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Highly Stereoselective Molecularly Imprinted Polymer Synthetic Receptors for Cinchona Alkaloids

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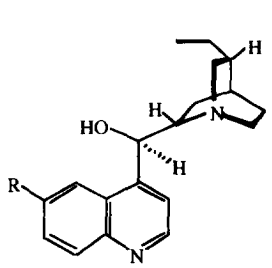
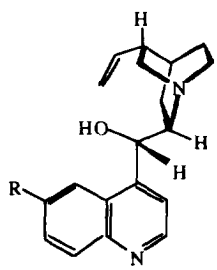
Abstract: Molecularly imprinted polymer (MIP) receptors for the cinchona alkaloids (-)-cinchonidine **1** and (+)-cinchonine **2** have been prepared and characterised using the polymers as HPLC chiral stationary phases (CSPs). Stereoseparation factors of up to 31 were obtained. The resolution of the ß-adrenergic blocking agent propranolol **8** and studies with related structures of pharmaceutical significance has permitted conclusions to be drawn regarding the origin of recognition phenomena.

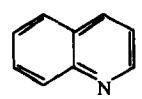
Molecular imprinting[1] has been shown to be a most useful technique for the preparation of tailor-made chiral stationary phases (CSPs)[2] and antibody mimics.[3] Two members of the cinchona class of alkaloids, (-)-cinchonidine **1** and (+)-cinchonine **2**, both antimalarial agents, were selected as candidates for study using molecular imprinting. Their relatively rigid structures, high degree of molecular asymmetry and the presence of hetero-atom functionalities capable of interaction with the functional monomer employed, methacrylic acid **3**, are factors conducive to good molecular imprint site formation,[4,5] and a requirement for producing the large separation factors necessary for their potential use in preparative scale chromatography.[5]



1 (R = H)
5 (R = CH_3O)



2 (R = H)
6 (R = CH_3O)



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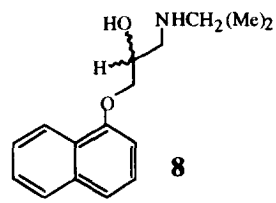

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MIPs were prepared against **1** and **2** by low temperature (4°C) photopolymerisation of methacrylic acid **3** and ethylene glycol dimethacrylate (EGDMA) **4** in the presence of each template species, Fig. 1. This polymerisation system is of established utility in a range of molecular imprinting applications.[6] The functional monomer, methacrylic acid, was selected on the basis of its ability to form hydrogen bonds and ionic interactions with the functionalities present in the selected templates.[7] The bulk polymers were

processed to render them for use as CSPs for HPLC and packed into columns. Polymers were exhaustively washed under acidic conditions to remove residual template material yielded recognition sites of complementary steric and functional topography. The induced *molecular memory* is reflected in the differing binding affinities of stereoisomers at MIP recognition sites. A non-imprinted polymer was prepared in an identical fashion.

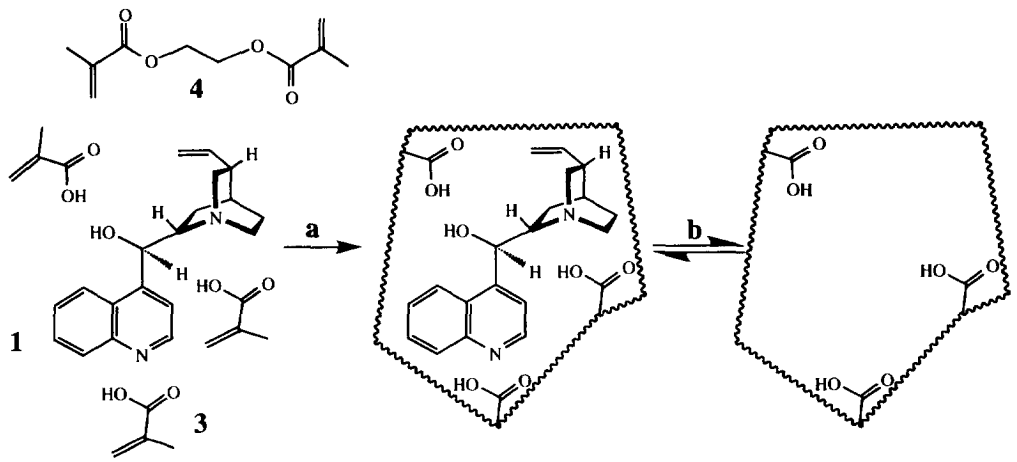

Fig. 1. Highly schematic representation of the molecular imprinting of (-)-cinchonidine **1** in a methacrylic acid **3** - ethylene glycol dimethacrylate **4** copolymer. **a**. Polymerisation in the presence of the crosslinking agent, **4**, captures the topographical relationship between the template structure **1** and attendant functional monomer units **3**. **b**. Template extraction yields a recognition site of complementary shape and functionality, and is capable of selectively rebinding the template.

The ligand selectivities of the *anti*-alkaloid MIP synthetic receptors, **P(1)** and **P(2)**, were examined in the HPLC mode using the template structures, the related cinchona alkaloids quinine **5** and quinidine **6**, and quinoline **7**, Table 1. Affinities for the non-imprinted polymer, **P(B)**, were similarly examined. Chloroform - acetic acid mixtures were used for examining ligand affinity to produce conditions similar to those employed during the polymerisation process, thus allowing more direct conclusions to be drawn regarding the origin of the imprinting effect. The imprinting templates, **1** and **2**, were the most strongly retained ligands on **P(1)** and **P(2)**, respectively, with stereoseparation factors (α-values) greater than 31 in the case of **P(1)**. Base line separation was readily achieved for co-injected samples, Fig. 2. This system demonstrates the most pronounced diastereoselectivity yet reported using a MIP CSP, which is indicative of extraordinary MIP receptor fidelity. It is notable that **1** and **2**, even though diastereomeric, displayed similar affinities for **P(B)**. The template : functional monomer : cross-linking monomer ratio employed in this case (1:4:28), similar to that used in conjunction with other template structures[6], provides a similar number of hetero-atoms for non-covalent interaction with the functional monomer. We attribute the superior resolving power of this matrix to the production of more uniform recognition sites due to the greater rigidity of these template species than those used in previous studies. This provides support for a recently presented thermodynamic based theory for the design of molecularly imprinted polymer systems.[4]

Table 1: Cinchona Alkaloid Receptor Stereoselectivities and Cross-reactivity Profiles

Column	P(1)		P(2)		P(B)	
Analyte	k'	α	k'	α	k'	α
1	34.24	31.70	1.46	23.05	0.29	1.17
2	1.08		33.65		0.34	
5	5.35	3.45	0.97	4.85	0.34	2.0
6	1.55		4.70		0.17	
7	0.19	-	0.15	-	0.08	-

Ligand capacity factors (k') and separation factors (α-values) for the diastereomeric pairs (10 µg of analyte was injected on each run). Analyses were run in $CHCl_3$/AcOH (95:5) in duplicate. α-values were calculated from individual sample injections.

The diastereomeric cinchona alkaloids quinine **5** and quinidine **6** are structurally identical to **1** and **2**, respectively, except for the presence of a methoxy substituent at the 6-position on the quinoline ring. Quinine **5** demonstrated a significantly greater preference for **P(1)** than the stereoisomeric **6**, though still with a substantially lower affinity than the template structure **1**. A similar effect was observed in the case of **P(2)**, whereby **6** showed a higher affinity than **5**. Thus, ligands with similar molecular geometry and functionality to the template are preferentially recognised. The fact that the presence of the bulky methoxy group does not totally inhibit binding is indicative of receptor site heterogeneity, a consequence of the molecular imprinting method, in particular when relying upon non-covalent interactions to define recognition site geometry. Analogy may here be drawn to the heterogeneity of receptor site populations in polyclonal antibody samples. It is significant that the location of the methyl ether moiety corresponds to a region of the template structure not expected to be involved in complex formation with the functional monomer, thus recognition sitegeometry corresponding to this region of the template is less precisely defined.[5]

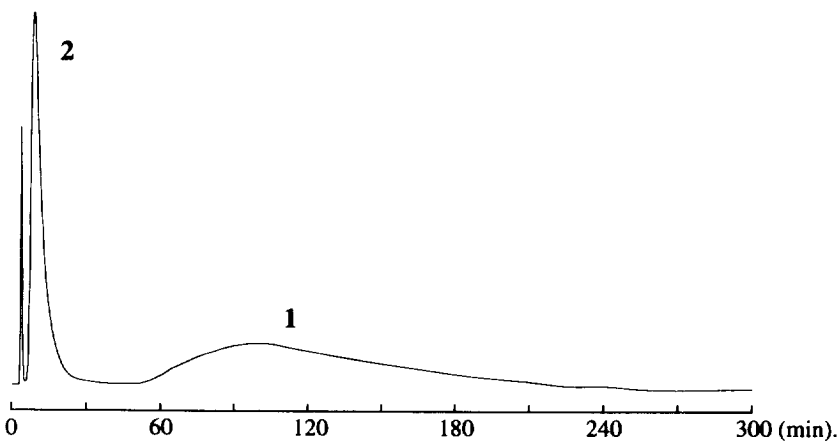

Fig. 2. HPLC separation of an equimolar mixture of (-)-cinchonidine **1** and (+)-cinchonine **2** on an *anti*-**1** MIP CSP, **P(1)** (10 µg of analyte mixture was injected). Detector attenuation was increased 4-fold after 45 min to accentuate the broad peak arising from **1**.

The capacity factors for quinoline **7**, a structural element present in the imprinting templates, on **P(1)** and **P(2)** was somewhat higher than for **P(B)**. This implies that some selective recognition of quinoline by the MIPs, even though **7** possesses only one possible point for hydrogen bonding, and limited structure for shape recognition.

The high stereoselectivity of this system suggested its use as a "general" CSP. Initial attempts to resolve racemates of other similar molecular weight samples, *e.g. N*-acetyl tryptophan, produced only slight enantiomer dependent differences in affinity (α-values less than 1.1), further underscoring the high selectivity of these alkaloid receptor mimics. The ß-adrenergic receptor blocking agent propranolol **8**, possesses structural and stereochemical elements quite similar to those of the template structures. A racemate of **8** was partially resolved on **P(1)** with an α-value of 1.5 ($\alpha = 1.0$ on **P(B)**, 1 µL injection volumes). That such levels of separation can be achieved supports our arguement in favour of rigid template structures being useful for producing more selective recognition sites. Furthermore, these data collectively imply a balance of steric and electronic effects as critical to optimal recognition at MIP receptor sites.

In conclusion, synthetic receptors for the cinchona alkaloid antimalarial agents (-)-cinchonidine **1** and (+)-cinchonine **2** were prepared by molecular imprinting in methacrylic acid - EGDMA copolymers and have demonstrated unprecedented diastereoselectivity, with stereoseparation factors of up to 31. Recognition studies using related alkaloid structures and racemates of pharmaceutical significance reflected the high fidelity of these synthetic receptor preparations and support the use of rigid template structures for generating better defined receptor sites. The selectivity of these systems, together with the general mechanical and chemical stability of MIPs, bodes well for their use as substitutes for biologically derived recognition elements in diagnostic and biosensor type applications, such as fluorescence based (non-isotopic) binding analyses.

EXPERIMENTAL

Chemicals and solvents were of analytical or HPLC grade. Monomers and chloroform were purified prior to use.[8] The template substance (2.45 mmoles), methacrylic acid (9.87 mmoles) and ethylene glycol dimethacrylate (69.05 mmoles) were dissolved in chloroform (20 g) in a borosilicate glass reaction vial. Reaction mixtures were sparged with dry nitrogen gas (0 °C, 15 min) and irradiated with a 360 nm light source (4 °C, 17.5 h). Bulk polymers were ground mechanically (Nitto AMN 200, Japan) and were wet sieved (water and methanol) through a 45 µm mesh filter. Material of $\leq$ 45 µm was sedimented (5 x 20 min) from acetonitrile (250 mL), packed (acetonitrile) into stainless steel HPLC columns (150 x 4.6 mm) and washed with methanol/acetic acid (7:3) for 12 h at 1.0 mL min^{-1} until a stable baseline was achieved. A blank, non-imprinted reference polymer, **P(B)**, was prepared identically, though in the absence of a template.

HPLC measurements were conducted on a system comprised of a Gilson 231 XL autoinjector coupled to a Hitachi L-5090 degasser, L-6000 pump system, L-4200 UV-VIS detector and D-2500 Chromato-Integrator. Analyses were run (isocratic) with flow rates of 0.5 mL min^{-1} and detection at 280 nm. Capacity factors (k') were determined from: $k' = (v\text{-}v_0)/v_0$, where v is the retention volume of analyte and v_0 that of the

void marker (acetone). Separation factors (α) were calculated from the relationship: $\alpha = k'_1/k'_2$, where k'_1 is the capacity factor of the more retained analyte.

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