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New CSPs based on peptidomimetics: efficient chiral selectors in enantioselective separations

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Summary

Two different families of peptidomimetics have been synthesized and used as chiral selectors for enantioselective chromatography. The functionalization of compounds with multiple nitrogen atoms allows their use in the preparation of chiral stationary phases (CSPs), with acrylic or styril comonomers, in both bead and monolithic formats. Some of these separation media, having the appropriate morphological properties for their use in chromatographic columns, were able to efficiently discriminate enantiomers of aminoacid derivatives and pharmaceuticals such as Oxazepam.

Introduction

Nature provides systems with a great versatility that can play a role in a number of different biological processes such as catalysis, physiological regulation, transport, structural composition and defense. Design of compounds able to mimic this behavior remains one of the main challenges of today's synthetic chemists. The preparation of peptidomimetics is an example of an attractive and simple approach. One fascinating feature emerging in this field is the enormous structural diversity and creativity that can be used to mimic the ingenious simplicity of nature [1].

Different peptidomimetic structures have been synthesized and employed to mimic the secondary structure in proteins, in particular folding [1], molecular recognition [2], catalysis [3], etc.

In recent years we have been involved in the design of simple peptidomimetics that could act as versatile ligands for a number of different processes. Such compounds are based in the presence of an aliphatic polyamine chain connecting two aminoacids. This simple design, and the possibility of the selective functionalization of the different nitrogen atoms in the molecule, enables a large molecular diversity and the creation, in an easy way, of libraries of compounds for different applications [5]. For example, polymers containing those chiral moieties have already been prepared by

their grafting onto preformed poly(styrene-divinylbenzene) (PS-DVB) polymers, and have been used as chiral catalysts in several asymmetric reactions [5a]. A very attractive application for those compounds is their use as selectors for chiral recognition and separation [6,7,8]. Here we present our initial results on the preparation of different Chiral Stationary Phases (CSPs) based on those simple peptidomimetics.

Experimental

*General synthetic procedure for the preparation of acrylic derivatives of peptidomimetics. Synthesis of **12a***

To a solution of compound **9a** (2.2 g, 6 mmol) in THF were added Et_3N (4.1 mL, 14.4 mmol) and a catalytic amount of DMAP. The solution was cooled to −35°C and methacryloyl chloride (1.41 mL, 14.4 mmol) was added dropwise. After stirring for 2 h, a saturated solution of $NaHCO_3$ (40 mL) was added and the phases separated. The aqueous phase was extracted three times with Et_2O, the combined organic phases dried over anh. $MgSO_4$ and the solvent vacuum evaporated. The crude product was crystallized (hexanes/DCM) to give **12a** as a white solid (2.3 g, 76%). Mp 202-205°C. $[\alpha]^{20}_D$= -4.86(c=0.01, THF). ^{13}C NMR ($CDCl_3$, δ): 18.6, 29.1, 38.3, 38.6, 56.2, 120.0, 129.6, 130.3, 131.0, 135.6, 139.8, 168.1, 170.3. MS (MALDI-TOF): 505 (M^++1).; (TOF-ES+): 527 (M+Na^+); (TOF-ES-): 502 (M-2). Anal calcd for $C_{29}H_{36}N_4O_4.H_2O$: C, 66.9; H, 6.9; N, 10.8; Found: C, 66.4; H, 7.1; N, 10.5.

*General synthetic procedure for the preparation of particulate polymers. Preparation of **P2***

Monodisperse polystyrene seeds (10% in water, 0.744 mL) were activated with the use of dibutyl ftalate (0.521 mL) and were emulsified overnight in an aqueous solution containing 0.25% SDS (75 mL). To this suspension were added styrene (0.78 g), DVB (0.96 g), monomer **13a** (1.16 g), cyclohexanol (6.45 g), DMSO (0.83 g) and AIBN (40 mg), previously emulsified by sonication in an aqueous solution (75 mL) containing 0.25% SDS. The mixture was stirred for two days and then an 4% aqueous solution of PVA was added, the mixture was purged with N_2 for 20 min and the polymerization was carried out in a closed flask at 75°C for 16 h using a stirring speed of 225 rpm. The resulting beads were decanted with water and methanol, then dried at the air and extracted with toluene for 30 min and with DCM for 4 h. The resulting beads were dried first at the air and then at the vacuum oven at 50°C for 12 h to obtain 0.9 g of dried polymer **P2**.

General synthetic procedure for the preparation of monolithic polymers

The corresponding chiral monomer and the respective acrylic comonomers (MMA, EDMA, TRIM...) were dissolved in an appropriate ternary mixture of porogens (DMSO, cyclohexanol, 1-dodecanol). Then AIBN (1 wt% with respect to the monomers) was added and the mixture stirred to obtain a homogeneous solution. The polymerization mixture was purged with nitrogen for several min and poured into a

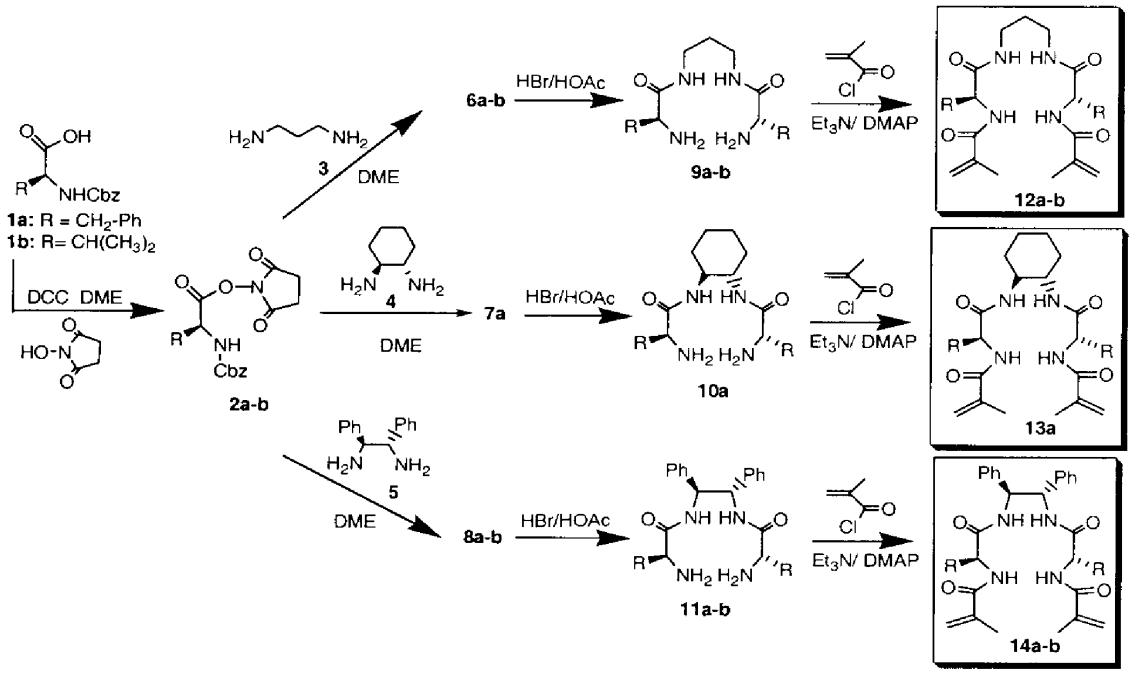
mold. The sealed mold was introduced in a bath and the polymerization was allowed to proceed at 80°C for 24 h. After this period, the polymer was exhaustively washed with THF to remove the porogenic agents and any other soluble compounds.

General procedure for chromatographic separations

Several chromatographic separations were assayed using 100x4.6 mm id columns containing the different polymers. In most cases mixtures hexane:dichloromethane (H:DCM) were used as the eluent (flow rate 1 mL/min), the exact composition of the mixture being selected, for each polymer to optimize the selectivity.

Results and discussion

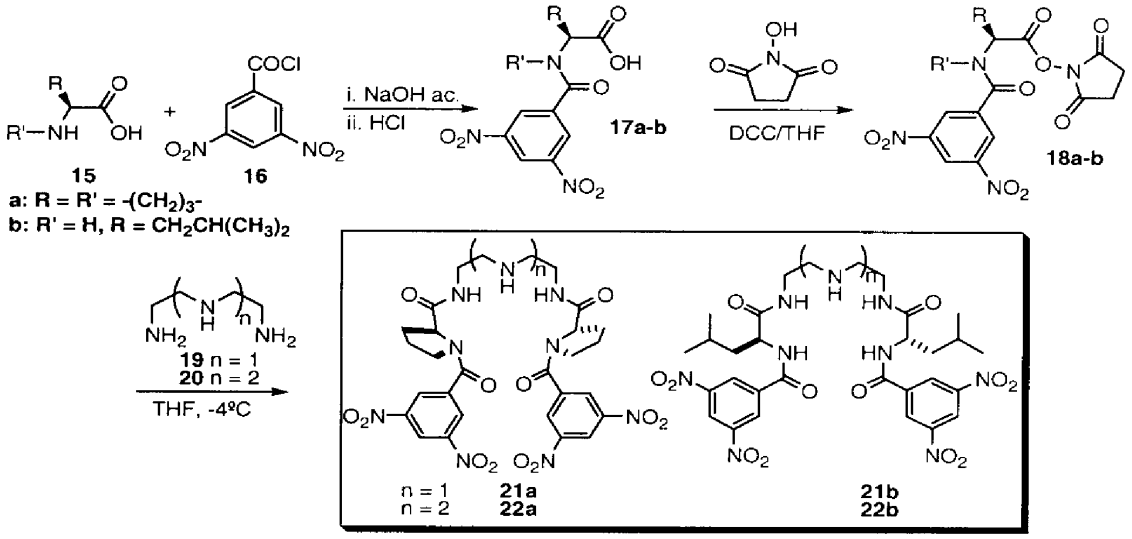
Initially, peptidomimetics **12-14** containing two vinyl groups were synthesized (*Scheme 1*) in order to be employed as crosslinking agents in the preparation of different CSPs by polymerization procedures. Yields for the individual steps were high (70-95% in most cases) so that compounds **12-14** were obtained in 35-63% overall yields.



Scheme 1. Synthesis of monomers **12-14**

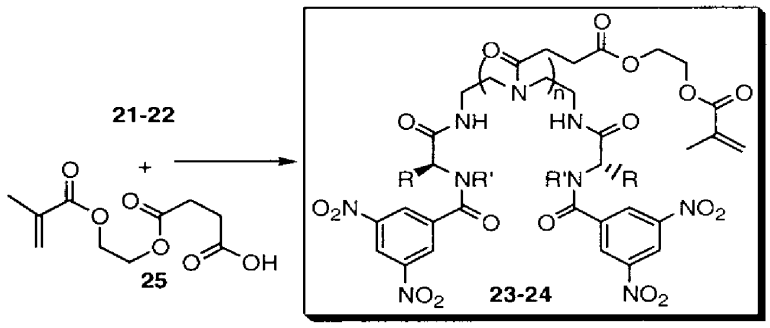
An alternative synthetic route was also developed to obtain a second family of peptidomimetics (*Scheme 2*) using a polyamine bridge bearing one or more additional nitrogen atoms in the chain (**19-20**). These molecules contain multiple anchoring points making their immobilization onto a polymeric backbone easier and also allowing the introduction of additional functionalities. Indeed, the amino groups of the amino acid fragment could be used to introduce different π-groups required for the preparation of Pirkle-type CSPs [7,8]. In our case, π-acceptor functionalities based on 3,5-dinitrophenyl derivatives were selected.

As shown in the *Scheme 2*, the α-amino acids were initially reacted with 3,5-dinitrobenzoyl chloride (60-73%) and the resulting products (**17a-b**) were used as the starting materials instead of **2a-b**. Despite of the presence of additional secondary amino groups, the condensation reactions to afford **21-22** are rather clean, with yields ranging from 66 to 80 %.
Reaction of **21-22** with methacryloyl chloride afforded monomers related to **12-14**. However, they exhibited a low reactivity and led to low polymerization yields. Molecular mechanics calculations suggested the presence of non-coplanar π-clouds in the acrylate moiety, due to intramolecular H-bonding between the C=O group of the acrylic fragment and the N-H amide groups. This prevents radical delocalization required during polymerization. Accordingly, a vinylic moiety with a longer spacer was selected to avoid such interactions.



Scheme 2. Synthesis of monomers **21-22**

Thus, compounds **21-22** were reacted with 2-(methacryloyloxy)ethylsuccinic acid (**25**) to give monomers **23-24** (50-81%) with the appropriate reactivity (*Scheme 3*).



Scheme 3. Synthesis of monomers **23-24**

The incorporation of the different monomers (**12-14**, **23-24**) into a polymeric insoluble backbone was achieved by the use of the different polymerization techniques [9,10].

The 'Staged templated suspension polymerization' was used as a first approach for the preparation of the corresponding macroporous beads [9]. The chemical and porous structures of the spherical CSPs were characterized by mercury porosimetry, BET, FT-IR spectroscopy and elemental analysis (*Table 1*).
The chromatographic efficiency of those CSPs was evaluated for the separation of 3,5-dinitrobenzamido derivatives of some racemic α-amino acids [10c] and for some commercially available racemic drugs such as benzodiazepines.
By the use of the CSP prepared with MMA, TRIM and the chiral selector **12a** (**P1**) it was possible to obtain an efficient enantioseparation of the leucine derivative **26** with a selectivity up to α=4.0 (H:DCM, 50:50 v/v; k'_1=1.62, k'_2=6.56; number of theoretical plates N=329). An even better selectivity (α=5.4; k'_1=4.80, k'_2=16.56) was achieved with the chiral selector derived from **13a** (**P3**) (H:DCM, 60:40 v/v; N=520) (*Figure 1*). This selectivity was sufficient to use this column in a preparative scale. When the VB-DVB based CSP **P2** was used (H:DCM, 30:70 v/v; N=548), the chiral separation of **26** was possible again, but the selectivity was lower (α=1.2; k'_1=8.92, k'_2=11.67), most likely due to the presence of non-specific aromatic-aromatic interactions.

Table 1. Polymerization conditions and properties of macroporous CSPs **P1-P4** in bead format.[a]

CSP	P1	P2	P3	P4
Monomer	12a	13a	13a	23a
Monomer (%)[b]	30.3	40.3	38.1	30.0
VB (%)[b]		26.8		
DVB (%)[b]		32.9		
MMA (%)[b]	29.9		21.9	
EDMA (%)[b]				70.0
TRIM (%)[b]	39.8		40.0	
DOH (%)[c]			50.9	70.0
DMSO (%)[c]	39.3	31.0	32.0	
COH (%)[c]	60.7	69.0	17.1	30.0
V_p (mL/g)[d]	0.9	0.8	0.9	0.6
S_g (m^2/g)[e]	174.3	97.3	144.2	198.3
D_{pm} (nm)[f]	34.3	21.7	34.0	61.8
FD (%)[g]	45	40	50	41
Part. size (μm)[h]	6	6.5	6	5.5

[a.] Polymerization temperature 60 °C. [b.] VB: styrene, DVB: divinylbenzene, MMA: methyl methacrylate, EDMA: ethylene dimethacrylate, TRIM: trimethylolpropane trimethacrylate. [c.] Porogenic mixture (DOH: 1-dodecanol, COH: cyclohexanol). [d.] Pore volume determined by mercury intrusion porosimetry. [e.] Specific surface area, calculated from the BET isotherm of nitrogen. [f.] Median pore diameter determined by mercury intrusion porosimetry. [g.] Functionalization degree determined by elemental analysis of nitrogen. [h.] Particule size determined by microscopy.

However, a rather surprising result was obtained when CSPs **P1**-**P4** were used for the separation of commercial racemic drugs, particularly benzodiazepines. The VB-DVB based CSP **P2** was able to separate racemic Oxazepam (α=1.25) (*Figure 2*), whilst the acrylic CSPs (**P1, P3** and **P4**) did not show any selectivity for this racemate (α=1). These results demonstrate that the presence of aromatic rings in the polymeric backbone does not necessarily play a negative role for enantioseparation. In any case,

the nature of the polymeric network plays an important role in the whole process, as has been previously observed in other applications of chiral polymers [11].
A second approach used for the incorporation of the chiral selectors included bulk polymerization to form CSPs in monolithic format [9a,10]. Thus, monolithic columns **M1** (MMA and EDMA as comonomers), **M2** (VB, DVB) and **M3** (MMA, TRIM) were prepared from compound **12a**. Polymers **M4** (MMA, EDMA), **M5** (VB, DVB) and **M6-M8** (MMA, TRIM) were obtained from monomer **13a**. Finally, monoliths **M9** (EDMA) and **M10** (TRIM) were prepared from **23a**. It is worth noting that due to the high concentrations of monomers required for this technique, we faced problems with their limited solubility. In addition, the preparation of monoliths with a desired porous structure featuring suitable pore size distribution required for their use in HPLC, was difficult to achieve with some specific polymerization mixtures.

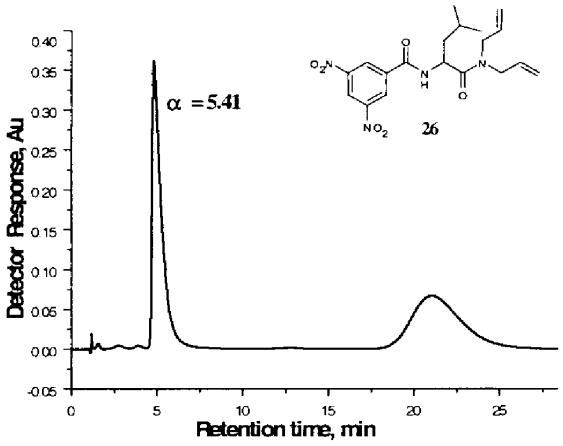


Figure 1. Chiral separation of **26** with **P3** using 60:40 hexane:dichloromethane

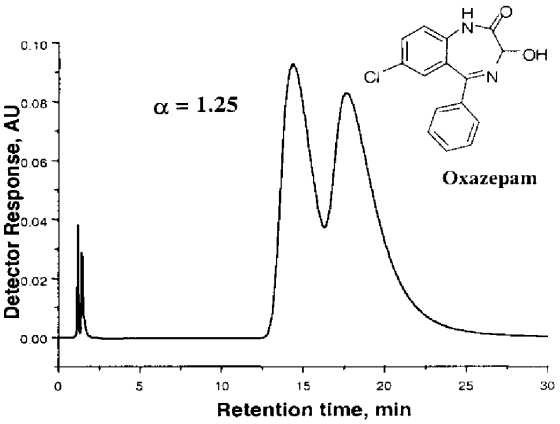


Figure 2. Chiral separation of racemic Oxazepam with **P2** using 30:70 hexane:dichloromethane

Initial evaluation of the acrylic based monolithic CSPs was promising. A reasonable enantioseparation of **26** (α=2.2, H:DCM 70:30 v/v; k'_1=0.91, k'_2= 2.18, N=154) could be achieved with **M4** (Dpm= 2554 nm, FD 41%). According to our knowledge, this is the first reported enantioselective HPLC separation using a monolithic CSP. More work is being carried out in order to optimize the separation process and the structural morphology of these chiral monoliths.

Conclusions

In conclusion, the present results clearly indicate that the design of polymeric stationary phases for enantioselective chromatography requires a delicate balance of different factors. In addition to the structure of the chiral selector that plays an important role in the resolution of enantiomers, other factors such as the nature and morphology of the polymers also greatly affect the selectivity. The mechanism that enables chiral recognition of the enantioselective media is complex and need to be re-evaluated continuously. Peptidomimetics appear to be well suited as chiral selectors due to their large molecular diversity attainable and the ease of tailoring their structures. CSPs in the bead format seem to be currently more efficient for enantioseparations that monolithic materials. However, results obtained with **M4**

indicate a promising way for the development of new CSPs in monolithic format. Further work is in progress in order to both better understand the factors affecting enantioseparation using these materials and to establish the scope of their potential applications.

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