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Polymer 41 (2000) 5583–5590

polymer

Enhancement of selectivity of imprinted polymers via post-imprinting modification of recognition sites

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Received 31 August 1999; received in revised form 20 October 1999; accepted 25 October 1999

Abstract

Imprinted polymers were synthesized for the recognition of small nitrogen heterocycles such as pyridine and quinoline using a new variant of the sacrificial spacer methodology employing silyl ether derivatized templates designed to act as N–H–O 'isosteres' for binding the targets. The cleavage of the labile silyl ether bond led to the formation of sites bearing phenolic residues. The polymers prepared with DVB as the cross-linker were capable of discriminating between pyridine, quinoline and acridine in hexane but the effect did not exceed 20%. In order to improve the recognition properties of these materials, a post-imprinting modification of the polymers was performed using acyl chlorides of varying size. This simple approach, carried out after the removal of the templates, resulted in an enhanced selectivity (by factors of up to 5-fold) for binding of pyridine and quinoline by the modified polymers. Similar effects were observed with EGDMA-based imprinted polymers. The results obtained suggest that post-imprinting chemical modification can be an effective tool to engender 'size' selectivity in binding of even small molecules containing a paucity of functional groups. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Molecular imprinting; Imprinted polymer; Selectivity

1. Introduction

Molecular imprinting [1–6] is a method for creating recognition sites in synthetic polymers. The technique involves carrying out polymerization around a template molecule, which is attached to a monomer by covalent or non-covalent bonds, such that a rigid cross-linked material is formed. After removal of the template, a recognition cavity in the polymer remains, retaining shape and functionality complementary to the molecule that was imprinted. The range of templates used to date in imprinted polymers is wide and varies from biomolecules, such as sugars [7], amino acids [8,9], peptides [10,11], steroids [12–14], nucleosides [15], dyes [16], drugs [17] and pesticides [18], to proteins [19], bacterial cells [20] and even inorganic crystals [21].

Three general strategies of imprinting have been developed: covalent [22,23], non-covalent [24] and a combination of the two, which we have termed the 'sacrificial spacer' methodology [25,26]. The relative merits of each method have been discussed extensively elsewhere [27–31] but all three have been essentially developed to deal with a particular set of functional groups present in specific templates. Indeed, with a few notable exceptions, e.g. glyceric acid esters [32], β-blockers [33], cholesterol [25,34], hydrophobic interactions [35,36] and 'solvent' imprinting [37–39], the templates that have been targeted in imprinting research are generally those which bear multiple functional groups. As a result, there is a need to develop general methodologies to address the imprinting of more poorly functional templates, and/or to design alternative strategies to enhance the selectivities of such imprinted polymers.

The objective of this investigation was to introduce a variant of the sacrificial spacer method using silyl ether derivatized templates designed to act as N–H–O 'isosteres' for binding nitrogen heterocycles, and also to assess the importance of 'size' discrimination in the recognition properties of polymers imprinted with templates containing a single functional group. More specifically, we aimed to determine whether a single hydrogen bond, in conjunction with steric constraints in the polymer recognition site, would be sufficient to discriminate between small heterocyclic aromatic ligands and, if not, to devise a strategy to enhance the selectivity of such

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PII: S0032-3861(99)00782-X

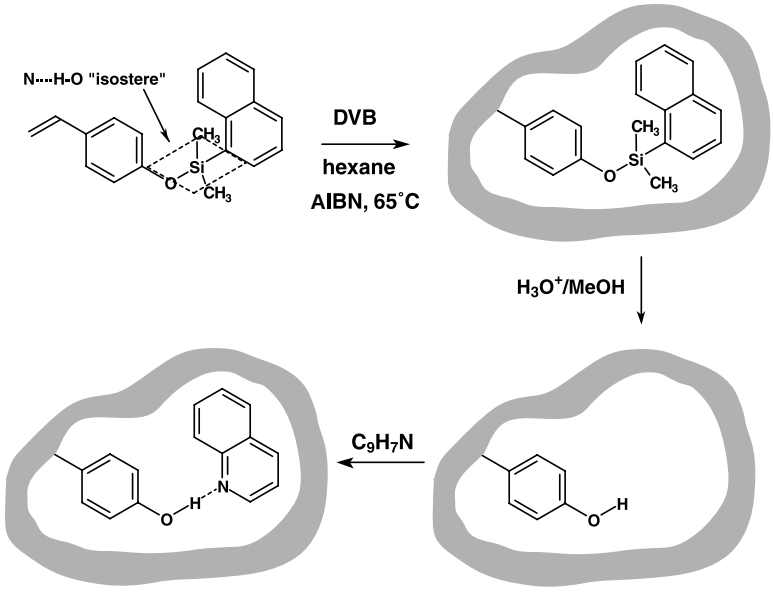

Fig. 1. Schematic diagram of the imprinting procedure using a silyl ether as an N···H–O isostere and single atom sacrificial spacer to create recognition sites for nitrogen heterocycles.

recognition. Three heterocycles, pyridine, quinoline and acridine were chosen as suitable model ligands for the purpose of this investigation.

2. Results and discussion

The imprinting of heterocyclic compounds has generally been carried out using the non-covalent strategy, typically via hydrogen-bonding of carboxylic acid monomers to aromatic nitrogen residues, and molecules containing pyridine groups have been selectively retained in chromatographic columns of polymers prepared by this method [40]. However, the use of single hydrogen-bonds to hold the template and monomer together limits the flexibility in choosing polymerization conditions as the template–monomer equilibrium must lie strongly in favor of the complex [41]. To overcome this limitation we planned to link the template and monomer via a covalent bond and thus required an appropriate structural analogue to use at the imprinting stage. It was also important to ensure that cleavage of the template from the resultant polymer would leave a suitable functional group, i.e. phenolic residue, specifically placed to form a hydrogen bond with the desired heterocycle ligands. Inspection of standard bond lengths suggested the N–H–O moiety used in the non-covalent re-binding step could be replaced by an 'isosteric' C–Si–O grouping (Fig. 1). Preliminary molecular modelling predicted a bond length for an C(Ar)–*Si* bond of ~1.9 Å, which we believed would be a better match for the pyridyl-N–H–O system, which is typically ~2.0 Å in length rather than the 1.49 Å bond distance of the C(Ar)–*C* moiety. In addition, the use of silyl ether chemistry offered the possibility of facile template removal via mild acidic hydrolysis or nucleophilic displacement with fluoride ion, and importantly for condensed aromatic templates, enhanced solubility in apolar solvents used as porogens in imprinted polymer synthesis.

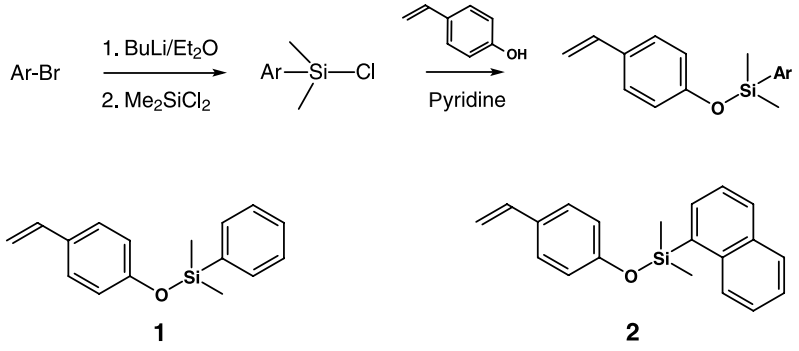

Fig. 2. General synthetic scheme for the preparation of silyl ether templates and the structures of templates (4-vinylphenoxy)dimethylphenylsilane (**1**) and (4-vinylphenoxy)dimethyl-(1-naphthyl)silane (**2**).

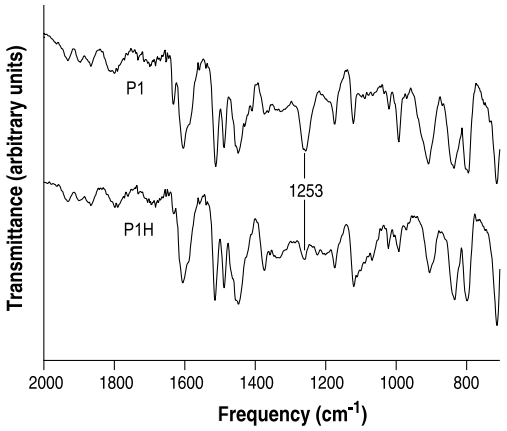

Fig. 3. IR spectra of DVB polymers imprinted with template **1**, before (**P1**, top) and after (**P1H**, bottom) removal of template by acidic hydrolysis, showing the loss of the band at 1253 cm^{-1}, attributed to silyl ether stretch.

We accordingly prepared two templates, reacting bromonaphthalene with butyllithium followed by in situ treatment with excess dichlorodimethylsilane. The intermediate chlorodimethylsilylarenes were reacted with 4-vinylphenol in the presence of pyridine to generate polymerizable aryloxydimethylsilanes. The structures of the 'pyridine' and 'quinoline' analogue monomers **1** and **2** are shown in Fig. 2. Yields for these syntheses were typically between 40–45% but were not optimized. Imprinted polymers **P1** and **P2** were then synthesized with 5 mol% of template using divinylbenzene (DVB) as a cross-linker. A DVB-based control polymer (**P0**) was prepared under the same conditions as **P1** and **P2** by replacing the template with styrene. Following polymerization for 24 h, the resultant highly cross-linked materials were crushed to a fine powder (<30 μm) and washed thoroughly. Cleavage of silyl ether groups to liberate the templates and expose phenolic hydroxyl residues in the sites was carried out using refluxing 5 M HCl/CH_3OH (conditions under which low molecular weight aryldimethylsilylethers were fully hydrolysed). As it was difficult to obtain quantitative data relating to template removal by chemical analysis owing to the volatility of the cleavage products, FT-IR spectroscopy was used to provide evidence of the release of template from the imprint sites. IR spectra of the imprinted DVB polymers obtained after hydrolysis clearly showed a substantial reduction in the Si–O stretch at 1253 cm^{-1} with a concomitant increase in OH stretches at 3500 cm^{-1} relative to the untreated polymers (Fig. 3). This confirmed qualitatively that the hydrolysis of silyl ethers took place as anticipated under the reaction conditions used.

Once obtained, the polymers were assessed for binding of *N*-heterocycles in uptake experiments. These were carried out in batch mode, shaking a suspension of polymer (10 mg, 3.68 μmol theoretical binding sites for **P1H**, **P2H**) with 1 ml of 2 mM ligand solutions in isohexane to facilitate the formation of hydrogen bonds between the polymer-bound phenolic residues and the aromatic nitrogen moiety of the heterocycles. Preliminary experiments established that no further uptake occurred after 12 h: thus we were able to evaluate ligand binding under equilibrium conditions and assess qualitatively the relative energetics of the heterocycle recognition process. After 16 h, the suspensions were filtered and the amount of ligand remaining in solution assayed by HPLC. As can be seen in Table 1, the overall pattern of binding to polymer **P1H** was as expected based on the size of the templates, with pyridine binding to the greatest extent, followed by quinoline and acridine. Similarly, for the 'quinoline-imprinted' polymer **P2H**, quinoline was found to exhibit the greatest uptake, followed by pyridine and acridine. The non-hydrolysed polymers **P1** and **P2** as well as the non-imprinted polymer **P0** showed considerably less uptake (54–75% reduction compared to **P1H** and **P2H**), as expected, thus implying that the binding of ligands was indeed driven by the formation of hydrogen bonds. However, the discrimination between the three ligands (Fig. 4) by **P1H** and **P2H** was not high with the difference in binding being 20% or lower in all cases.

On reflection this result is not too surprising perhaps given the nature of the imprinting process and the ligands used. It seems intuitively obvious that during the polymerization, a wide range of 'pockets' should be created around the template with the smallest of them being only slightly bigger than the dimensions of the template and the rest having a wide distribution of sizes. It is arguable then that a reduction in the size distribution of sites should result in

Table 1
Uptake of aromatic nitrogen heterocycles (2 mM in isohexane) by DVB-based imprinted polymers (10 mg ml^{-1})(non H = nonhydrolysed polymer, H = hydrolysed polymer. Polymers were prepared in hexane.)

Polymer	Template	Surface area ($m^2\ g^{-1}$)	Sites (μmol g^{-1})[a]	Uptake (mM)					
				Acridine		Quinoline		Pyridine	
				Non H	H	Non H	H	Non H	H
P0	Styrene	286	0	0.03 ± 0.01		0.23 ± 0.02		0.22 ± 0.03	
P1	**1**	177	386	0.14 ± 0.01	0.55 ± 0.10	0.18 ± 0.09	0.67 ± 0.11	0.36 ± 0.03	**0.78 ± 0.12**
P2	**2**	108	386	0.12 ± 0.06	0.45 ± 0.07	0.20 ± 0.01	**0.60 ± 0.01**	0.33 ± 0.02	0.54 ± 0.09

[a] Theoretical maximum number of binding sites after hydrolysis.

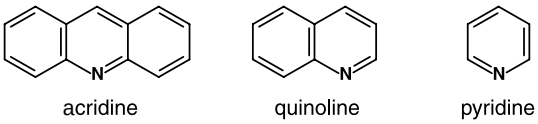

Fig. 4. Structures of the nitrogen heterocycles used in uptake experiments with imprinted polymers.

better discrimination. The real question then is how 'narrow' a distribution can be achieved for ligands as small as pyridine or quinoline, especially when the molecular dimensions of the template are comparable to those of the cross-linker. The results presented in Table 1 suggest that in the best case only ~20% of the sites were small enough to accommodate pyridine and exclude quinoline. It should also be noted that this result was not necessarily at odds with our previous work [25] when a significant difference between uptake of cholesterol to phenol- and cholesterol-imprinted polymers bearing single phenolic hydroxyl groups in binding sites was observed. Indeed, cholesterol is much bigger than phenol and the results in Table 1 indicate that **P1H**, for example, showed better discrimination between pyridine and acridine than between pyridine and quinoline. Nevertheless, the degree of selectivity observed was judged insufficient for practical applications and a method to enhance the specificity was sought.

To this end, **P1H** was reacted with a series of acyl chlorides of decreasing size in the presence of triethylamine with the hope that this simple chemical modification should, at least to some extent, enable us to discriminate 'small' from 'large' sites. A number of groups have reported post-imprinting modifications to reduce non-specific binding [42], but we reasoned that the use of a range of modification reagents differing in their size should increase selectivity still further. Thus, any given reagent would be able to occupy only those sites that are sufficiently large to accommodate it in the correct orientation to react with the phenolic hydroxyl group. These modified sites would therefore be 'switched off' and no longer available for the binding of ligands. A small reagent such as acetyl chloride should be capable of modifying a greater number of sites than anthracene-9-carbonyl chloride, the latter being excluded from all but the largest of sites. The outcome of this simple procedure would be an increase in selectivity of the imprinted polymers for the smallest ligand. Table 2 shows the results of these modifications of **P1H** and **P2H** with acyl chlorides varying in size from anthracene-9-carbonyl chloride to acetyl chloride. It should be noted that chemical modification unavoidably increases the mass of the polymers as well as blocking some of the binding sites, however, any mass change alone could account for no more than an 8% decrease in the capacity of these polymers.

As is evident from the data, this modification strategy worked well, and the observed selectivities were in the expected order. Thus, polymers reacted with anthracene-9-carbonyl chloride displayed greatly decreased uptake of acridine (similar to, or less than, that of non-hydrolysed polymers **P1** and **P2**), but retained the ability to bind pyridine and quinoline. Modification with 1-naphthoyl chloride reduced both acridine and quinoline binding (by 53–100%), whilst pyridine uptake fell to a lesser extent (17–48%). Complete suppression of pyridine binding was not achieved even with acetyl chloride, suggesting that some pockets were large enough to accommodate pyridine, but too small to allow access of more bulky *N*-acyl intermediates. Additional attempts at *O*-alkylation using NaH followed by iodomethane caused a further reduction in the pyridine uptake, but not complete suppression of binding (not shown).

Finally, we decided to verify that the strategy of post-imprinting modification would work well for polymers other than those based on DVB. Consequently, an analogous series of ethyleneglycoldimethacrylate (EGDMA)-based polymers imprinted with templates **1** (**P3**) and **2** (**P4**) was

Table 2
Uptake of aromatic heterocyclic bases by DVB-based polymers modified with different sized acyl chlorides (H = hydrolysed, A = modified with anthracene-9-carbonyl chloride; N = modified with 1-naphthoyl chloride; Ac = modified with acetyl chloride.)

Polymer	Uptake (mM)		
	Acridine	Quinoline	Pyridine
P1H-A	0.10 ± 0.01	0.37 ± 0.01	0.50 ± 0.01
P1H-N	0.10 ± 0.01	0.29 ± 0.01	0.41 ± 0.04
P1H-Ac	0.08 ± 0.03	0.32 ± 0.09	0.41 ± 0.02
P2H-A	0.06 ± 0.08	0.34 ± 0.01	0.56 ± 0.01
P2H-N	−0.04 ± 0.04	0.18 ± 0.04	0.45 ± 0.01
P2H-Ac	−0.06 ± 0.06	0.11 ± 0.05	0.43 ± 0.02

Table 3
Uptake of pyridine, quinoline and acridine by EGDMA-based polymers (Polymer **P3** imprinted with template **1**, Polymer **P4** imprinted with template **2**, Polymer **P5** styrene-EGDM copolymer. H = hydrolysed, A = modified with anthracene-9-carbonyl chloride: N = modified with 1-naphthoyl chloride; Ac = modified with acetyl chloride. All polymers were prepared in toluene/hexane 1/4 (v/v) with EGDMA as the cross-linker present at 95 mol%.)

Polymer	Uptake (mM)		
	Acridine	Quinoline	Pyridine
P3	0.14 ± 0.03	0.12 ± 0.01	0.18 ± 0.01
P3H	0.74 ± 0.08	0.69 ± 0.05	0.54 ± 0.09
P3H-A	−0.01 ± 0.06	0.18 ± 0.08	0.52 ± 0.11
P3H-N	−0.01 ± 0.03	0.03 ± 0.07	0.45 ± 0.16
P3H-Ac	−0.07 ± 0.07	0.09 ± 0.01	0.28 ± 0.03
P4	0.01 ± 0.02	0.02 ± 0.04	0.28 ± 0.05
P4H	0.45 ± 0.12	0.53 ± 0.07	0.44 ± 0.14
P4H-A	0.14 ± 0.01	0.31 ± 0.08	0.44 ± 0.01
P4H-N	0.10 ± 0.08	−0.11 ± 0.08	0.51 ± 0.04
P4H-Ac	−0.15 ± 0.01	0.22 ± 0.04	0.22 ± 0.02
P5	0.06 ± 0.12	0.09 ± 0.15	0.16 ± 0.06
P5H	0.53 ± 0.10	0.61 ± 0.07	0.39 ± 0.08

prepared and characterized (Table 3). Despite several literature reports of the superiority of EGDMA over DVB [43,25], the former was not our first choice of cross-linker as DVB may be more compatible with the aromatic templates used thus potentially favouring ligand recognition via additional π–π stacking interactions in the binding sites. Further, some degradation of the polyester matrix might be expected under the acidic hydrolysis conditions used for template removal, generating hydroxyl and carboxyl groups capable of non-specific interactions with basic ligands. This indeed appeared to be the case and in general EGDMA-imprinted polymers showed higher overall uptake of all the ligands. Significantly, the binding to a non-imprinted EGDMA/styrene (95/5 mol%) copolymer (**P5**) increased substantially on hydrolysis from 0.16 to 0.39 mM for pyridine and 0.09 to 0.61 mM for quinoline. Virtually no differences in the uptake of these ligands were observed with these partially degraded polymers in preliminary experiments (Table 3). However, the chemical modification still worked rather well; blocking the phenol groups (and presumably any hydroxyl and carboxyl groups liberated by hydrolysis) via acylation and methanol work-up, once again resulted in the formation of materials, which were noticeably more selective to quinoline and pyridine (compare Table 2). Thus, the molecular 'sorting' by post-imprinting modification proved to be successful in this case too.

The results described above pose the question of whether an imprinting step alone is sufficient for the preparation of polymers specific to small, poorly functionalized ligands. The evidence in the literature points in two opposite directions. For example, Yoshikazo et al [37] reported the solvent memory of imprinted polymers, thus implying a reasonable level of size or shape discrimination, even for hydrocarbon 'templates'. Also, the imprinting of anthracene [39] and our earlier data on the binding of cholesterol [25] suggest that a moderate degree of size discrimination can be achieved with large templates bearing either no reactive functionality or only a single binding point, respectively. On the other hand, Wulff has stated that, for a series of sugar derivatives at least, the arrangement of functional groups in the cavity plays the dominant role in determining selectivity, with the shape of the binding site being less important [44]. However, if the arrangement of functional groups is the same, the size and shape of re-binding ligands become more significant, although again this applies to multi-functional templates [45]. The results obtained in this study can probably be used to advocate some form of middle ground. On balance, there is little doubt that a degree of selectivity can be introduced into polymers imprinted with relatively small monofunctional ligands. However, whether it is necessary, from a practical point of view, to imprint with a precisely matched template for the generation of selective materials in this case is another matter. Indeed Fréchet and co-workers have conclusively shown that efficient 'molecular sorting' based on the size of derivatizing reagents can be successfully accomplished with conventional polymers or supports bearing suitable functionality [46,47]. It would seem sensible therefore to consider this strategy as a complementary alternative to conventional imprinting of monofunctional templates or, perhaps, even as a general tool to improve the selectivity of imprinted polymer materials. It should be stressed, however, that more research is necessary to better define the scope and limitation of this post-imprinted polymer derivatization strategy.

3. Conclusions

A new variant of the sacrificial spacer methodology was used to imprint pyridine and quinoline via isosteric silyl ether chemistry. Polymers prepared with DVB as the cross-linker showed a limited degree of selectivity for their respective templates, and this was further enhanced for pyridine binding via the post-imprinting modification. EGDMA-based polymers showed no discrimination for pyridine and quinoline following acidic hydrolysis, however, by using the same range of acylation reagents employed to modify the DVB polymers, similar selectivities were observed. The results suggest that combining a conventional imprinted polymer preparation with molecular sorting by post-imprinting chemical modification is an attractive new approach to enhancing the selectivity of imprinted materials towards ligands, which are generally considered to be too small, or contain too few functional groups.

4. Experimental

4.1. Materials and methods

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Jeol EX 270 Fourier transform spectrometer at 67.8 MHz (^{13}C) and 270.05 MHz (^{1}H). IR spectra were recorded on a Perkin–Elmer 1600 series spectrometer by the diffuse reflectance method using KBr as dispersant. All standard reagents were purchased from Aldrich or BDH and used as received. Solvents used for chromatography were purchased from Fisher Scientific and were at least HPLC grade. Anhydrous solvents were prepared by standard methods [48].

Dichlorodimethylsilane, chlorodimethylphenylsilane, 1-bromonaphthalene, *n*-butyllithium, pyridine, *p*-acetoxystyrene, quinoline, acridine, 1-naphthoyl chloride, acetyl chloride, 9-anthracenecarboxylic acid and thionyl chloride were purchased from Aldrich Chemical Co. Ltd. and used as received except acridine, which was recrystallized from isohexane and dichlorodimethylsilane which was redistilled before use. Azo-bis-isobutyronitrile (AIBN) was obtained from Fluka and recrystallized from methanol. Divinylbenzene (DVB tech 80% Aldrich Chemical Co.

Ltd.) was purified by extraction with NaOH solution to remove inhibitors, dried over $CaCl_2$ and filtered through activated Al_2O_3. EGDMA was obtained from Aldrich Chemical Co. Ltd. and was purified with NaOH solution to remove inhibitors, dried over $MgSO_4$ and filtered through activated Al_2O_3. Polymers were filtered from solutions prior to HPLC analysis using 2 μm membrane filter cartridges. HPLC analyses were performed on a Gilson HPLC system using a reversed phase C18 column and a Milton Ray UV detector. EI–MS spectra were obtained on a VG Autospec spectrometer. Polymer surface areas were determined from multi point N_2 adsorption isotherms and calculated using the BET equation. Polymers were degassed in vacuo over night at room temperature before measurement.

4.2. Synthesis of 4-vinylphenol

4-Vinylphenol was prepared by hydrolysis of *p*-acetoxystyrene with aqueous potassium hydroxide according to the method of Corson et al [49]. To an aqueous solution of KOH (21.5 g; 0.38 mol, 210 ml) solution and THF (1 ml) acetoxystyrene (26 g; 0.16 mol) was added. The mixture was stirred until homogenous, the solution was filtered and CO_2 was bubbled through the solution. The white crystals obtained were washed with water, dissolved in diethyl ether and dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from isohexane to obtain the title compound, (10 g; 51%).^{1}H NMR ($CDCl_3$) δ(ppm): 7.28–7.24 (d, 2H, $J = 8.6$ Hz, CH_2=CH–C_6H_4–OH), 6.78–6.75 (d, 2H, $J = 8.6$ Hz, CH_2=CH–C_6H_4–OH), 6.67–6.57 (dd, 1H, $J = 10.9$, 17.5 Hz, *CH*=CH_2), 5.61–5.54 (d, 1H, $J = 17.5$ Hz, *trans*-CH=*CH*$_2$), 5.13–5.09 (d, 1H, $J = 10.9$ Hz, *cis*-CH=*CH*$_2$); ^{13}C NMR ($CDCl_3$) δ(ppm): 154.89 (O–*C*; C^6), 136.08 (*C*H=CH$_2$; C^2), 130.76 (*C*; C^3), 127.65 *C*H; C^5), 115.49 (*C*H; C^4), 111.79 (CH=*C*H$_2$; C^1).

4.3. Synthesis of (4-vinylphenoxy)dimethylphenylsilane (**1**)

An ice-cold reaction mixture of 4-vinylphenol (3 g; 25 mmol) and pyridine (2 ml; 25 mmol) in diethyl ether (20 ml) was stirred during the dropwise addition of chlorodimethylphenylsilane (4.2 ml; 25 mmol) under an atmosphere of nitrogen. After further stirring for 2 h at room temperature a precipitate formed, which was removed by filtration and washed with diethyl ether. The solvent was evaporated and distillation under reduced pressure gave the pure compound as a colourless liquid, (2.65 g; 41.7%): b.p. 104–106°C/0.2 mbar; ^{1}H NMR ($CDCl_3$) δ(ppm): 7.67–7.63 (m, 2H, Si–C_6H_5), 7.43–7.38 (m, 3H, Si–C_6H_5), 7.28–7.24 (d, 2H, $J = 8.6$ Hz, CH_2=CH–C_6H_4–O), 6.80–6.76 (d, 2H, $J = 8.6$ Hz, CH_2=CH–C_6H_4–O), 6.70–6.60 (dd, 1H, $J = 10.9$, 17.5 Hz, *CH*=CH_2), 5.64–5.57 (d, 1H, $J = 17.5$ Hz, *trans*-CH=*CH*$_2$), 5.15–5.11 (d, 1H, $J = 10.9$ Hz, *cis*-CH=*CH*$_2$), 0.54 (s, 6H, Si*CH*$_3$); ^{13}C NMR ($CDCl_3$) δ(ppm): 154.88 (O–*C*; C^6), 137.41 (*C*; C^8), 136.23 (*C*H=CH$_2$; C^2), 132.97 (*C*H; C^{11}), 131.12 (*C*; C^3), 129.92 (*C*H; C^9), 127.93 (*C*H; C^{10}), 127.30 (*C*H; C^5), 120.02 (*C*H; C^4), 111.81 (CH=*C*H$_2$; C^1), −1.18 (Si*C*H$_3$; C^7). IR (NaCl-disc) 3049 (CH=CH$_2$), 2960 (–*C*–*H*), 1628 (H*C*=*C*H$_2$), 1603 (Ar*C*=*C*), 1506 (Ar*C*=*C*), 1427 (Ar*C*=*C*), 1253 (*Si*–*C*H$_3$), 1170, 1119 (Si–*O*), 913, 838, 789, 700 cm^{-1}; EI–MS [*m*/*z*]: 254 (M+), 238, 178, 161, 135, 119, 105, 77.

4.4. Synthesis of chlorodimethyl-(1-naphthyl)silane

To a solution of 1-bromonaphthalene (7.45 g, 36 mmol) in diethyl ether (15 ml) at 0°C, *n*-butyllithium (15 ml, 2.5 M in hexane) was added dropwise under an atmosphere of nitrogen. After stirring for 15 min the mixture was added to dichlorodimethylsilane (6.45 g, 50 mmol) via syringe. The mixture was stirred for 2 h and the resultant precipitate was removed by filtration under nitrogen and the solvent removed in vacuo. The residue was distilled under reduced pressure to give the pure product as a colourless liquid (4.8 g; 67.1%): b.p. 92°C/0.2 mbar (Lit [50]: 108–110°C/0.15 Torr) ^{1}H NMR ($CDCl_3$) δ(ppm): 8.29–8.28 (d, 1H, $J = 8.6$ Hz, Si–$C_{10}H_7$), 7.98–7.85 (m, 3H, Si–$C_{10}H_7$), 7.62–7.51 (m, 3H, Si–$C_{10}H_7$), 0.91 (s, 6H, Si*CH*$_3$); ^{13}C NMR ($CDCl_3$) δ(ppm): 135.92 (*C*), 133.75 (*C*H), 133.46 (*C*), 131.43 (*C*H), 129.90 (*C*), 129.20 (*C*H), 127.71 (*C*H), 126.31 (*C*H), 125.79 (*C*H), 124.91 (*C*H), 3.49 (Si*C*H$_3$).

4.5. Synthesis of (4-vinylphenoxy)dimethyl-(1-naphthyl)silane (**2**)

The same method as for (**1**), starting with chlorodimethyl-(1-naphthyl)silane and stirring overnight, gave, after distillation under reduced pressure, a colourless oil: b.p. 164–167°C/0.2 mbar. The yield after distillation was 37%; ^{1}H NMR ($CDCl_3$) δ(ppm): 8.36–8.32 (d, 1H, $J = 9.6$ Hz, Si–$C_{10}H_7$); 8.14–7.77 (m, 3H, Si–$C_{10}H_7$), 7.56–7.43 (m, 3H, Si–$C_{10}H_7$), 7.22–7.19 (d, 2H, $J = 8.6$ Hz, CH_2=CH–C_6H_4–O), 6.80–6.77 (d, 2H, $J = 8.6$ Hz, CH_2=CH–C_6H_4–O), 6.65–6.55 (dd, 1H, $J = 10.9$; 17.8 Hz, *CH*=CH_2), 5.57–5.50 (d, 1H, $J = 17.5$ Hz, *trans*-CH=*CH*$_2$) 5.10–5.06 (d, 1H, $J = 10.9$ Hz, *cis*-CH=*CH*$_2$), 0.66 (s, 6H, Si*CH*$_3$); ^{13}C NMR ($CDCl_3$) δ(ppm): 154.90 (O–*C*; C^6), 136.53 (*C*), 136.25 (*C*H=CH$_2$; C^2), 135.22 (*C*), 133.69 (*C*H), 133.35 (*C*) 131.12 (*C*; C^3), 130.84(*C*H), 128.98 (*C*H), 128.08 (*C*H), 127.33 (*C*H; C^5), 126.27 (*C*H), 125.68 (*C*H), 125.05 (*C*H), 119.84 (*C*H; C^4), 111.79 (CH=*C*H$_2$; C^1), −0.03 (Si*C*H$_3$; C^7). IR (NaCl-disc) 3038 (CH=CH$_2$), 2960 (–*C*–*H*), 1627 (H*C*=*C*H$_2$), 1603 (Ar*C*=*C*), 1507 (Ar*C*=*C*), 1406 (Ar*C*=*C*), 1254 (*Si*–*C*H$_3$), 1170, 1148 (Si–*O*), 912, 842, 788, cm^{-1}; EI–MS [*m*/*z*]: 304 (M+), 289, 271, 243, 185, 169, 161; ^{1}H NMR showed the presence of starting material as a minor contaminant.

4.6. Synthesis of anthracene-9-carbonyl chloride [51]

9-Anthracenecarboxylic acid (10 g; 45 mmol) and thionyl chloride (10 ml; 90 mmol) were added to a dry nitrogen purged two neck flask equipped with condenser connected to two wash-bottles, the second of which

contained NaOH solution to trap HCl and SO_2. The mixture was stirred at 76°C for 2 h (reaction completed when no further gases were evolved). Residual HCl and SO_2 were removed by a nitrogen purge, and the excess thionyl chloride removed in vacuo. Dry toluene (40 ml) was added to the residue, and the insoluble (starting) material was removed by filtration. Removal of the solvent yielded the product as yellow crystals (10.12 g; 93.4%): ^{1}H NMR ($CDCl_3$) δ(ppm): 8.57 (s, 1H), 8.13–8.02 (m, 4H), 7.65–7.50 (m, 4H); ^{13}C NMR ($CDCl_3$) δ(ppm): 130.67 (*C*H), 128.75 (*C*H), 127.94 (*C*H),125.89 (*C*H),123.91 (*C*H), 126.13 (*C*).

4.7. General polymer synthesis

The concentration of template was 5% relative to cross-linking monomer (DVB or EGDMA) (95 mol%) and all polymerizations were initiated with AIBN (1 mol%). The solvents used are listed in Tables 1 and 3. Theoretical numbers of sites were 386 μmol g^{-1} for DVB polymers after hydrolysis (**P1H**, **P2H**), and 257 μmol g^{-1} for EGDMA polymers (**P3H**, **P4H**).

The monomer to be imprinted, the cross-linking monomer, AIBN and solvent were placed in a reaction tube fitted with a ground glass joint for connection to a vacuum line. After degassing the mixture by a series of freeze–thaw cycles, polymerization was carried out in a water-bath at 65°C for 24 h.

The polymer was obtained as a solid bulk, which was broken up with a spatula and washed with methanol. The polymer was then ground in an agate mortar on a Fritsch Pulverisette 'O' grinding mill, before being extracted sequentially with methanol and diethyl ether in a soxhlet apparatus for 8–12 h and dried in vacuo at 80°C.

4.8. Hydrolysis of polymers

The polymers were suspended in 5 M HCl in methanol and refluxed for 16 h. The polymer was removed by filtration, washed with methanol/water, methanol and THF. After extracting with diethyl ether the polymers were dried in a vacuum oven as before.

4.9. Chemical modification of polymers

To the polymer in triethylamine the acid chloride (0.1 mol l^{-1} for **P1**–**P2** polymers, 2 mol l^{-1} for **P3**–**P4**) was added in hexane (**P1**,**P2**) or toluene/isohexane 1/4 (**P3**,**P4**) with a solution to polymer ratio of 1 ml/50 mg. The mixture was heated to 65°C and stirred for 3 h. In the case of acetyl chloride, the mixture was reacted at 65°C for 6 h (or 16 h in all cases with **P1** and **P2**). At the end of this time, reaction mixtures were quenched by addition to methanol and the polymers obtained by filtration. The polymers were washed with methanol/water, methanol, THF, extracted with diethyl ether and dried at 80°C in vacuo.

4.10. Uptake measurements

A solution of ligand (0.5–1 ml, 2 mM) was added to control or imprinted polymer (10 mg ml^{-1}) and the resultant suspension was shaken overnight. The polymer was filtered and the concentration of ligand remaining in the solution determined by HPLC. The samples were diluted with ethanol to ensure miscibility with the mobile phase. Elution was carried out in acetonitrile/water at a flow rate of 1 ml min^{-1}, isocratically. Detection was via UV adsorption monitoring at 257 nm (pyridine), 270 nm (quinoline), or 350 nm (acridine). The results are quoted are from three replications with duplicate analytical runs.

Acknowledgements

We would like to thank Professor R. Burch, Mr D. Gleeson and Miss V. Caps of the University of Reading for carrying out surface area measurements and BBSRC for funding this work.

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