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Short communication

New 1,3,5-triazine based chiral stationary phase for the high-performance liquid chromatographic separation of enantiomers

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Abstract

2-Chloro-4-(*S*)-1-(1-naphthyl)ethylamino–6-L-Val-L-Val-L-valine isopropyl ester 1,3,5-triazine (I), a triazine derivative containing two different chiral moieties, was linked to 3-aminopropylsilanized silica, affording chiral stationary phase (CSP) II, in order to verify its enantiodiscriminating capability and to make a comparison with similar triazine derivative CSPs containing only one kind of chiral moiety. CSP II was successfully employed for the HPLC separation of N-3,5-dinitrobenzoyl amino acid alkylesters and 2,2′-disubstituted-1,1′-binaphthyl compounds. Compound I was also used to prepare a different CSP by in situ derivatization of a prepacked 3-aminopropylsilanized silica column: the obtained CSP exhibited the same characteristics of the CSP prepared by conventional methods. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Triazine-based stationary phases; Amino acids

1. Introduction

During the last decade the preparation of new chiral stationary phases (CSPs) for enantiomer separations by high-performance liquid chromatography (HPLC) has shown a progressive trend in organic chemistry [1]. Amongst the most successful CSPs are the "brush type" phases, which consist of a silica matrix with covalently bonded chiral groups. The most relevant contribution to the development of this type of CSPs is due to Pirkle and co-workers, who have prepared chiral stationary phases of wide applicability and good accessibility [2].

Many other research groups have addressed their efforts toward the achievement of new CSPs: therefore the literature reports a great number of CSPs obtained by linking to silica optically active compounds either coming from the "chiral pool" such as quinine [3], amino acids [4,5], tartaric [6] and lactic acid [7] derivatives, or synthetic products such as 1,2-cyclohexanediamine [8] or biaryl derivatives [9].

Nowadays an important goal in this research field is the achievement of chiral selectors which have satisfactory enantiodiscriminating capability over a wide range of racemic compounds [10].

An interesting approach to this problem could be the synthesis of a new chiral polyfunctional selector by linking different chiral moieties to the same achiral unity. We think 2,4,6-trichloro-1,3,5-triazine, which has three chlorine atoms which can be displaced in succession by chiral nucleophilic reagents, employing adequate experimental conditions, is very

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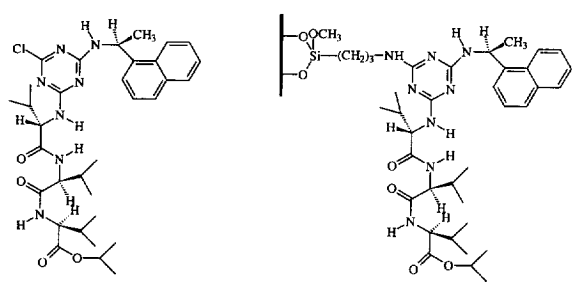
suitable for this purpose [11,12]. In fact, by displacing two chlorine atoms by two chiral nucleophiles having different structures, a polyfunctional selector is obtained, which can be linked to aminopropylsilanized silica using the last chlorine atom.

The literature reports some examples of CSPs prepared starting from a triazine derivative containing only one kind of chiral moiety[1], namely amino acid [14–17] and peptide [18] derivatives and 1-(1-naphtyl)ethylamine [18]. Ôi et al. [18] have obtained two CSPs by linking to silica monochloro *s*-triazine derivatives of L-Val-L-Val-L-valine isopropyl ester (CSP A) or (*S*)-1-(1-naphthyl)-ethylamine (CSP B), which separate the enantiomers of 3,5-dinitrobenzoyl (DNB) derivatives of alkylaryl amines, amino acids and carboxylic acids: when comparing CSPs A and B, CSP A provides the best separations of amino acid derivatives, whereas CSP B provides better separations of the amine DNBs.

The aim of this work was the preparation of a new CSP starting from the monochloro *s*-triazine derivative I (Fig. 1), containing both the tripeptide and 1-(1-naphthyl)ethylamine moieties, in order to verify if this new CSP is able to separate the racemic compounds resolved by CSP A as well as those resolved by CSP B.



I II

Fig. 1. Chiral selector I and chiral stationary phase II.

[1]During the preparation of the present manuscript, we found in the literature a paper which describes the preparation and the use of a CSP, obtained starting from a triazine derivative containing two different chiral moieties, namely 1-(1-naphthyl)ethylamine and an amino acid derivative [13].

2. Experimental

2.1. Analysis

Liquid chromatography was carried out using a Jasco PU 980 pump equipped with a Jasco 075 UV detector. Elemental analyses were performed in the Microanalytical Laboratory of the Institute of Pharmaceutical Chemistry, University of Pisa. Optical rotations were measured on a Jasco DIP 360 automatic digital polarimeter in a 1 dm cell. Melting points were measured on a Kofler Reichert–Jung apparatus and are uncorrected. UV spectra were recorded on a Jasco UVIDEC 710 in a 1 cm cell.

2.2. Materials

3-Aminopropylsilanized silica gel was a Merck product (LiChrosorb-NH_2, 10 μm). It was dried by means of azeotropic distillation with benzene. The solvent was removed under vacuum and the material was dried at 0.1 mmHg for 6 h (1 mmHg=133.322 Pa).

2-Chloro-4-(*S*)-1-(1-naphthyl)ethylamino–6-L-Val-L-Val-L-valine isopropyl ester-1,3,5-triazine (I) was prepared as described elsewhere [12]: m.p. 206–210°C, $[\alpha]_D^{20}=-50.09$ ($c=1$, $CHCl_3$).

N-3,5-Dinitrobenzoyl derivatives of aminoacids were prepared by standard literature procedures.

The binaphthyl derivatives were prepared according to literature procedures: 2-isopropoxy-2′-hydroxy-1,1′-binaphthyl [19], 7,7′-bis(1-propen-3-oxy)-2,2′-dihydroxy-1,1′-binaphthyl [20], 2-amino-2′-hydroxy-1,1′-binaphthyl [21], 2,2′-bis(methylamino)-1,1′-binaphthyl [22].

All the other chemicals were purchased from Fluka or from Merck.

2.3. Preparation of CSP II

A solution of I (4.13 g, 6.46 mmol) in dry tetrahydrofuran (THF) (40 ml), containing triethylamine (1.19 g, 11.93 mmol) was mixed with a slurry of LiChrosorb-NH_2 (5 g) in dry THF (30 ml).

The mixture was stirred under reflux for ten days. After cooling at room temperature, the material was centrifuged and washed with THF and acetonitrile until the absorbance at 220 nm (transition of the

naphthalene and triazine chromophores) was negligible. The material was then washed with ether and pentane and dried at reduced pressure. The elemental analysis showed that the content of I was 0.15 mmol/g.

Elemental analysis for the aminopropylsilanized silica: N 0.48%, C 1.60%, H 0.45%.

Elemental analysis for the derivatized material: N 1.63%, C 6.92%, H 1.03%.

With this material a stainless-steel column (250×46 mm) was slurry packed by conventional techniques.

2.4. Preparation of CSP II in situ

A Si-NH_2 column (250×46 mm) was activated by pumping through it a 3% solution of triethylamine in dry THF. A solution of I (1.5 g, 2.35 mmol), triethylamine (0.303 g, 3 mmol) and naphthalene (0.256 g, 2 mmol) in dry THF (100 ml) was pumped through the activated column, kept at 50°C by means of a thermostat, (flow 0.5 ml/min) in recycle mode. The progress of the derivatization was monitored by HPLC [C_{18} column; eluent: acetonitrile–water (85:15), flow 0.2 ml/min] using the naphthalene as internal standard. After five days the CSP, reported at room temperature, was washed with THF, methanol, acetonitrile, ethylether, then hexane until the baseline was steady.

3. Results and discussion

The covalent CSP II (Fig. 1) was prepared by reacting 3-aminopropylsilanized silica with a solution of I in dry THF in the presence of triethylamine. The reaction was carried out under reflux of the solvent for ten days, as the same time was required in order to obtain the displacement of the chlorine atom of I by hexylamine [12]. The reacted material, washed and dried, was used for packing a stainless-steel column (250×46 mm). Compound I was also used to derivatize a commercial HPLC column of 3-aminopropylsilanized silica. The derivatization was performed by pumping a solution of I and triethylamine in THF, containing naphthalene as internal standard into the column.

These columns were used to separate, at first, racemic 3,5-dinitrobenzamides of alkylaryl amines and racemic 3,5-dinitroanilides of carboxylic acids resolved by CSP B and racemic N-3,5-dinitrobenzoyl derivatives of amino acids resolved by CSP A.

As far as the amine and carboxylic acid derivatives are concerned, high values of k' (ranging from 4.52 to 5.65) are observed, employing the eluent used by Ôi et al. [18] to elute the same compounds on CSP A, which are quite similar to those obtained on CSP A; however, these compounds are not resolved on CSP II. This chromatographic behaviour indicates that the compounds strongly interact with the tripeptide–triazine moiety of the CSP (high retention times) but these interactions are not enantiodiscriminating.

Since these compounds are resolved on CSP B, which has only the 1-(1-naphthyl)ethylamine moiety, we feel that the lack of enantiodiscrimination of CSP II could be attributed to the predominance of non enantioselective interactions of this kind of analytes with the tripeptide–triazine moiety. This hypothesis seems to be reasonable considering that these racemic compounds are not resolved by CSP A, which has only the tripeptide moiety. In order to confirm that these compounds do not interact with the 1-(1-naphthyl)ethylamino moiety of CSP II, we eluted the analytes employing the mobile phase which Ôi et al. [18] used to separate the same compounds on CSP B. This eluent is more polar than the previous one, because CSP B retains the racemic compounds more strongly than CSP A. In Table 1 the reported k' values obtained with CSP II are compared with the corresponding ones reported for CSP B.

The data reported in Table 1 show that these

Table 1
k' values[a] on CSP II and on CSP B [18] for alkylarylamine[b] and carboxylic acid[c]

Compound	k' on CSP II	k' on CSP B
1-Phenylethylamine	0.50	1.76
1-(1-Napthtyl)ethylamine	0.62	1.46
2-Phenylpropionic acid	0.43	2.17

[a] Flow-rate: 1 ml/min, UV detector (λ=254 nm), t=25°C, eluent: hexane–CH_2Cl_2–ethanol (100:40:10).
[b] As 3,5-dinitrobenzamides.
[c] As 3,5-dinitroanilide.

compounds are hardly retained on CSP II compared to CSP B; we think that this behaviour can be attributed to a lack of interaction with the 1-(1-naphthyl)ethylamino moiety of CSP II.

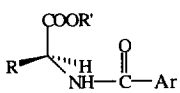
At the same time, CSP II is able to separate the enantiomers of amino acid derivatives as CSP A.

Table 2 lists the chromatographic resolution data of amino acid derivatives on CSP II.

The phase separates only the N-3,5-dinitrobenzoyl derivatives of amino acid alkyl esters, whereas other aroyl derivatives are not resolved (runs 4 and 5). The capacity factors k' are related not only to the nature of the amino acids but also to the kind of alkoxy group: increasing the bulkiness of this group the retention time of the analyte is lowered (runs 2 and 3). As far as the structure of the amino acid is concerned, we observe that the amino acids having an aromatic group (phenylglycine and phenylalanine, runs 9 and 10), as well as those having a β-branched alkyl group (leucine) are more retained than substrates having an α-branched alkyl group (runs 1, 7, 8). The most retained compounds are the alanine and methionine derivatives. All the N-3,5-dinitrobenzoyl amino acid alkyl esters are resolved on CSP II with α values ranging from 1.19 (run 9) to 1.54 (run 2): the enantioseparations, quite similar to those obtained by Ôi et al. [18] with CSP A, are dependent on the structure of the amino acid. The highest separation factors are observed for the α-branched amino acids (runs 1 and 7) and the most retained amino acids exhibit the lowest α values (runs 6, 9, 11 and 12). The elution order has been determined for five compounds using enantiomerically enriched mixtures: the first eluted enantiomer has the *R* configuration. In order to check the efficiency of the CSP derivatized in situ all the runs reported in Table 1 were repeated: the same values of k', α and the same elution order were obtained with this CSP.

Recently Ôi et al. [23], reported on a technique where CSP A is used to separate racemic compounds which can interact with the phase only by formation of hydrogen bonding, such as binaphthol: this prompted us to check the enantiodiscriminating capabilities of CSP II toward 2,2′-disubstituted 1,1′-

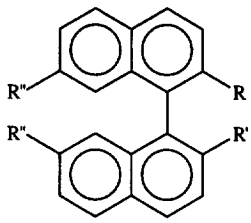
Table 2
Chromatographic resolution[a] on CSP II of aminocid derivatives having the general structure:



Run	Amino acid	Ar	R′	k'^{b}	α^{b}	Eluent[d]	A.C.[e]
1	Valine	3,5 DNP[f]	CH_3	2.79	1.47	A	R
2	Valine	3,5 DNP	^{n}Bu	1.43	1.54	A	
3	Valine	3,5 DNP	^{i}Pr	1.45	1.52	A	
4	Valine	4-NP[g]	CH_3	1.13	1	A	
5	Valine	C_6F_5[h]	CH_3	2.56[i]	1	A	
6	Leucine	3,5 DNP	CH_3	4.22	1.25	A	
7	Isoleucine	3,5 DNP	CH_3	2.61	1.39	A	
8	t-Leucine	3,5 DNP	CH_3	1.80	1.31	A	
9	Phenylglycine	3,5 DNP	CH_3	4.85	1.19	A	R
10	Phenylalanine	3,5 DNP	CH_3	4.57	1.31	A	R
11	Methionine	3,5 DNP	CH_3	3.73	1.28	B	R
12	Alanine	3,5 DNP	CH_3	4.09	1.21	B	R

[a] Flow-rate: 1 ml/min, UV detector (λ=254 nm), t=25°C.
[b] Capacity factor for the first eluted enantiomer.
[c] Chromatographic separation factor.
[d] A=hexane–CH_2Cl_2–ethanol (100:20:1); B=hexane–CH_2Cl_2–ethanol (100:20:2).
[e] Absolute configuration of the first eluted enantiomer.
[f] 3,5-DNP=3,5-dinitrophenyl.
[g] 4-NP=4-nitrophenyl.
[h] C_6F_5=pentafluorophenyl.
[i] Flow-rate: 0.5 ml/min.

Table 3
Chromatographic resolution[a] on CSP II of 1,1′-binaphthyl derivatives having the general structure:



Run	R	R′	R″	k'^{b}	α^{c}	A.C.[d]	Eluent[e]
1	OH	OH	H	4.09	1.09	R	A
2	OH	O′Pr	H	0.76	1		B
3	OH	OH	O-CH_2-CH=CH_2	3.9	1.10		A
4	$NHCH_3$	$NHCH_3$	H	2.68	1		C
5	NH_2	OH	H	2.29	1		B

[a] Flow-rate: 1 ml/min, UV detector (λ=254 nm), t=25°C.
[b] Capacity factor for the first eluted enentiomer.
[c] Chromatographic separation factor.
[d] Absolute configuration of the first eluted enantiomer.
[e] A=hexane–CH_2Cl_2–ethanol (100:20:2); B=hexane–CH_2Cl_2–ethanol (100:20:1); C=hexane–CH_2Cl_2 (100:20).

binaphthyl compounds. The chromatographic results concerning the separations of these compounds are reported in Table 3.

Only 7,7′-bis(1-propen-3-oxy)-2,2′-dihydroxy-1,1′-binaphthyl and 2,2′-diidroxy-1,1′-binaphthyl are resolved on CSP II; the elution order has been determined for binaphthol (run 1), using an enantiomerically enriched sample. The chiral recognition between the phase and the binaphthols should take place by means of the formation of hydrogen bonding between the hydroxyl protons of the substrate and the amide oxygen atoms of the peptide moiety: π–π donor–acceptor interaction seems unlikely, since both the triaminotriazine group and the naphthalene rings are supposedly π-basic. The presence of two groups capable of hydrogen bonding formation is necessary for the enantiodiscrimination: when an hydroxyl group is replaced with an ether functionality (run 2) the compound is not resolved. Moreover, when hydroxyl group are replaced by anilinic amino groups, the separation does not take place (runs 4 and 5): this could be ascribed to the weaker hydrogen bonding provided by the amino group with respect to the hydroxyl group one. The same chromatographic results were obtained using the column derivatized "in situ" in terms of retention time, elution order and enantiomer separation.

In conclusion the results obtained demonstrate that the chiral 1,3,5-triazine selector I resolves racemic compounds by means of interactions taking place only with the triazine–peptide moiety, since these interactions overcome those which could be provided by the triazine-1-(1-naphtyl)ethylamino moiety.

It is worthy of note that we have obtained, derivatizing in situ a commercial HPLC column of aminopropylsilanized silica, a CSP having the same characteristics of the CSP obtained by derivatization of aminopropyl silica and successive packing of a stainless-steel column: this constitutes a new and simple method to obtain CSPs starting from monochlorotriazine selectors.

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