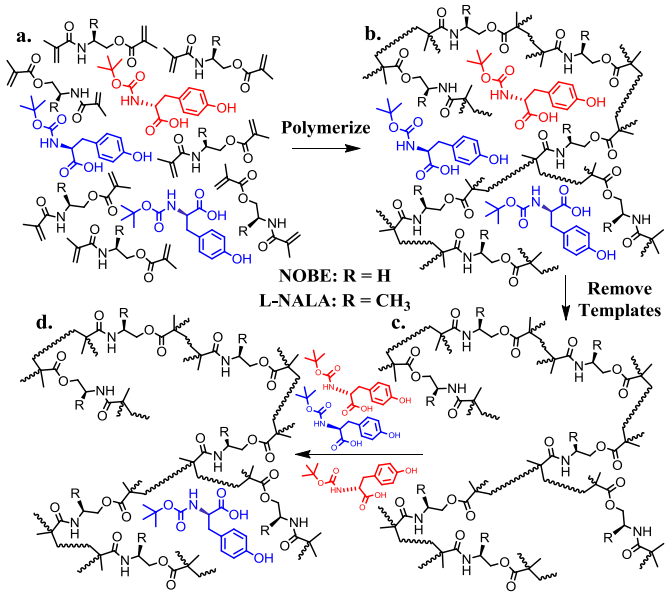

Scheme 1. Outline of the process for molecular imprinting a scalemic template mixture using L-NALA or NOBE crosslinkers. (a) Pre-polymerization complex; (b) imprinted polymer; (c) templates removed from polymer; (d) selective template rebinding.

There have been three reports of racemic imprinting that make use of a chiral functional monomer that have shown potential for racemic imprinting [8–10]; however there have not yet been any reports investigating polymers imprinting scalemic templates. Therefore based on previous development of chiral crosslinkers in our group [10], a study on the use of scalemic and racemic mixtures for imprinting was investigated using the achiral crosslinker N,O-bisacrylamide ethanolamine (NOBE) or the chiral crosslinker N-α-bismethacryloyl-L-alanine (L-NALA) in an OMNiMIP (one monomer molecularly imprinted polymer). OMNiMIPs, which imprint using one crosslinking monomer, have shown increased performance over traditional multi-monomer imprinted polymers toward both chiral selectivity and separation of a target compound in a mixture [11–14].

There has always been a paradox with the traditional development of molecularly imprinted chiral stationary phases (MIP-CSP). In virtually all cases (with the exception of the three references noted above), a pure chiral template has been used for the formation of a MIP- CSP. The paradox is that it is necessary to have the enantiopure target compound before the MIP-CSP can be fabricated; however, the enantiopure target may not be available and that may even be the driving force for fabricating the MIP-CSP in the first place. Most chiral compounds synthesized provide scalemic or racemic products, even if a stereoselective process is used [6]. Therefore, development of molecular imprinting methods that can use the scalemic or racemic compounds as the template would provide a solution to the traditional MIP-CSP paradox. Torres et al., demonstrated the use of a chiral monomer that forms diastereomeric complexes with each enantiomer of a racemic template, thereby forming binding sites in the MIP-CSP that exhibit chiral selective binding [9]. The same principles can be employed for scalemic templates that are used for forming MIP-CSPs.

In the racemic imprinting method presented by Torres et al., a non-crosslinking chiral carboxylate monomer was developed that is primarily limited to imprinting amine based targets, such as the bis(1-phenylethyl)amine. The group of Hosoya also synthesized a non-crosslinking functional monomer that could form Pirkle-type diastereomeric interactions with nitro-aromatic derivatized chiral amines [8]. A third example by our group introduced the chiral crosslinking monomer L-NALA, which is further investigated here toward scalemic and racemic imprinting which can ultimately be used either for enantiomeric separations, or for simply determining enantiomeric excess (% ee) of scalemic mixtures [10]. A primary advantage of scalemic/racemic imprinted polymers is that the process time for polymerization-to-operation of the MIP-CSP can be as fast as 1–2 days; whereas commercial columns are not only expensive, but also require mobile phase development that may take longer.

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma–Aldrich, with the exception of D-Boc-Tyr purchased from Chem-Impex, and used without further purification. The solvents were used as received from either Sigma or Fischer Scientific. Reactions carried out under anhydrous conditions were performed in oven dried glassware under N_2 atmosphere. Synthesized compounds were purified by flash chromatography using flash silica gel (32–63 μm) from Science Adsorbents Inc. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DPX-250 and a Bruker AVIII-400 spectrometer for compounds dissolved in $CDCl_3$. Empty stainless steel HPLC columns were purchased from Grace Davison Discovery Sciences.

2.2. Synthesis of crosslinking monomers

The synthesis of NOBE and L-NALA followed the published protocols [10,14]. NOBE: ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 6.41 (1H, br, NH), 6.06 (1H, s), 5.64 (1H, s), 5.54 (1H, s), 5.27–5.25 (1H, d, J = 8 Hz), 4.25–4.22 (2H, t, J = 6 Hz), 3.58–3.54 (2H, q), 1.90 (3H, s), 1.88 (3H, s.) ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 168.46, 167.65, 139.78, 135.94, 126.20, 119.76, 63.36, 39.22, 18.58, 18.29. NALA: ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 6.12 (s, 1H), 6.01 (br, 1H), 5.99 (s, 1H), 5.66 (s, 1H), 5.59 (s, 1H) 4, 4.39–4.36 (m, 1H), 4.16–4.09 (m, 2H), 1.94 (s, 6H), 1.24 (d, 3H, 4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 171.14, 167.89, 140.02, 135.93, 126.16, 119.51, 67.10, 44.89, 18.56, 18.27, 17.31.

2.3. Synthesis of imprinted polymers

The monomer (1.0 g) was added to a 13 × 100 mm glass tube along with solutions of Boc-Tyr (5 mol% with respect to monomer) in 1.3 mL acetonitrile. The initiator AIBN (1.0 mol% with respect to monomer) was added into the solution and purged with nitrogen for five minutes. To seal the system, the glass tube was capped, wrapped with Teflon tape, and overlayed with Parafilm. The glass tube was inserted into a photoreactor apparatus and submerged in a water bath where the temperature was maintained at 21 °C. The tube with the solution mixture was then exposed to a 450 W mercury arc lamp surrounded by a borosilicate jacket for 8 hours immersed in the water bath along with the polymer mixture. To remove the polymer, the glass test tube was broken with a hammer and the particle monolith removed. The resulting polymer was lightly crushed into pieces in the 1–5 mm size range and placed in a Soxhlet extraction apparatus charged with methanol for two days to remove the template(s). Using U.S.A. Standard Testing Sieves the polymer was further sized to 25–38 μm after grinding with mortar and pestle and slurried with acetone. The sized polymer was slurry-packed into a stainless steel column (100 mm × 2.1 mm i.d.) for analysis by HPLC (Hitachi L-7000 series equipped with L-7100 pump, L-7400 detector and L-7500 integrator) in a 99/1 acetonitrile/acetic acid mobile phase.