Fluorescent imprinted polymer sensors for chiral amines†

T. Hien Nguyen‡ and Richard J. Ansell*

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Molecularly imprinted polymer (MIP) based fluorescent sensors require suitable fluorescent moieties which respond to the binding event with significant fluorescence changes. Two novel polymerisable coumarins: 6-styrylcoumarin-4-carboxylic acid (SCC) and 6-vinylcoumarin-4-carboxylic acid (VCC) have been designed and synthesised. These functional monomers allow for the preparation of fluorescent sensors of chiral amines, an important class of pharmaceutical compounds. MIPs were prepared with SCC and VCC, using (–)-ephedrine as a template and ethylene glycol dimethacrylate as a cross-linker. In MeCN, the polymers exhibited a decrease of fluorescence in response to amines, with some selectivity for the template over its enantiomer (+)-ephedrine and other structural analogues. Interestingly the response of SCC to (–)-ephedrine in the MIP occurs in the opposite direction to the change when recognition occurs in solution. The control polymers (NIPs) exhibited a lesser response to (–)-ephedrine, and no resolving power, suggesting that imprinting has been successful and selective recognition sites exist in the MIPs. Recognition in aqueous buffers at different pHs has also been investigated.

Introduction

Molecular imprinting has been demonstrated over the last three decades as a versatile technique for the preparation of molecular receptors capable of the selective recognition of given target molecules. The approach is based on the self-assembly of a template molecule with polymerisable monomers possessing functional group(s) interacting with the template.[1] After polymerisation, the template is removed, leaving vacant recognition sites which are complementary in shape and functional groups to the original template. Molecularly imprinted polymers (MIPs) provide an exciting alternative to biological receptors as recognition elements in chemical sensors.[2] Sensors based on electrochemical,[3] mass-sensitive,[4] optical[5] and surface plasmon resonance spectroscopic (SPR)[6] transduction combined with MIPs as the recognition element have all been fabricated.

Fluorescence is a promising detection method for MIP sensor systems due to its high sensitivity and non-destructive nature.[7,8] The combination of fluorescence and molecular imprinting techniques for the sensing of analytes has been based either on the use of fluorescent[9]/fluorescent-labeled analytes[10] or, in case analytes are non-fluorescent, on the formation of fluorescent complexes between added reagents and functional groups on the polymers. Several examples of MIPs containing fluorophores and exhibiting fluorescence modulation on analyte binding have been reported for analytes including cAMP,[8] D-fructose,[11] cyclododecylidene pyridine-2-carboxamidrazone,[12] and cyclobarbital[13] among others.[14] However, selectivity was mostly demonstrated in comparison to structurally related compounds, not towards enantiomers. Indeed, the only true evidence for selectivity in MIPs arising from specific recognition sites is chiral selectivity because, otherwise, differences in the response of fluorescent MIPs may be due to various factors such as different quenching abilities or acidity/basicity of the analyte and competitors.

Therefore, this work aimed to develop fluorescent sensors capable of demonstrating enantioselectivity. Novel fluorescent functional monomers suitable for the preparation of amine selective MIPs were designed and synthesised. The fluorescent monomers should interact as strongly as possible with the templates, ideally on a stoichiometric basis, since any randomly arranged fluorophores outside recognition sites in the resulting MIPs may reduce the sensitivity and/or interact with competing ligands, resulting in the MIPs showing false positive responses.

(–)-Ephedrine was used as the model template for this study because bulk MIPs based on methacrylic acid (MAA), imprinted with (–)-ephedrine, have been shown to exhibit very good recognition properties in the HPLC mode.[15,16] A bridged hydrogen bond interaction of the carboxylic acid with both the amine lone pair and the hydroxyl hydrogen of ephedrine, in the organic solvent used to prepare the MIP, was postulated. Like many other chiral amines, (–)-ephedrine is an important drug and it offers the opportunity for chiral selectivity to be demonstrated by comparing the sensor response towards (–)-ephedrine with that for the opposite enantiomer, (+)-ephedrine. The imprinting strategy is illustrated in Fig. 1.

Results and discussion

Synthesis of fluorescent monomers

To be applied successfully in molecular imprinting as a fluorescent functional monomer, a fluorophore must include a polymerisable

School of Chemistry, University of Leeds, Leeds, UK LS2 9JT. E-mail: chmrja@leeds.ac.uk; Fax: +44 (0)113 343 6565; Tel: +44 (0)113 343 6415

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‡ Current address: School of Engineering and Mathematical Sciences, City University, London, EC1V 0HB, UK

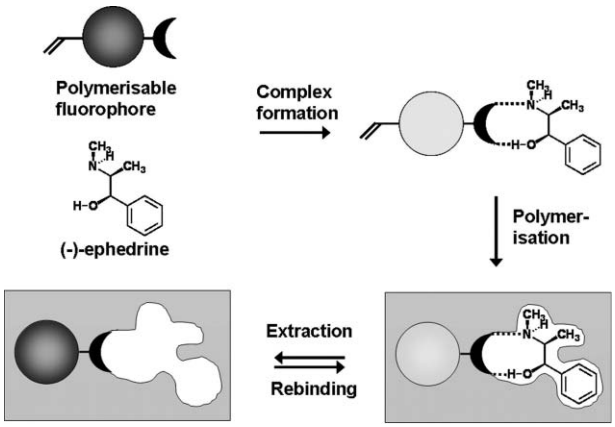
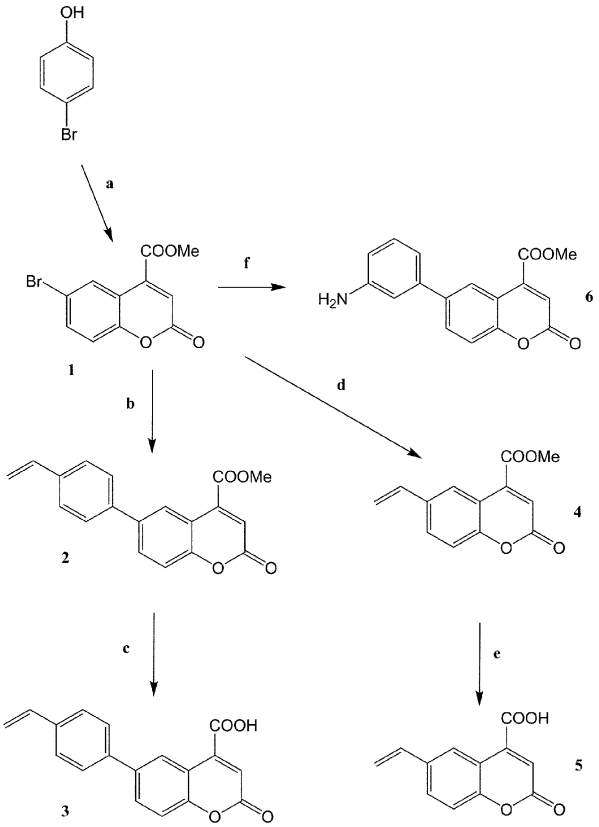


Fig. 1 The preparation of an (–)-ephedrine-sensing MIP which exhibits fluorescence changes upon template binding.

group, interact with the template covalently or non-covalently, exhibit fluorescence changes upon binding, and have a rigid structure, minimizing conformational flexibility around binding sites and maintaining the specific orientation of the functional group(s) originally positioned by the imprinting process.

Two novel polymerisable coumarins **3** and **5** suitable for molecular imprinting were prepared in three steps starting from 4-bromophenol as outlined in Scheme 1. The reaction of phenols with dimethyl acetylene dicarboxylate is a versatile approach for the synthesis of coumarin-4-carboxylates. **1** was prepared similarly to the method reported by Hekmatshoar *et al.*[17] The synthesis of **2** was achieved from **1** in a good yield *via* a Suzuki coupling[18,19] with vinylphenylboronic acid using K_2CO_3 in dioxane as a base/solvent mixture. **4** was prepared from **1** *via* a Stille reaction,[18,20] following the method described by Farina and Krishnan.[21] The reaction initially gave a mixture of starting material **1** and product **4** which were inseparable by normal or reverse phase chromatography. However, this problem was overcome by performing a Suzuki reaction on the inseparable mixture with 3-amino-benzeneboronic acid that gave **6** containing an amino group which has a high affinity to silica gel and thus was easily removed from **4**. The synthesis of **3** and **5** was completed by hydrolyzing esters **2** and **4** under mild basic conditions.



Scheme 1 Preparation of fluorescent monomers. (a) $C_2(COOMe)_2$, Ph_3P, CH_2Cl_2, –5 °C to 55 °C, 100 h, 52%; (b) CH_2=$CHC_6H_4B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, dioxane, 80 °C, 48 h, 42%: (c) 1 M NaOH, EtOH/THF (2.5:1), 40 °C, 12 h, 84%; (d) Bu_3SnCH=CH_2, Ph_3As, Pd_2dba_3, THF, 70 °C, 125 h, 38%; (e) 1 M NaOH, EtOH/THF (2.5:1), 40 °C, 14 h, 79%; (f) $NH_2C_6H_4B(OH)_2 \cdot H_2O$, $Pd(PPh_3)_4$, K_2CO_3, dioxane, 80 °C, 50 h.

Absorption and fluorescence behaviours of fluorophores

The absorption spectra of VCC (**5**) and SCC (**3**) in MeCN show two main bands, at about 280 and 340–345 nm (Fig. 2). The shorter wavelength bands with higher absorbances can be attributed to the $S_o \rightarrow S_2$ transition whereas the longer-wavelength bands with weaker absorbances may be attributed to the $S_o \rightarrow S_1$ transition. These absorption patterns of VCC and SCC are fairly similar to those of other 6-substituted coumarins as well as the parent coumarins reported in the literature,[22,23] whose π systems in the excited states are less conjugated than *e.g.* 7-substituted ones. Emission spectra for each compound recorded in the same solvent using excitation at the absorbance maxima include only one band in the 510–530 nm region (Fig. 2). Although both compounds absorb more at 280 nm than at longer wavelengths, the emission of VCC is actually higher with $\lambda_{ex} = 345$ nm than it is with $\lambda_{ex} = 280$ nm, and the fluorescence of SCC is only slightly lower with

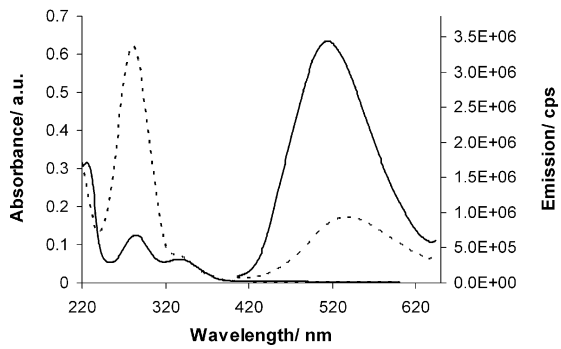


Fig. 2 Absorption and emission spectra of VCC (solid lines) and SCC (dotted lines) (10^{-5} M) in MeCN. Emission spectra were recorded with $\lambda_{ex} = 340$ nm for VCC and 345 nm for SCC, using the same slit widths.

$\lambda_{ex} = 340$ nm than it is with $\lambda_{ex} = 280$ nm This may be because not all molecules excited to the S_2 state using $\lambda_{ex} = 280$ nm fall to the S_1 state, from which fluorescence occurs, during the internal conversion process, energy may be lost in other ways. Therefore, excitation wavelengths of 340 nm and 345 nm were used in all experiments for VCC and SCC, respectively.

Interestingly, although the absorbances of the monomers at the excitation wavelengths used are roughly the same, VCC is more

fluorescent than SCC, and its emission maximum is observed at a shorter wavelength. Coumarin molecules have close lying π,π* and n,π* states which are easily perturbed by changes in solvents, substituents and other factors,[22,24] and hence the S_1 state probably has both π,π* and n,π* character. It is proposed that in VCC a π,π* state may lie below the n,π* state and in SCC vice versa, as shown in Fig. 3. This could account for the less intense fluorescence of SCC, n←π* transitions being generally less intense than π←π* transitions because they characteristically have longer lifetimes which enhance intersystem crossing.[25] The red-shifted fluorescence of SCC with respect to VCC may be due to a greater degree of charge separation in the excited state of SCC, characteristic of n,π* states, which would therefore be more likely to be stabilised by solvent prior to fluorescence.

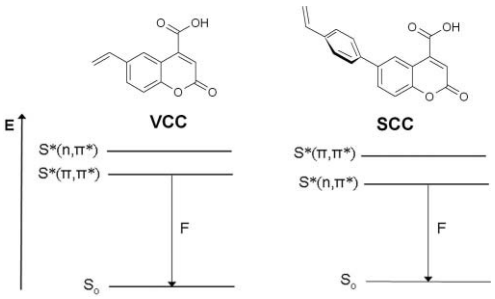


Fig. 3 Possible energy diagrams for VCC and SCC. S_0: ground state, S*: excited single state, E: energy, F: fluorescence.

Adding ephedrine to VCC or SCC in MeCN affected the fluorescence of both compounds (Fig. 4). The fluorescence of VCC was quenched and shifted to a shorter wavelength whilst that of SCC increased and shifted to a shorter wavelength. The data in Fig. 4 can be converted to a plot of emission at a single wavelength, but it is non-trivial to fit this to an association model because neither reagent is in excess so the data cannot be linearised. Nonetheless, we have used the software package Maple™ to fit the data manually to a 1:1 association (see ESI†). This gives an extremely high association constant of ~10^6 M^{-1}. Even given the strong association previously noted for ephedrine with MAA in MeCN[16] this is clearly unrealistic. There are a number of complicating factors, including self-association of the monomer and self-quenching of the fluorophore which mean the true association constant is likely to be less than this. A full series of NMR titrations would be required to arrive at an accurate value. Similar although less pronounced changes were observed when acetic acid was added. This behaviour must be due to interactions of the added amine (or carboxylic acid) with the carboxylic acid group on VCC and SCC, as shown in Fig. 5, which was confirmed when the corresponding esters (**4** and **2**) of VCC and SCC did not respond to the same addition (data not shown). The large change in fluorescence intensity observed for VCC and SCC upon adding ephedrine, which approximately saturates after the addition of just one equivalent of ephedrine, suggests that the interactions with ephedrine are very strong and roughly on a 1:1 basis. The shifts to lower λ_{em} suggest a destabilisation of the excited state: for VCC it may gain more n,π* character (causing quenching) and for SCC more π,π* character (causing the increase in emission).

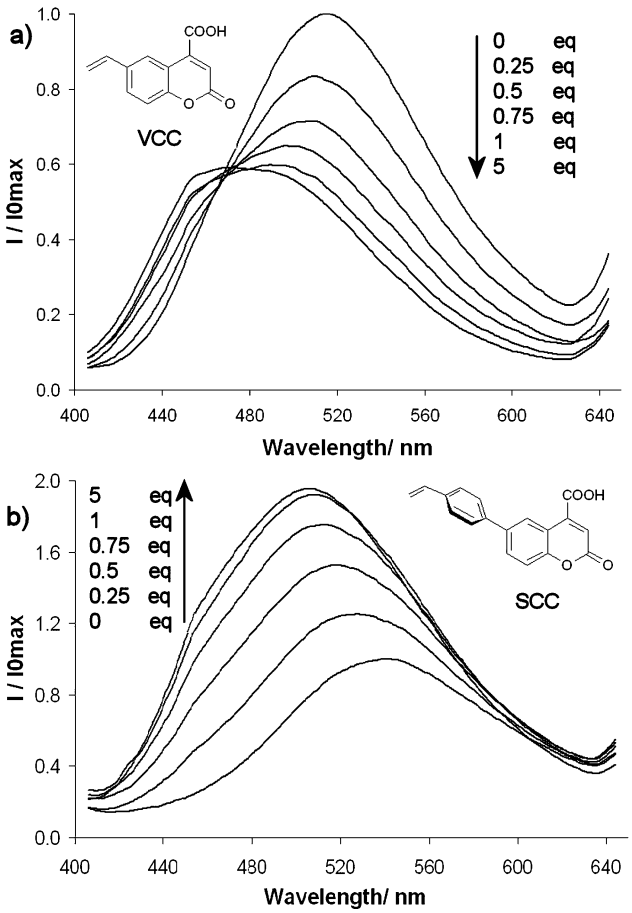


Fig. 4 Fluorescence spectra of a) VCC (50 μM, λ_{ex} = 340 mm) and b) SCC (50 μM, λ_{ex} = 345 nm) in MeCN with the addition of (−)-ephedrine. I/I0max is the emission intensity in cps divided by the maximum intensity for the fluorophore alone, in the absence of added ephedrine. Different slit widths were used for a) and b) to optimise the signal.

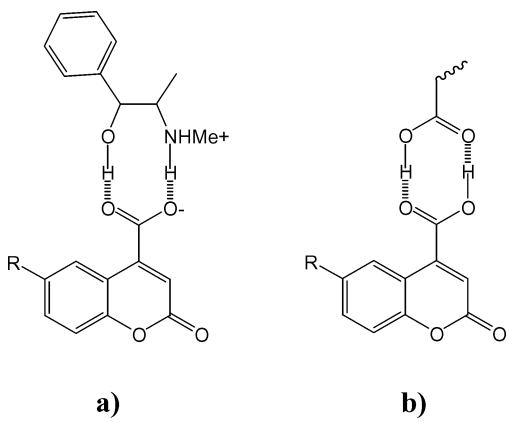


Fig. 5 Hydrogen bonding between a) fluorophore–ephedrine, b) fluorophore–carboxylic acid or fluorophore–fluorophore.

Fluorescence studies of polymers in MeCN

Imprinted polymers and their controls were prepared in MeCN. These were prepared as monolithic MIPs and ground into small particles, since these are easier to produce than spherical beads which can be made by precipitation polymerisation

and since a minimal amount of solvent is required. To make a dispersion polymer, excess solvent is required and the equilibrium will disfavour complex formation, hence dispersion MIPs usually exhibit a lower density of good binding sites. In previous studies using fluorescence[26] ground monolithic MIP particles have been used successfully. There is no problem with scattering from the irregular-shaped particles because with the coumarin used here the Stokes shift is sufficient that emission can be measured well above the scattered wavelengths. The molar amount of fluorescent monomers used was fixed at 1:40 of the cross-linker for all polymers. Additional 'inert' monomer, MMA, was included so that the MIP should not be too rigid (MIPs with >90% cross-linker tend to show impaired performance).[27] More fluorescent monomers would be expected to give more recognition sites but too high a concentration of fluorophore could also result in fluorescence quenching by the inner filter effect. A template:fluorescent monomer ratio of 1:1 or higher was employed to avoid 'false positives' caused by randomly incorporated fluorophores.

It was also interesting to see whether the inclusion of MAA would affect the recognition properties of the MIPs. MAA can increase the hydrophilicity of the polymers and hence may improve recognition in aqueous buffers. In addition, it may yield higher-order complexes in the prepolymerisation mixture which are expected to give stronger and more selective binding sites,[15] but should not out-compete the fluorescent monomers for the amine template since its pK_a is higher than that for coumarin-4-carboxylic acid (4.58 compared to 2.52[28]). The PV and PS polymers differed in the type of fluorescent monomer used (i.e. either VCC or SCC). Different PV polymers differed in the amount of template and the amount as well as the type of co-monomer (MAA or MMA). Control polymers were prepared under identical conditions, without the addition of the template (–)-ephedrine. The polymer concentrations used in the fluorescence experiments were fixed at 0.443 mg mL^{-1} for PV/CV-1:1 and PV/CV-1:8, 0.437 mg mL^{-1} for PV/CV-1:1-MAA and PV/CV-1:8-MAA and 0.446 mg mL^{-1} for PS/CS-1:1. These amounts of polymers were calculated to give the same fluorophore concentration of 50 μM, assuming that the polymerisation yield was the same for all polymers and equal to 100% and the template was completely removed from the MIPs.

Fig. 6 shows emission spectra of different polymers in MeCN. It can be seen that both fluorophores emit at lower wavelengths in the polymers than in their free forms, though PS-1:1 and CS-1:1 emit at higher λ_{em} than PV-1:1 and CV-1:1, consistent with the higher λ_{em} for SCC than VCC. The blue shift could be because when fixed in a rigid polymer network the excited states of both fluorophores undergo less stabilisation from solvent rearrangement. However, PV-1:1 and CV-1:1 exhibit less intense fluorescence than PS-1:1 and CS-1:1, despite VCC being more fluorescent than SCC. Possibly the π,π* state of VCC is destabilised due to the vinyl group undergoing addition, causing loss of conjugation with the coumarin ring. The higher fluorescence yields observed for CV-1:1 and CS-1:1 compared to PV-1:1 and PS-1:1 are most likely because of residual ephedrine left in the imprinted polymers (ephedrine causes quenching as shown below). The fluorescence yield of PV-1:1-MAA may be lower than that of the corresponding NIP because the fluorophore in the NIP is self-associated or associated with MAA, and the interaction

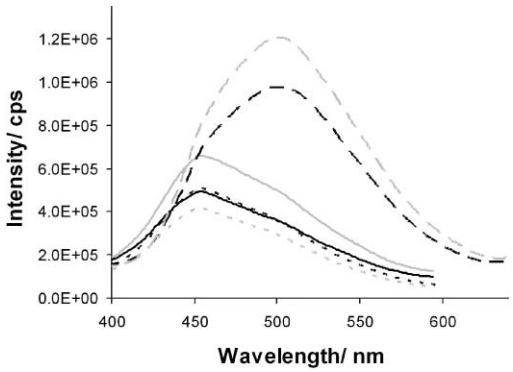


Fig. 6 Emission spectra* in MeCN of PV-1:1 (lower continuous line), CV-1:1 (upper continuous line), PS-1:1 (lower broken line), CS-1:1 (upper broken line), PV-1:1-MAA (upper dotted line) and CV-1:1-MAA (lower dotted line) with excitation at 340 nm for PVs, CVs and at 345 nm for PS, CS (same slit widths used in all cases). Polymer concentrations are as discussed. * The discontinuities on all spectra at 450 nm were due to an artefact of the instrument.

with a carboxylic acid group decreases the fluorescence of VCC as discussed previously.

Initially, the fluorescence responses of all polymers to (–)-ephedrine in comparison to the opposite enantiomer, (+)-ephedrine, were studied in MeCN, the same solvent used for MIP preparation, since re-binding has often been demonstrated to be most selective in the same solvent as that in which the MIP was prepared.[29] Each measurement was performed in duplicate or triplicate and data presented are average values. Although the low density of MeCN (0.782) might present a problem as it allows polymer particles to settle, by shaking the cuvette vigorously before each measurement the problem can be overcome. No significant settling out was observed on the timescale of the measurements and the results were found to be reproducible.

The addition of (–/+)-ephedrine resulted in a decrease in fluorescence of all polymers. However, the degree of quenching was different for each polymer and different for the (–)-enantiomer and (+)-enantiomer in some cases (Fig. 7). Interestingly, SCC polymers responded to ephedrine in the opposite direction to the free fluorophore. This might be because an inversion of the lowest-lying n,π* and π,π* states of the SCC molecule occurred when SCC was incorporated in the polymers, resulting in the lowest excited state of SCC being more π,π* in character and hence the fluorophore behaving more similarly to VCC. The data in Fig. 7 might be interpreted to show that the association of ephedrine to the polymers is weaker than to the monomer itself (shown in Fig. 4). Application of the Maple™ model for association to these data (ESI†) yields apparent association constants for PV and PS of ~10^5 M^{-1}. The poor fits demonstrate that the situation is more complex than a simple 1:1 model with fluorescence dependent only on the number of empty and number of occupied sites.

To demonstrate imprinting effects, it is desirable that a difference between enantiomers or at least a difference between MIPs and NIPs is achieved. All MIPs, except PV-1:1-MAA, showed a greater decrease in fluorescence upon ephedrine addition than did the corresponding controls, suggesting that the analyte bound to the MIPs more strongly than to the NIPs. Moreover, PV-1:1 and PS-1:1 exhibit a small discrimination between (–)-ephedrine and

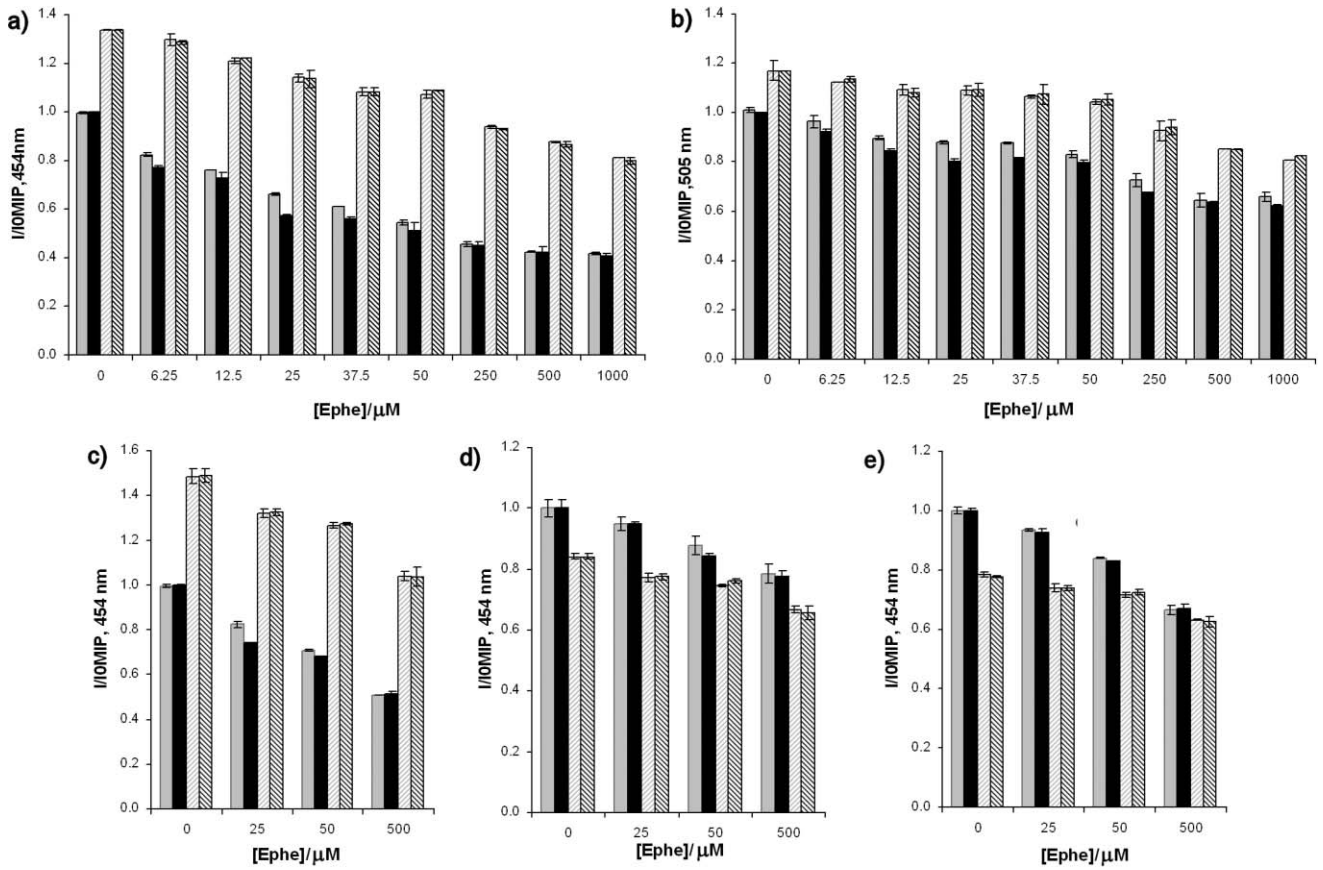


Fig. 7 Fluorescence responses of a) PV-1:1 and CV-1:1, b) PS-1:1 and CS-1:1, c) PV-1:8 and CV-1:8, d) PV-1:1-MAA and CV-1:1-MAA, e) PV-1:8-MAA and CV-1:8-MAA to (–/+)-ephedrine in MeCN with excitation and polymer concentrations as in Fig. 6. MIP + (+)-ephedrine grey; MIP + (–)-ephedrine black; NIP + (+)-ephedrine grey hatched; NIP + (–)-ephedrine black hatched. I/I0MIP is the emission intensity in cps divided by the intensity for the imprinted polymer alone, in the absence of added ephedrine. Data recorded at 454 nm except b) recorded at 505 nm. Different slit widths were used for a), b), c), d) and e) to optimise the signal. Error bars represent standard deviations in the calculated values.

(+)-ephedrine while the respective control polymers CV-1:1 and CS-1:1 show no resolving power, indicating that the MIPs contain chiral recognition sites. At high concentrations of ephedrine, no chiral selectivity and no further fluorescence quenching are seen due to the saturation of all available binding sites.

A slight selectivity for (–)-ephedrine over (+)-ephedrine is also observed for PV-1:8 at the low analyte concentration of 25 μM (Fig. 7c). PV-1:8 was made with a large excess of template, such that residual template in the MIP may account for its much lower initial fluorescence than CV-1:8 and its limited response to added analyte. PV-1:8-MAA responds more strongly than CV-1:8-MAA, but chiral selectivity is not apparent (Fig. 7d). Similar results are obtained with PV-1:1-MAA and CV-1:1-MAA (Fig. 7e), suggesting MAA does little to enhance the selectivity of the fluorophore-based binding sites, whether the ratio of template:fluorophore:MAA is 1:1:7 or 8:1:7.

The highest enantioselectivity, the greatest difference in fluorescence response between MIP and NIP and also the biggest decrease of fluorescence upon the addition of ephedrine (up to 59% at 1000 μM (–)-ephe) were observed for PV-1:1. The superiority of PV-1:1 over PV-1:8 may indicate that the interaction between the fluorescence monomer and ephedrine is very strong so that a 1:1 ratio of template:monomer is sufficient to complex all monomer and none is left non-specifically incorporated throughout the polymer. The lower response of PS-1:1 and CS-1:1 to ephedrine compared to PV-1:1 and CV-1:1 may be due simply to the photophysical properties of the fluorophore as discussed previously and does not necessarily suggest that binding of ephedrine to these polymers is weaker.

Since PV-1:1 and PS-1:1 were the most promising MIPs, their selectivity towards different ephedrine-related amines was further investigated (Fig. 8). The responses of PV-1:1, PS-1:1 and the corresponding NIPs to the different amines were recorded at 25 μM and 50 μM where the greatest difference between (–)-ephedrine and (+)-ephedrine had been observed (Fig. 9). It can be seen that both MIPs respond more to all amines investigated than do the NIPs, suggesting that there are more binding sites available in the MIPs than in the NIPs (since some of the fluorophore is self-associated during preparation of the NIPs). Moreover, the MIPs respond less to any of these compounds than to the template, (–)-ephedrine whereas the NIPs respond similarly to (–)-ephedrine, (+)-ephedrine and (+)-pseudoephedrine and less to the other compounds. This once again indicates successful imprinting and selective recognition sites in the MIPs.

The fluorescence responses of the NIPs to different amines can be explained in terms of pK_a values. Since there are no specific

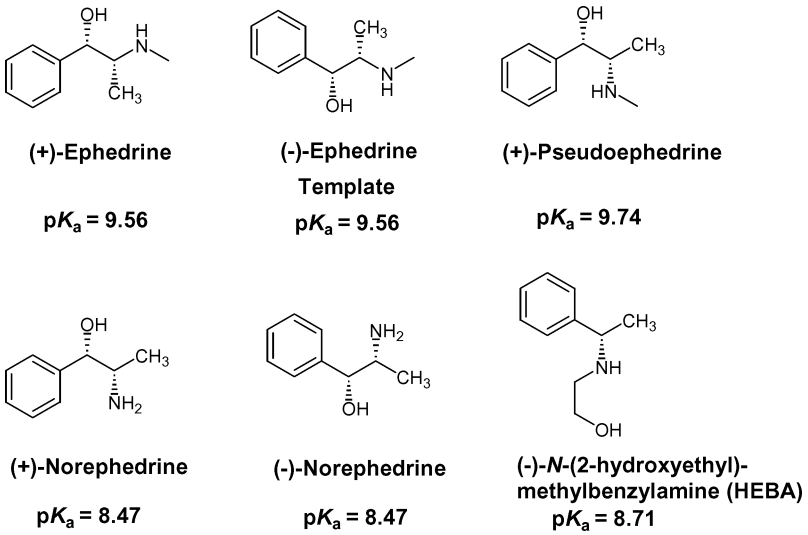


Fig. 8 Structures and pK_a values of different amines used for fluorescence studies.

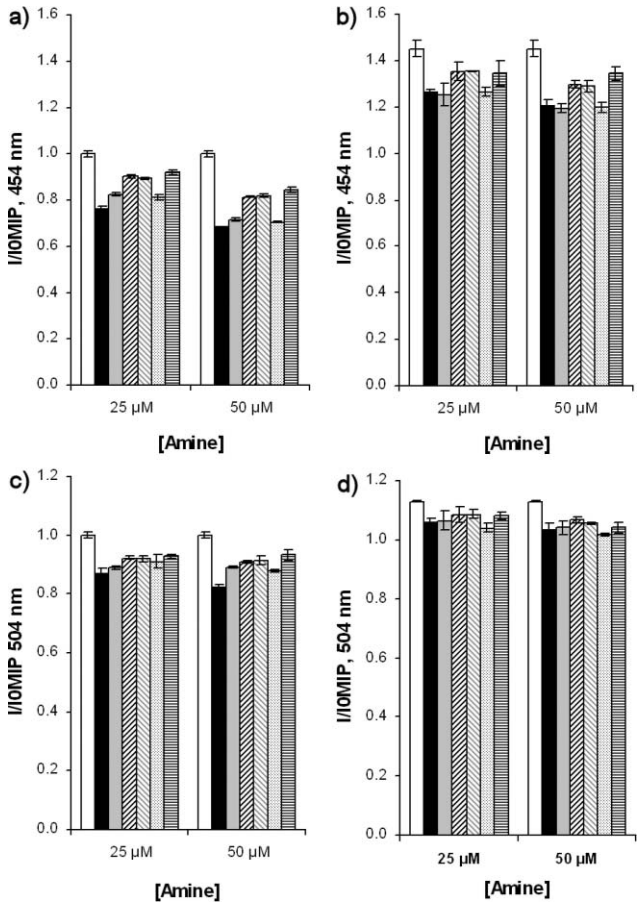


Fig. 9 Fluorescence responses of a) PV-1:1, b) CV-1:1, c) PS-1:1, d) CS-1:1 to different amines in MeCN. Polymer alone white; polymer + (–)-ephedrine black; polymer + (+)-ephedrine grey; polymer + (–)-norephedrine black hatched; polymer + (+)-norephedrine grey hatched; polymer + (+)-pseudoephedrine dotted; polymer + (–)-HEBA horizontal hatched. I/I0MIP is the emission intensity in cps divided by the intensity for the MIP alone, in the absence of added amine. Same slit widths used for a) and b) (with emission measured at 454 nm) and for c) and d) (at 504 nm).

binding sites in the NIPs, they respond to compounds with higher pK_a values more strongly than to less basic ones. The basicities of (–)-ephedrine, (+)-ephedrine and (–)-pseudoephedrine are similar, so these compounds bound similarly to the NIPs and to a greater degree than (–/+)-norephedrine and (–)-HEBA whose pK_a values are lower. This trend was also seen partly with the MIPs. However, due to the presence of binding sites selective for the template, the MIPs bound most strongly to (–)-ephedrine even though the pK_a for pseudoephedrine is slightly higher than that for (–)- ephedrine (9.74 compared with 9.56[28]).

Fluorescence studies of polymers in aqueous buffer

It was also interesting to see if selective responses of the MIPs could be observed in buffered aqueous solutions, resembling the conditions in which biological recognition occurs. The fluorescence of PV-1:1, PS-1:1 and the corresponding controls was investigated over a range of pH. 25 mM citrate buffer at various pHs ranging from 2 to 6 was employed. A small amount of surfactant Triton X-100 (0.5% (w/v)) was added to reduce non-specific binding but also to enable the dispersion of the polymers. As preliminary experiments suggested that polymers showed little response to ephedrine in aqueous buffer, a high analyte concentration of 1000 µM was used. Fig. 10 shows the response of PV-1:1, CV-1:1 and PS-1:1, CS-1:1 to 1000 µM (+/–)-ephedrine at different pHs. It can be seen that the polymers show a decrease in fluorescence with increasing pH, and the decrease is greater for the MIPs than for the NIPs. A decrease is also observed on adding ephedrine, which is more apparent at pH 5 and 6, and slightly bigger for the MIPs. However, selectivity for (–)-ephedrine over (+)-ephedrine is not apparent. The results suggest firstly that the protonated form of the fluorophore is most fluorescent, and its pK_a in the polymer is higher than the value in free solution of 2.52.[28] Since ephedrine in aqueous solution is protonated below pH 9.38[28] it interacts better with the polymer at pH 5–6 than at pH 2–3. The interaction is an ion-exchange process with ephedrine replacing the protons and buffer cations (sodium)—such that the number of sites occupied only by protons, and hence the fluorescence, falls.

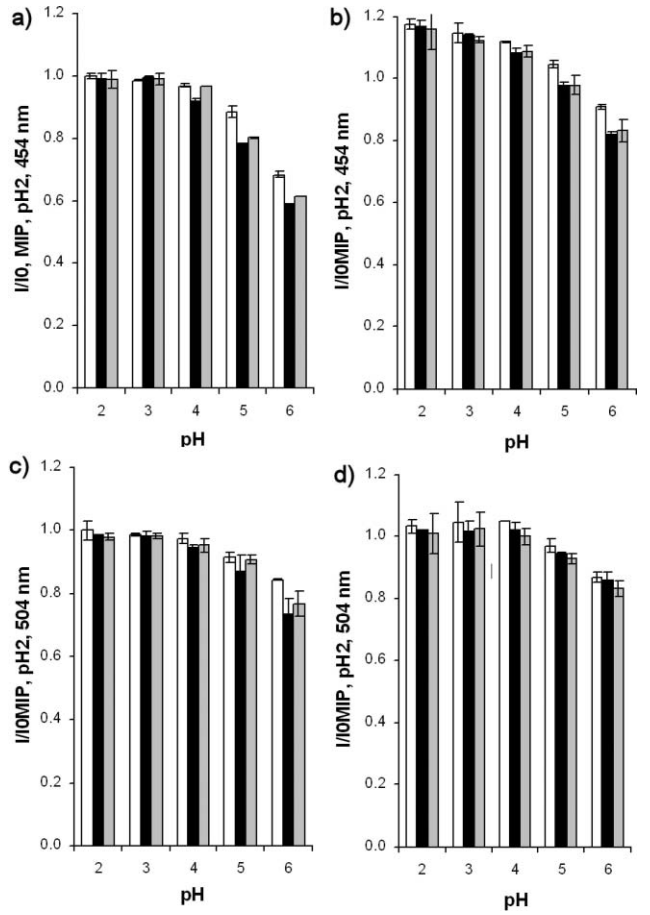


Fig. 10 Fluorescence responses of a) PV-1:1, b) CV-1:1, c) PS-1:1, d) CS-1:1 to 1000 μM (+/–)-ephedrine at different pHs (25mM citrate + 0.5% Triton X-100). Polymer alone white; polymer + (–)-ephedrine black; polymer + (+)-ephedrine grey. I/I0MIP, pH2 is the emission intensity in cps divided by the intensity for the MIP alone, in the absence of added amine, at pH2. Same slit widths used for a) and b) (with emission measured at 454 nm) and for c) and d) (at 504 nm).

Conclusions

Novel coumarin-based fluorescent functional monomers containing a carboxylic acid functionality, 6-vinyl-coumarin-4-carboxylic acid (VCC) and 6-vinylphenyl-coumarin-4-carboxylic acid (SCC), have been synthesised. These functional monomers allow for the preparation of fluorescent sensors of amines. Their fluorescence behaviours have been investigated in MeCN. Both monomers exhibited a significant degree of fluorescence intensity change, but in opposite directions, upon ephedrine addition. VCC decreased fluorescence whereas SCC showed a fluorescence enhancement in response to the binding event. The monomers were found to respond also to carboxylic acids in the same manner as to ephedrine but to a lesser extent. The photophysical properties of the fluorophores have been postulated to explain these phenomena. Although photobleaching has not been studied, coumarins are frequently used as laser dyes for single-molecule spectroscopy so are not expected to suffer from excessive photobleaching under the conditions used here.

Different polymers were prepared with VCC and SCC using (–)-ephedrine as a template and EDMA as a cross-linker. Either MAA or MMA was used as a co-monomer. The polymers differed in the type of fluorescent monomer used (i.e. either VCC or SCC), the amount of template and the amount as well as the type of co-monomer (MAA or MMA). All polymers showed a quenching of fluorescence with the addition of ephedrine. It was found that the MIPs prepared with a 1:1 ratio of template:fluorescent functional monomer and without the addition of MAA demonstrated the best recognition properties. These MIPs exhibited a decrease of fluorescence in response to amines, with some selectivity for the template over its enantiomer, (+)-ephedrine. The control polymers (NIPs) exhibited a lesser response to (–)-ephedrine, and no resolving power, suggesting that imprinting has been successful and selective recognition sites exist in the MIPs. At high concentrations of ephedrine, no chiral selectivity was seen with the MIPs due to the saturation of binding sites. Selectivity to structural analogues was also demonstrated in MeCN. However, little response to ephedrine was observed in aqueous buffer.

The fluorescent monomers developed in this study are potentially suited for use in the preparation of fluorescent sensors for other templates containing amine functionalities.

Experimental section

Materials

Ethylene glycol dimethacrylate (EDMA), methacrylic acid (MAA), methylmethacrylate (MMA), (1*R*,2*S*)-(–)-ephedrine, (1*S*,2*R*)-(+)-ephedrine hemihydrate, (+/-)-norephedrine, (+)-pseudoephedrine, 4-bromophenol, tetrakis-(triphenylphosphine) palladium(0), 4-vinylphenylboronic acid, dimethyl acetylenedicarboxylate, triphenylarsine, triphenylphosphine, 4-dimethylaminopyridine were from Aldrich (Dorset, UK). Tris(dibenzylideneacetone)dipalladium (0) was from Strem Chemicals. 3-aminobenzenboronic acid monohydrate was from Avocado. 6-Bromocoumarin-3-carboxylic acid and tri-n-butyl(vinyl)tin were from Alfa Aesar. 2,2′-Azobis-(2-methylpropionitrile) (AIBN), (*S*)-(–)-*N*-(2-hydroxyethyl)-methylbenzylamine (HEBA) were from Acros Organics (Geel, Belgium). All solvents used were of HPLC grade. Monomers were distilled under reduced pressure prior to use to remove inhibitors. Ephedrine isomers were dried under vacuum over phosphorous pentoxide for 24 h prior to use. The optical purity of each isomer was checked by polarimetry (t = 25 °C): (–)-ephedrine $[\alpha]_D = -43.47°$ (c = 0.5, 1M HCl); (+)-ephedrine $[\alpha]_D = -43.30°$ (c= 0.5, 1 M HCl).

Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX300 and Bruker Avance 500 spectrometers. Mass spectra were run by positive ion Electrospray (ES) mode on a LCT Micromass (TOF) or a Bruker Daltonics (MicroTOF) mass spectrometers. IR spectra were recorded on a PerkinElmer Spectrum One FT-IR spectrophotometer and were run neat. Optical rotations were recorded on an AA-1100 automatic polarimeter manufactured by Optical Activity Ltd. Melting points were recorded on a Reichert melting point apparatus and were uncorrected. Elemental analyses were carried out at the Microanalytical Laboratory, Department of Chemistry, University of Leeds. UV measurements were carried out on a Perkin-Elmer Lambda 900 UV-Vis-nIR

spectrophotometer. Fluorescence spectra were measured on a Jobin-Yvon-Horiba Fluorolog-3 Model FL3–21 spectrofluorometer system with DataMax for Windows™ software.

Synthesis of fluorophores

Methyl 6-bromocoumarin-4-carboxylate (1). **1** was prepared similarly to the method reported by Hekmatshoar *et al.*[17] (the compound was not properly purified or fully characterised by those authors). A solution of 4-bromophenol (3.46 g, 20 mmol) and triphenylphosphine (5.25 g, 20 mmol) in dichloromethane (80 mL) was stirred at −5 °C in an ice–salt mixture under argon and treated dropwise with a solution of dimethyl acetylenedicarboxylate (2.46 mL, 20 mmol) in CH_2Cl_2 (40 mL). After 1 h, the reaction mixture was refluxed for 100 h. The solvent was then removed under reduced pressure to give a red–brown solid which was recrystallised from ethanol to give a crude mixture of the desired product and triphenylphosphine. This solid was purified by flash chromatography on silica gel using CH_2Cl_2-petrol (95:5, v/v) as eluent to afford the coumarin ester **1** (2.9 g, 52%) as an off-white solid that was used in the next step. A sample of **1** was further purified by recrystallisation from ethanol to give **1** as fine needles, mp 117–119 °C [lit.[17] mp 129–131 °C (ethanol)]; IR (neat) ν_{max} (cm^{-1}) 3439, 3075, 2906, 2772, 2330, 1740–1732 (C=O), 1598, 1554, 1426, 1264, 1182; ^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 8.49 (d, 1H, H5, $J_{5,7}$ = 2.3 Hz), 7.67 (dd, 1H, H7, $J_{7,5}$ = 2.3 Hz, $J_{7,8}$ = 8.8 Hz), 7.25 (d, 1H, H8 $J_{8,7}$ = 8.8 Hz), 7.01 (s, 1H, H3), 4.02 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ(ppm): 164.1 (***C***OOMe), 159.7 (C2), 153.5 (C9), 141.3 (C4), 135.7 (C7), 129.9 (C5), 121.1 (C3), 120.9 (C8), 119.2 (C6), 118.2 (C10), 53.8 (OCH_3); MS (ES^+): Calcd. m/z = 282.9606 ($C_{11}H_8O_4{}^{79}Br$), 284.9585 ($C_{11}H_8O_4{}^{81}Br$). Found m/z = 282.9595, 284.9581 ($M + H^+$); Elem. Anal. Calcd. for $C_{11}H_7O_4Br$ (283.075): C 46.67, H 2.49, Br 28.23. Found: C 46.70, H 2.65, Br 28.05.

Methyl 6-vinylphenylcoumarin-4-carboxylate (2). A mixture of **1** (3.11 g, 11 mmol), 4-vinylphenylboronic acid (2.44 g, 16.5 mmol, 1.5 mol equiv.), potassium carbonate (5.7 g, 41.25 mmol, 3.75 mol equiv.) and dioxane (80 mL) was stirred at room temperature under argon for 0.5 h. Tetrakis(triphenylphosphine)palladium(0) (636 mg, 0.55 mmol, 5 mol%) was added. The reaction was heated to 80 °C and left at reflux in the dark for 48 h. After cooling to room temperature, dioxane was removed and the resulting residue was redissolved in CH_2Cl_2 (100 mL), washed with 1M aqueous HCl (2 × 100 mL) and saturated aqueous NaCl (100 mL). The organic phase was dried over $MgSO_4$, filtered, concentrated *in vacuo* to give the crude product as an orange semicrystalline oil. The crude product was purified by flash chromatography on silica gel with CH_2Cl_2-EtOAc (95:5, v/v) as eluent to give **2** as a yellow solid which was further purified by recrystallisation from ethanol to afford **2** (1.4 g, 42%) as yellow fine crystals, mp 91–92 °C; IR (neat) ν_{max} (cm^{-1}) 3435, 3073, 2905, 2455, 2312, 2108, 1916, 1742 (C=O), 1614, 1567, 1429, 1360, 1303, 1244, 1193; ^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 8.51 (d, 1H, H5, $J_{5,7}$ = 2.2 Hz), 7.80 (dd, 1H, H7, $J_{7,5}$ = 2.2 Hz, $J_{7,8}$ = 8.6 Hz), 7.57 (d, 2H, aromatic H J = 8.4 Hz), 7.51 (d, 2H, aromatic H J = 8.4 Hz), 7.43 (d, 1H, H8, $J_{8,7}$ = 8.6 Hz), 6.99 (s, 1H, H3), 6.77 (dd, 1H, C***H***=CH_2, J = 10.9 Hz, J = 17.6 Hz), 5.81 (d, 1H, CH=C***H***$_a$H$_b$ J = 17.6 Hz), 5.31 (d, 1H, CH=CH_a***H***$_b$,J = 10.9 Hz), 4.02 (s, 3H, OCH_3); ^{13}C-NMR ($CDCl_3$) δ(ppm): 164.6 (COOMe), 160.3 (C2), 154.0 (C9), 142.6 (C4), 139.2 (***C***C6), 138.0 (C6), 137.6 (***C***CH=CH_2), 136.6 (***C***H=CH_2), 131.5 (C7), 127.7 (aromatic C), 127.3 (aromatic C), 125.4 (C5), 120.3 (C3), 118.0 (C8), 116.5 (C10), 114.8 (CH=***C***H_2), 53.6 (OCH_3); MS (ES^+): Calcd. m/z = 307.0970 ($C_{19}H_{15}O_4$). Found m/z = 307.0962 ($M + H^+$); Elem. Anal. Calcd. for $C_{19}H_{14}O_4$ (306.10): C 74.55, H 4.61. Found: C 74.50, H 4.80.

6-Vinylphenylcoumarin-4-carboxylic acid (3). **2** (1.06 g, 3.44 mmol) was dissolved in THF (22 mL)-EtOH (56 mL), and 1M aqueous NaOH (14 mL) was added. The reaction mixture was heated to 40 °C for 12 h. The organic solvents were removed *in vacuo* and water (50 mL) was added to the remaining aqueous solution. The solution was then washed with CH_2Cl_2 (100 mL), ethylacetate (100 mL), filtered to remove any insoluble material and acidified with concentrated aqueous HCl. The mixture was extracted with ethyl acetate. The organic extracts were washed with H_2O and saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The obtained crude solid was recrystallised from ethyl acetate to give **3** (844 mg, 84%) as an yellow solid, mp 211–212 °C; IR (neat) ν_{max} (cm^{-1}) 3814, 3150–2700 (COOH), 1791, 1732(C=O), 1522, 1428, 1364, 1215; ^{1}H-NMR (500 MHz, DMSO-d_6) δ(ppm): 8.47 (d, 1H, H5, $J_{5,7}$ = 2.2 Hz), 7.98 (dd, 1H, H7, $J_{7,5}$ = 2.2 Hz, $J_{7,8}$ = 8.6 Hz), 7.67 (d, 2H, aromatic H J = 8.3 Hz), 7.62 (d, 2H, aromatic H J = 8.3 Hz), 7.56 (d, 1H, H8, $J_{8,7}$ = 8.6 Hz), 6.93 (s, 1H, H3), 6.81 (dd, 1H, C***H***=CH_2, J = 11.0 Hz, J = 17.7 Hz), 5.92 (d, 1H, CH=C***H***$_a$H$_b$ J = 17.7 Hz), 5.33 (d, 1H, CH=CH_a***H***$_b$, J = 11.0 Hz); ^{13}C-NMR (DMSO-d_6) δ(ppm): 165.6 (COOH), 159.8 (C2), 153.6 (C9), 144.0 (C4), 138.6 (***C***C6), 137.0 (***C***CH=CH_2), 136.5 (C6), 136.4 (***C***H=CH_2), 131.1 (C7), 127.3 (aromatic C), 124.8 (C5), 119.0 (C3), 117.8 (C8), 116.5 (C10), 115.1 (CH=***C***H_2); MS (ES^+): Calcd. m/z = 293.0814 ($C_{18}H_{13}O_4$). Found m/z = 293.0821 ($M + H^+$); Elem. Anal. Calcd. for $C_{18}H_{12}O_4$ (292.1): C 74.01, H 4.15. Found: C 74.25, H 4.20.

Methyl 6-vinylcoumarin-4-carboxylate (4). **1** (1.86 g, 6.58 mmol) was dissolved in dry THF (40 mL) and the solution was treated with triphenylarsine (403 mg, 0.2 equiv.) and tris(dibenzylideneacetone)dipalladium(0) (Pd_2dba_3) (151 mg, 0.05 equiv. of Pd) under argon. After stirring at r.t for 10 min, tributylvinyltin (2.4 mL, 8.24 mmol) was added. The solution was heated to 70 °C and left under reflux in the dark for 125 h. The reaction was allowed to cool to r.t. Saturated aqueous sodium fluoride (20 mL) and H_2O (10 mL) were added and left to stir for 1 h. The mixture was filtered and extracted with CH_2Cl_2. The organic extracts were washed 2 times with saturated aqueous NaCl (50 mL each), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting dark brown residue was chromatographed on silica gel using CH_2Cl_2-EtOAc (95:5, v/v) as eluent to give a yellow solid (951 mg). ^{1}H NMR analysis of this solid showed that it was a mixture of **4** and the starting material **1**, in a 4:1 ratio.

The above mixture (951 mg, containing 0.79 mmol of **1**) was dissolved in dioxane (14 mL). 3-aminobenzeneboronic acid monohydrate (183 mg, 1.185 mmol, 1.5 equiv.) and K_2CO_3 (409 mg, 2.96 mmol, 3.75 equiv.) were added and the mixture was stirred at room temperature under argon for 0.5 h. Tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) (636 mg, 0.55 mmol, 5 mol%) was added. The reaction was heated to 80 °C under argon and left at reflux in the dark for 50 h. After cooling to room temperature, dioxane was removed and the resulting residue redissolved in CH_2Cl_2 (25 mL), washed with 1M aqueous HCl

Table 1 Composition of (–)-ephedrine MIPs and corresponding NIPs

Polymer	(–)-ephedrine/mmol	Fluorescent monomer type/mmol		EDMA/mmol	MMA/mmol	MAA/mmol	MeCN/mL
PV-1:1	0.2	VCC	0.2	8.0	1.4	–	2.21
CV-1:1	–	VCC	0.2	8.0	1.4	–	2.21
PV-1:1-MAA	0.2	VCC	0.2	8.0	–	1.4	2.17
CV-1:1-MAA	–	VCC	0.2	8.0	–	1.4	2.17
PV-1:8	1.6	VCC	0.2	8.0	1.4	–	2.21
CV-1:8	–	VCC	0.2	8.0	1.4	–	2.21
PV-1:8-MAA	1.6	VCC	0.2	8.0	–	1.4	2.17
CV-1:8-MAA	–	VCC	0.2	8.0	–	1.4	2.17
PS-1:1	0.2	SCC	0.2	8.0	1.4	–	2.21
CS-1:1	–	SCC	0.2	8.0	1.4	–	2.21

Note that CV-1:1 is identical to CV-1:8 and CV-1:1-MAA is identical to CV-1:8-MAA but were prepared on different days.

(3 × 25 mL) and saturated aqueous NaCl (2× 25 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated in *vacuo* to give the crude product as an orange semicrystalline oil. The crude product was purified by flash chromatography on silica gel with CH_2Cl_2-EtOAc (95:5, v/v) as eluent to give **4** as a yellow solid which was further purified by recrystallisation from ethanol to afford **4** (574 mg, 38%) as yellow crystals (fine needles), mp 106–108 °C; IR (neat) ν_{max} (cm^{-1}) 3437, 3074, 2963, 2885, 2326, 1916, 1747(C=O), 1629, 1569, 1428, 1400, 1378, 1274, 1249, 1197; ^{1}H-NMR (500 MHz, $CDCl_3$) δ(ppm): 8.27 (d, 1H, H5, $J_{5,7} = 2.0$ Hz), 7.66 (dd, 1H, H7, $J_{7,5} = 2.0$ Hz, $J_{7,8} = 8.6$ Hz), 7.33 (d, 1H, H8, $J_{8,7} = 8.6$ Hz), 6.96 (s, 1H, H3), 6.76 (dd, 1H, C***H***=CH_2, $J =$ 10.9 Hz, $J = 17.6$ Hz), 5.79 (d, 1H, CH=C$\boldsymbol{H_a}H_b$, $J = 17.6$ Hz), 5.34 (d, 1H, CH=$CH_a\boldsymbol{H_b}$, $J = 10.9$ Hz), 4.02 (s, 3H, OCH_3); ^{13}C-NMR ($CDCl_3$) δ(ppm): 164.6 (***C***OOMe), 160.3 (C2), 154.1 (C9), 142.5 (C4), 135.7 (***C***H=CH_2), 134.9 (C6), 130.2(C7), 125.1 (C5), 120.1 (C3), 117.8 (C8), 116.2 (C10), 115.6 (CH=***C***H_2), 53.6 (OCH_3); MS (ES^+): Calcd. m/z = 231.0657 ($C_{13}H_{11}O_4$). Found m/z = 231.0660 (M + H^+); Elem. Anal. Calcd. for $C_{13}H_{10}O_4$ (230.07): C 67.86, H 4.38. Found: C 67.75, H 4.55.

6-vinylcoumarin-4-carboxylic acid (5). **5** was prepared in a manner similar to the preparation of **3**. **4** (250 mg, 1.086 mmol) was dissolved in THF (7 mL)-EtOH (18 mL), and 1M aqueous NaOH (4 mL) was added. The reaction mixture was heated to 40 °C for 14 h under argon in the dark. The organic solvents were removed *in vacuo* and water (25 mL) was added to the remaining aqueous solution. The solution was then washed with CH_2Cl_2 (2 × 20 mL) and ethyl acetate (2 × 20 mL), filtered to remove any insoluble material and acidified with concentrated HCl. The mixture was extracted with ethyl acetate. The organic extracts were washed with H_2O and saturated NaCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give fairly pure **5** as a yellow solid which was further purified by recrystallisation from chloroform to give **5** (185 mg, 79%) as a yellow solid, mp 174–176 °C; IR (neat) ν_{max} (cm^{-1}) 3811, 3150–2700 (COOH), 1921,1785, 1733(C=O), 1668, 1567, 1443, 1370, 1305, 1233, 1190; ^{1}H-NMR (500 MHz, DMSO-d_6) δ(ppm): 8.19 (d, 1H, H5, $J_{5,7} = 1.8$Hz), 7.86 (dd, 1H, H7, $J_{7,5} =$ 1.9 Hz, $J_{7,8} = 8.6$ Hz), 7.46 (d, 1H, H8, $J_{8,7} = 8.6$ Hz), 6.87 (s, 1H, H3), 6.84 (dd, 1H, C***H***=CH_2, $J = 11$Hz, $J = 17.6$Hz), 5.87 (d, 1H, CH=C$\boldsymbol{H_a}H_b$, $J = 17.6$ Hz), 5.35 (d, 1H, CH=$CH_a\boldsymbol{H_b}$, $J = 11.0$ Hz); ^{13}C-NMR (DMSO-d_6) δ(ppm): 165.6 (***C***OOH), 159.8 (C2), 153.7 (C9), 144.3 (C4), 135.7 (***C***H=CH_2), 134.0 (C6), 129.9(C7), 124.9 (C5), 118.5 (C3), 117.6 (C8), 116.2 (C10), 115.6 (CH=***C***H_2); MS (ES^+): Calcd. m/z = 217.0501($C_{12}H_9O_4$). Found m/z = 217.0505 (M + H^+); Elem. Anal. Calcd. for $C_{12}H_8O_4$ (216.05): C 66.71, H 3.73. Found: C 66.95, H 3.88

Preparation of polymers

The compositions of the different polymers are given in Table 1. For the imprinted polymers PS and PVs (except PV-1:8-MAA which was prepared by a slightly different procedure) §, (–)-ephedrine, coumarin, MMA and MAA were weighed into borosilicate glass vials and dissolved in MeCN. EDMA was added followed by azobis(isobutyronitrile) (AIBN). The vials were placed in a sonicating water bath until the AIBN was fully dissolved, then purged thoroughly with argon for about 2 min before being tightly capped and sealed. Polymerisation was carried out at 60 °C in the dark for approximately 17 h and at 80 °C for a further 2 h. The hard bulk polymers were then hand ground with a mortar and pestle until fine particles (< 50 μm by optical microscope) were obtained. The polymer particles were washed to remove the template (where applicable) by repeated incubation in MeOH-AcOH (8:2, v/v) (45 mL solvent each), centrifugation and re-suspension (11 × 2 h incubations), followed by the same procedure with MeOH alone (4 × 2 h incubations) and finally on a sintered filter with MeOH (250 mL). The filtrate was taken for UV measurement and it was confirmed that no species was detected from the recovered solution with a UV spectrophotometer. After washing, polymer particles were dried under vacuum for at least 24 h before doing fluorescence measurements.

Control polymers CS and CVs were prepared under identical conditions, with the same composition as PS and PVs, respectively but without the addition of the template (–)-ephedrine.

Fluorescence measurements in MeCN

Emission spectra of monomers and polymers were recorded using a front-faced (FF) setup with excitation at 340 nm (VCC) and

§ Excess ephedrine and carboxylic acids caused precipitation immediately after mixing, due to complex formation.[15] Therefore, PV-1:8-MAA was prepared by a slightly different procedure. (–)-Ephedrine, coumarin, EDMA and AIBN were dissolved in MeCN in a glass vial. The vial was degassed and purged with argon as described above. Before capping and sealing, MAA was added very quickly and the solution was bubbled with argon for a further 20 s. The solution was polymerised in the same manner as the others. By following this procedure, the mixture could be purged of oxygen more easily.

345 nm (SCC). A 0.8 mL (0.2 × 1 × 4 cm) quartz cuvette was used for rebinding experiments and a 4 mL (1 × 1 × 4 cm) quartz cuvette was used for all other measurements. Slit widths were adjusted to give emission below the saturating limit (1000000 cps) of the detector, and then the same settings were used for a complete set of experiments.

(−)-Ephedrine (18.5 mg, 0.112 mmol) or (+)-ephedrine hemihydrate (19.5 mg, 0.112 mmol) (dried over P_2O_5) was dissolved in 700 μL MeCN-d_3 (+0.3% TMS as internal standard). ^{1}H NMR spectroscopy was carried out to ensure that the concentrations of each enantiomeric solution were equal, by comparing the integrals of the ephedrine protons with that due to TMS. The samples were adjusted by adding more solvent, if required, until the concentrations were ≤ 2% of each other. The solutions were then diluted 1 in 64 and then 1 in 10 with MeCN to give the final stock solutions of 2.5 mM and 0.25 mM. (+/−)-Norephedrine and (+)-pseudoephedrine stock solutions were prepared in the same manner except that the concentrations were not checked by ^{1}H NMR spectroscopy.

PV-1:1, CV-1:1, PV-1:8, CV-1:8 (4.43 mg/mL, containing 0.5 μmol coumarin/mL), PS-1:1, CS-1:1 (4.46 mg/mL) and PV-1:1-MAA, CV-1:1-MAA, PV-1:8-MAA, CV-1:8-MAA (4.37 mg/mL) stock solutions were prepared in the same solvent. Various amounts of ephedrine stock solution and 120 μL of polymer stock solution were added to microcentrifuge tubes or small glass vials and the samples were made up to a total volume of 1.2 mL with MeCN. Each concentration was prepared in duplicate or triplicate. After incubating in the dark on a shaker for at least 4 h, the contents of the vials were transferred into a 0.8 mL cuvette and fluorescence spectra were acquired. The cuvette was shaken vigorously before each measurement.

Fluorescence measurements in aqueous buffers

(−)-Ephedrine (18.51 mg, 0.112 mmol) or (+)-ephedrine hemihydrate (19.52 mg, 0.112 mmol) was dissolved in 700 μL MeOH-d_4 (+0.3% TMS). ^{1}H NMR spectroscopy was carried out to ensure that the concentrations of each enantiomeric solution were equal, by comparing the integrals of the ephedrine protons with that due to TMS. The solutions were then diluted 3 in 8 with MeOH to give the final stock solutions of 60 mM. Polymer stock solutions of the same concentrations as those in MeCN were prepared in buffer. Samples were made up similarly to those in MeCN except that MeCN was replaced with buffer. It was also ensured that the amount of MeOH in each sample was the same by adding more pure MeOH and less buffer if required.

Mixtures were incubated and fluorescence measurements performed as described for samples in MeCN.

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Notes and references

1 G. Wulff, *Angewandte Chemie, International Edition*, 1995, **34**, 1812; K. Haupt and K. Mosbach, *Chemical Reviews*, 2000, **100**, 2495.
2 C. Alexander, H. S. Andersson, L. I. Andersson, R. J. Ansell, N. Kirsch, I. A. Nicholls, J. O'Mahony and M. J. Whitcombe, *Journal of Molecular Recognition*, 2006, **19**, 106.
3 M. C. Blanco-Lopez, M. J. Lobo-Castañón, A. J. Miranda-Ordieres and P. Tuñón-Blanco, *Trends in Analytical Chemistry*, 2004, **23**, 36; D. Kriz and K. Mosbach, *Analytica Chimica Acta*, 1995, **300**, 71.
4 K. Haupt, K. Noworyta and W. Kutner, *Analytical Communications*, 1999, **36**, 391; S. Stanley, C. J. Percival, T. Morel, A. Braithwaite, M. I. Newton, G. McHale and W. Hayes, *Sensors and Actuators, B: Chemical Sensors and Materials*, 2003, **89**, 103.
5 R. Levi, S. McNiven, S. A. Pilesky, S. H. Cheong, K. Yano and I. Karube, *Analytical Chemistry*, 1997, **69**, 2017; O. Y. F. Henry, D. C. Cullen and S. A. Pilesky, *Analytical and Bioanalytical Chemistry*, 2005, **382**, 947.
6 E. P. C. Lai, A. Fafara, V. A. VanderNoot, M. Kono and B. Polsky, *Canadian Journal of Chemistry*, 1998, **76**, 265.
7 A. Prasanna, de Silva, H. Q. Nimal Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chemical Reviews*, 1997, **97**, 1515.
8 P. Turkewitsch, B. Wandelt, G. D. Darling and W. S. Powell, *Analytical Chemistry*, 1998, **70**, 2025.
9 F. L. Dickert, M. Tortschanoff, W. E. Bulst and G. Fischerauer, *Analytical Chemistry*, 1999, **71**, 4559; D. Kriz, O. Ramström, A. Svensson and K. Mosbach, *Analytical Chemistry*, 1995, **67**, 2142; F. L. Dickert, H. Besenbock and M. Tortschanoff, *Advanced Materials*, 1998, **10**, 149; J. Matsui, H. Kubo and T. Takeuchi, *Analytical Chemistry*, 2000, **72**, 3286; J. L. Suárez-Rodríguez and M. E. Díaz-García, *Analytica Chimica Acta*, 2000, **405**, 67.
10 J. L. Suarez-Rodriguez and M. E. Diaz-Garcia, *Biosensors and Bioelectronics*, 2001, **16**, 955.
11 W. Wang, S. Gao and B. Wang, *Organic Letters*, 1999, **1**, 1209; S. Gao, W. Wang and B. Wang, *Bioorganic Chemistry*, 2001, **29**, 308.
12 D. L. Rathbone, D. Su, Y. Wang and D. C. Billington, *Tetrahedron Letters*, 2000, **41**, 123.
13 T. Takeuchi, T. Mukawa and H. Shinmori, *The Chemical Record*, 2005, **5**, 263; H. Kubo, N. Yoshioka and T. Takeuchi, *Organic Letters*, 2005, **7**, 359.
14 Y. Liao, W. Wang and B. Wang, *Bioorganic Chemistry*, 1999, **27**, 463; D. L. Rathbone, A. Ali, P. Antonaki and S. Cheek, *Biosensors and Bioelectronics*, 2005, **20**, 2353; T. Takeuchi, T. Mukawa, J. Matsui, M. Higashi and K. D. Shimizu, *Analytical Chemistry*, 2001, **73**, 3869; J. Matsui, M. Higashi and T. Takeuchi, *Journal of the American Chemical Society*, 2000, **122**, 5218; N. Lavignac, C. J. Allender and K. R. Brain, *Tetrahedron Letters*, 2004, **45**, 3625.
15 R. J. Ansell and K. Kuah, *Analyst*, 2005, **130**, 179.
16 R. J. Ansell, D. Wang and K. L. Kuah, *Analyst*, 2008, **133**, 1673.
17 R. Hekmatshoar, Y. S. Beheshtiha, M. M. Heravi and K. Asadollah, *Phosphorous, Sulfur and Silicon*, 2002, **177**, 703.
18 M. Beller and C. Bolm, eds., *Transition metals for organic synthesis*, Wiley-VCH, Weinheim, 1998.
19 F. Diederich and P. J. Stang, eds., *Metal-catalyzed cross-coupling reactions*, Wiley-VCH, Weinheim, 1998; N. Miyaura and A. Suzuki, *Chemical Reviews*, 1995, **95**, 2457.
20 V. Farina, V. Krishnamurthy and W. J. Scott, *The Stille reaction*, John Wiley & Sons, New York, 1998.
21 V. Farina and B. Krishnan, *Journal of the American Chemical Society*, 1991, **113**, 9585.
22 S. S. d. Melo, R. S. Becker and A. L. Macanita, *Journal of Physical Chemistry*, 1994, **98**, 6054.
23 X. Yu, D. Scheller, O. Rademacher and T. Wolff, *Journal of Organic Chemistry*, 2003, **68**, 7386; C. Kim, A. Trajkovska, J. U. Wallace and S. H. Chen, *Macromolecules*, 2006, **39**, 3817.
24 S. S. Rathi, M. K. Machwe, K. Gopalakrishanan and V. V. S. Murthi, *Current Science*, 1972, **41**, 326; J.-J. Aaron, M. Buna, C. Parkanyi, M. S. Antorious, A. Time and L. Cisse, *Journal of Fluorescence*, 1995, **5**, 337.
25 B. Valeur, *Molecular Fluorescence: Principles and Applicatons*, Wiley-VCH, 2002, D. M. Hercules, *Fluorescence and Phosphorescence Analysis*, Interscience Publishers, New York, 1966.
26 C. E. Hunt and R. J. Ansell, *Analyst*, 2006, **131**, 678.
27 B. Sellergren, *Makromol. Chem.*, 1989, **190**, 2703.
28 *SciFinder Scholar*, American Chemical Society, 2006 edn., 2006.
29 L. I. Andersson, *Analytical Chemistry*, 1996, **68**, 111.

