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Chromatographic separation of enantiomers on N,N'-diallyl-L-tartardiamide-based network–polymeric chiral stationary phases

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Abstract

A series of network–polymeric chiral stationary phases, based on *para*-substituted N,N'-diallyl-L-tartardiamide dibenzoates, has been investigated with respect to differences in the enantioselectivity displayed during liquid chromatography of varying racemates in the straight-phase mode. These phases are thought to operate via a different hydrogen bonding ability of the analyte enantiomers. Although a rationalization of retention data in terms of a chiral recognition model is not possible at the present stage, it seems clear that an alkyl or a phenyl *para*-substituent generally improves the enantioselectivity of the phase. Further, from an applications point of view it is of interest that the 4-phenylbenzoate gives a sorbent showing particularly high chromatographic enantioselectivity towards the series of profens (α-arylpropionic acids) investigated (α-values ranging between 1.22 and 1.80).

Keywords: Enantiomer separation; Chiral stationary phases, LC; Network–polymeric chiral stationary phases; α-Arylpropionic acids; N,N'-Diallyl-L-tartardiamide

1. Introduction

As a readily available, C_2-symmetric chiral starting material, (*R,R*)-tartaric acid has been used previously for the synthesis of suitable selectors in chiral phase systems. In particular, esters and amides have found interest as chiral additives to the mobile phase [1–5]. Further, some monoamides have been used as ionically bound selectors via adsorption to an aminopropylsilica support [6]. Covalently bound (*R,R*)-tartaric acid-based selectors have also been described, notably the isopropyltartramide [7] and diacetyl isopropyltartramide [8] selectors.

With the objective of creating a chiral sorbent useful for large scale preparative resolution of racemates, we recently described a new class of network–polymeric chiral stationary phases (CSP's) based on the chiral synthon N,N'-diallyl-L-tartardiamide (DATD) [9]. Modification of this monomer is achieved via functionalization of the free hydroxyl groups to acyl or carbamoyl derivatives. The DATD derivative is then allowed to react to form a network polymeric structure with subsequent covalent attachment to the vinyl-functionalized silica support. It was found that sorbents with substantial enantioselective properties, of high capacity and giving very satisfactory column efficiency when used under straight-phase conditions, could be obtained by aroylation of

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the hydroxyl groups. Further, the substituents in the aryl group were found to greatly influence the retention properties, yielding large effects on the α-values. Thus, replacement of benzoyl groups by 3,5-dimethylbenzoyl increased the α-value of binaphthol from 1.35 to 2.75 in hexane–1% 2-propanol with a simultaneous decrease in k_1' from 4.54 to 2.98. Other large substituent effects were found, indicating, for example, that bulky substituents in the para-position were favourable with respect to certain classes of analytes. This prompted us to carry out a comparative study of substituent effects with the use of analytes of different structural types. This paper mainly describes the results found on varying the para-substituent in the substituted DATD benzoates and their implication for useful applications.

2. Experimental

2.1. Chromatography

The liquid chromatography system was composed of a Waters HPLC pump (Model 600E) in conjunction with a Waters variable-wavelength UV detector (Model 484). The samples were introduced into the system using a Rheodyne Model 7125 injector equipped with a 20-μl loop. Chromatographic data was collected and processed by a Waters Model 745 integrator.

The chiral sorbent was packed into stainless steel columns (250×4.6 mm I.D.) using the balanced density slurry packing technique. Separation was carried out with hexane-based mobile phases.

2.2. Synthesis of the chiral monomers

The synthesis of the chiral monomers I and III has been reported earlier [9].

2.2.1. O,O'-Bis-(4-methylbenzoyl)-N,N'-diallyl-L-tartardiamide (II)

N,N'-Diallyl-L-tartardiamide (7 g, 31 mmol) was added to 50 ml of pyridine and cooled to ~5°C. To the stirred solution, 2.2 equivalents of 4-methylbenzoyl chloride was added drop by drop. The reaction mixture was allowed to stand at room temperature for 4 h, 200 ml of dichloromethane was added and

the reaction mixture washed twice with 50 ml of 10% hydrochloric acid, 5% sodium hydrogen carbonate solution and distilled water. The organic phase was dried over anhydrous magnesium sulphate and evaporated to dryness. The pale yellow residue was recrystallized from ethanol to give white crystals (87%), m.p. 216–217°C, $[\alpha]_D^{20} = -105°$ (THF, c. 1). ^{1}H NMR (DMSO-d_6) 250 MHz, δ-values: 3.66 (m, 4H), 4.96 (dd, 4H), 5.64 (m, 2H), 5.79 (s, 2H), 7.36 (d, 4H), 7.95 (d, 4H), 8.58 (t, 2H).

In general, the above method was used to synthesize all the following monomers. The acid chlorides necessary for the synthesis of monomers IV–VI were prepared from the corresponding acids using standard procedures.

2.2.2. O,O'-Bis-(4-phenylbenzoyl)-N,N'-diallyl-L-tartardiamide (IV)

Recrystallization from ethyl acetate (73%) gave white crystals, m.p. 227–228.5°C, $[\alpha]_D^{20} = -159°$ (THF, c. 1). ^{1}H NMR (DMSO-d_6) 250 MHz, δ-values: 3.67 (m, 4H), 4.95 (dd, 4H), 5.65 (m, 2H), 5.80 (s, 2H), 7.09 (m, 10H), 7.25 (m, 4H), 8.06 (d, 4H), 8.55 (t, 2H).

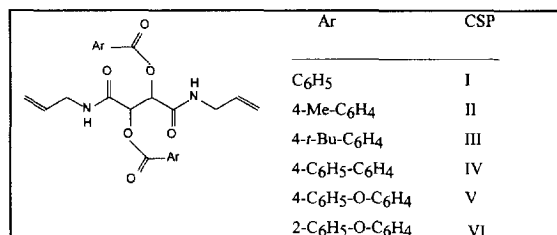#### 2.2.3. O,O'-Bis-(4-phenoxybenzoyl)-N,N'-diallyl-L-tartardiamide (V)

Recrystallization from ethanol gave white crystals (74%), m.p. 200–201°C, $[\alpha]_D^{20} = -97°$ (THF, c. 1). ^{1}H NMR (DMSO-d_6) 250 MHz, δ-values: 3.67 (m, 4H), 4.95 (dd, 4H), 5.65 (m, 2H), 5.80 (s, 2H), 7.09 (m, 10H), 7.25 (m, 4H), 8.06 (d, 4H), 8.55 (t, 2H).

2.2.4. O,O'-Bis-(2-phenoxybenzoyl)-N,N'-diallyl-L-tartardiamide (VI)

Recrystallization from ethanol gave white crystals (64%), m.p. 151–152°C, $[\alpha]_D^{20} = -23°$ (THF, c. 1). ^{1}H NMR (DMSO-d_6) 250 MHz, δ-values: 3.65 (m, 4H), 4.94 (dd, 4H), 5.63 (m, 2H), 5.80 (s, 2H), 6.98 (m, 10H), 7.39 (m, 4H), 7.62 (m, 2H), 7.96 (d, 2H), 8.40 (t, 2H).

2.3. Chiral stationary phases

The sorbents were prepared according to the procedure described earlier using vinyl-functionalized silica gel (Kromasil 5μm, 150Å; EKA Nobel

	Ar	CSP
	C_6H_5	I
	4-Me-C_6H_4	II
	4-t-Bu-C_6H_4	III
	4-C_6H_5-C_6H_4	IV
	4-C_6H_5-O-C_6H_4	V
	2-C_6H_5-O-C_6H_4	VI

Scheme. 1. Chiral stationary phases based on O,O'-diaroyl-DATD derivatives.

AB, Sweden) [9]. Depending on the monomer used, small variations in the amount of solvent and in the reaction time were made. Elemental analysis (C, H, N) of the chiral sorbents showed a loading of 0.7–0.9 μmol/m^2.

3. Results and discussion

In previous work, we have evaluated extensively several CSPs based on diaroyl derivatives of DATD [9]. Substitution of the aromatic ring at the 3- and 5-positions with methyl groups gave the most interesting CSP in terms of broad enantioselectivity. Further, CSP III (Ar: 4-t-Bu-C_6H_4-) gave a much larger α-value for the acidic compound ibuprofen (1) [9]. With the exception of CSP VI, the new chiral sorbents studied are all substituted in the *para* position of the phenyl ring (Scheme 1) and have been evaluated chromatographically using a large

Fig. 1. Separation of the enantiomers of flurbiprofen on CSP III. Mobile phase: hexane–2-propanol (99:1) with 0.1% trifluoroacetic acid as additional modifier, flow-rate 2.0 ml/min; injected amount 40 μg; UV detection at 258 nm.
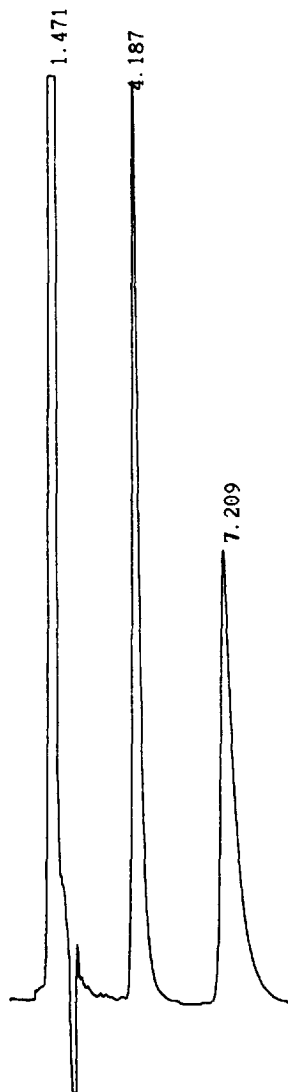
Fig. 2. Resolution of *rac*-bupivacaine on a column (250×4.6 mm I.D.) packed with a sorbent based on 4-phenylbenzoate of DATD. Mobile phase: hexane–2-propanol (95:5) containing 0.1% of trifluoroacetic acid, flow-rate 2.0 ml/min; injected amount 50 μg; UV detection at 238 nm.

number of structurally different racemates, a substantial number of which are acidic compounds. Since these phases operate by means of differential hydrogen bonding in the straight-phase mode, hexane-based mobile phase systems were used to evaluate the CSPs.
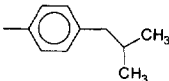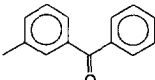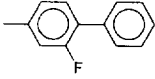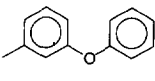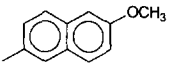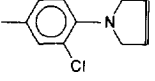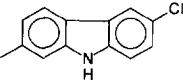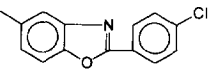
3.1. Influence of the aroyl substituent in the CSP on retention and enantioselectivity

Generally, substitution of the aromatic ring in the para position in the monomer molecule increases the selectivity of the CSPs. The resolution of a series of 2-arylpropionic acids on the chiral sorbents studied, shows that the phase prepared from the 4-phenylbenzoate (IV) gives the highest separation factors, Table 1. Replacing the phenyl group on the aromatic ring with a phenoxy group results in a marked decrease in selectivity, however, retention increases. The size of the alkyl substituent has a large influence on the enantioselectivity, which increases with increasing bulkiness of the alkyl group [$(CH_3)_3C > CH_3 > H$].

Table 1
Influence of the para substituent in the aryl moiety in the monomer on the chiral separation of 2-arylpropionic acids, $ArCH(CH_3)COOH$

Compound	Aryl substituent	C_6H_5		$4\text{-}CH_3C_6H_4$		$4\text{-}t\text{-}Bu\text{-}C_6H_4$		$4\text{-}C_6H_5C_6H_4$		$4\text{-}C_6H_5OC_6H_4$	
	CSP	I		II		III		IV		V	
		k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
	AR-group										
1		2.69	1.24	2.62	1.32	1.99	1.51	1.28	1.54	2.58	1.47
2		9.04	1.05	9.84	1.13	6.59	1.17	5.37	1.22	9.32	1.15
3		4.49	1.16	5.70	1.26	3.95	1.50	2.85	1.69	4.94	1.38
4		3.80	1.15	5.26	1.13	3.30	1.30	2.32	1.39	4.76	1.31
5		6.90	1.19	6.83	1.29	4.57	1.65	3.42	1.74	5.81	1.39
6		5.07	1.13	6.23	1.32	4.27	1.50	3.17	1.80	5.41	1.37
7		6.95[a]	1.06	12.49[a]	1.23	2.19[a]	1.20	5.19[a]	1.27	8.88[a]	1.16
8		5.59	1.17	7.13	1.28	5.02	1.48	4.23	1.63	7.06	1.40

Mobile phase: hexane–2-propanol (99:1, v/v) with 0.1% trifluoroacetic acid (TFA). Flow-rate: 2.0 ml/min; column: 250×4.6 mm I.D.
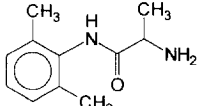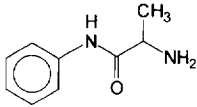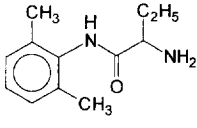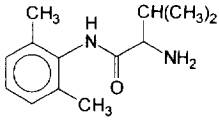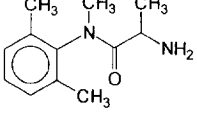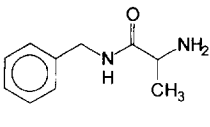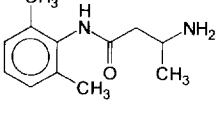[a] Mobile phase: hexane–2-propanol (95:5, v/v) with 0.1% TFA. .

The chromatographic behaviour of these CSPs is illustrated by Fig. 1, which shows the separation of rac-flurbiprofen (**3**) on CSP IV.

The antiarythmic drug tocainide and its structural analogues, containing a primary amino group, were in nearly all cases best separated on CSP III (Ar=4-t-Bu-C$_6$H$_4$-), Table 2. Small changes in the structure of the solute molecule had a great effect on selectivi-

ty and the largest α-value on all the CSPs studied was obtained for the compound **10** that has no substituents on the aromatic ring. Methylation of the amide nitrogen, compound **13** results in lower α-values, especially on CSP IV which showed no selectivity for this compound. In most cases, elongation of the carbon chain between the chiral centre and the aromatic ring in tocainide has a negative

Table 2
Separation of tocainide and structural analogues on CSPs II–V

Compound	Aryl substituent	4-CH$_3$C$_6$H$_4$		4-t-Bu-C$_6$H$_4$		4-C$_6$H$_5$C$_6$H$_4$		4-C$_6$H$_5$OC$_6$H$_4$	
	CSP	II		III		IV		V	
		k_1'	α	k_1'	α	k_1'	α	k_1'	α
	Structure								
9		6.56	1.17	4.66	1.33	3.15	1.24	3.85	1.10
10		7.71	1.40	4.96	2.00	2.72	1.61	4.32	1.84
11		5.71	1.28	3.75	1.61	2.73	1.38	3.52	1.24
12		4.81	1.00	2.61	1.69	2.35	1.14	3.25	1.11
13		5.25	1.10	2.84	1.23	0.21	1.00	3.37	1.07
14		6.89	1.00	4.78	1.00	3.17	1.00	4.91	1.00
15		10.77	1.06	5.30	1.00	3.58	1.12	5.33	1.13

Mobile phase: hexane–2-propanol (97:3, v/v) with 0.1% trifluoroacetic acid; flow-rate: 2.0 ml/min; column dimensions: 250×4.6 mm I.D..
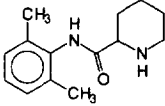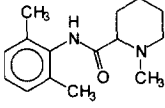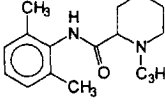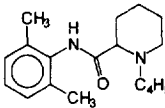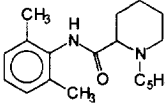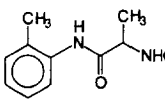
influence on the chiral discrimination ability of the sorbents. The largest effect is seen when the methylene group is inserted between the aromatic ring and the amide nitrogen, compound **14**, which indicates that the elongated π-system including the amide hydrogen plays an important role in the chiral discrimination process.

The related compounds, bupivacaine (**19**) and its structural analogues that are used as local anaesthetics, display an interesting trend as shown in Table 3. Enantioselectivity increases with the number of carbon atoms in the N-alkyl group incorporated in the piperidyl ring. However, when the alkyl substituent is larger than a butyl group (compound **20**), enantioselectivity decreases. Fig. 2 shows the resolution of bupivacaine on CSP IV.
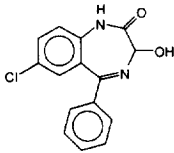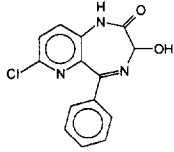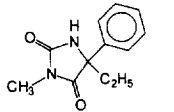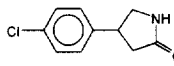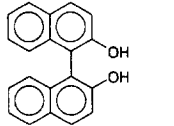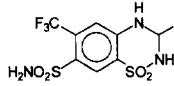
A number of racemates with varying chemical structures were resolved on CSPs II–V, the related chromatographic data is shown in Table 4. Under the conditions used, the largest α-values were achieved

Table 3
Variation in the separation of Bupivacaine and structural analogues with the aryl substituent

Compound	Aryl substituent	4-$CH_3C_6H_4$		4-t-Bu-C_6H_4		4-$C_6H_5C_6H_4$		4-$C_6H_5OC_6H_4$	
	CSP	II		III		IV		V	
		k'_1	α	k'_1	α	k'_1	α	k'_1	α
	Structure								
16		5.35	1.39	2.79	1.68	2.32	1.54	3.41	1.54
17		9.17	1.24	5.91	1.42	3.83	1.36	5.76	1.28
18		5.13	1.40	2.94	1.70	2.93	1.67	2.77	1.54
		(R)-(+)-		(R)-(+)-		(R)-(+)-		(R)-(+)-	
19		4.66	1.61	2.90	1.93	1.85	2.11	2.80	1.65
20		4.30	1.50	2.81	1.70	1.93	1.95	2.32	1.54
21		3.72	1.20	3.09	1.15	2.06	1.00	2.72	1.18

Mobile phase: hexane–2-propanol (95:5, v/v) with 0.1% trifluoroacetic acid; flow-rate: 2.0 ml/min; column dimensions: 250×4.6 mm I.D..

Table 4
Resolution of racemic compounds on CSPs II–V

Compound	Aryl substituent	4-$CH_3C_6H_4$		4-t-Bu-C_6H_4		4-$C_6H_5C_6H_4$		4-$C_6H_5OC_6H_4$	
	CSP	II		III		IV		V	
		k_1'	α	k_1'	α	k_1'	α	k_1'	α
	Structure								
22		6.36[a]	1.13	3.78[a]	1.35	2.71[a]	1.15	4.15[a]	1.00
23		9.77[a]	1.46	3.21[a]	1.49	3.84[a]	1.39	6.00[a]	1.38
24		2.57	1.16	2.28	1.14	1.27	1.06	2.60	1.12
25		4.52	1.32	3.54	1.53	2.50	1.35	3.32	1.31
26		4.26	1.32	5.71	1.21	2.13	1.20	3.56	1.14
27		7.41[b]	1.22	1.54[b]	1.24	1.00[b]	1.29	1.85[b]	1.16

Mobile phase: hexane–2-propanol (95:5, v/v); flow-rate: 2.0 ml/min; column dimensions: 250×4.6 mm I.D..
[a] Mobile phase: hexane–2-propanol (90:10, v/v).
[b] Mobile phase: hexane–2-propanol (75:25, v/v).

on the sorbent prepared from the 4-*tert.*-butylbenzoate of DATD.

The position of the substituent on the aromatic ring has a large effect on both retention and selectivity. Generally, substitution in the 2-position vis-à-vis the 4-position on the aromatic ring leads to depreciating α-values and in some cases to a large increase in retention, Table 5

3.2. Dependence of retention and selectivity on the composition of the mobile phase

The muscle relaxant, baclophen-lactam (**25**), was used as a model compound to study the effect of organic modifiers in the mobile phase on selectivity and retention. Since retention on these CSPs is mainly caused by the hydrogen bonding ability of the

Table 5
The effect of the substitution pattern on retention and selectivity

Aryl substituent	4-$C_6H_5OC_6H_4$		2-$C_6H_5OC_6H_4$	
CSP	V		VI	
	k_1'	α	k_1'	α
Analyte				
Lorazepam	4.19	1.29	5.76	1.41
Mephenytoin	2.60	1.12	2.79	1.00
Baclophenlactam	3.32	1.31	5.31	1.10
Bendroflumethiazide	1.85	1.16	10.26	1.12
Binaphthol	3.56	1.14	7.28	1.06
Bupivacaine	2.80	1.65	5.18	1.32
Ibuprofen	2.58	1.47	2.23	1.00
N-Benzoyl-DL-phenylglycine[a]	0.91	1.16	1.92	1.08

Mobile phase: as in Tables 1–4. Flow-rate: 2.0 ml/min; column dimensions: 250×4.6 mm I.D..

[a] Mobile phase: hexane–2-propanol (90:10, v/v) containing 0.1% TFA.
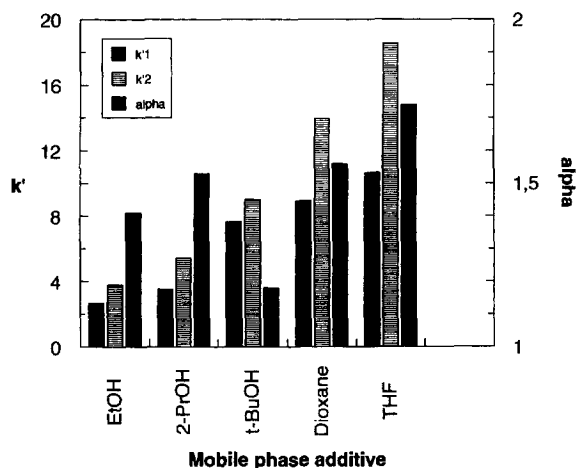
Fig. 3. The effect of various mobile phase additives on retention and enantioseparation of baclophen-lactam on CSP III. All mobile phase systems were hexane-based and contained 5% alkanol or 20% of the ether component. Flow-rate 2.0 ml/min; injected amount 40 μg; UV detection at 225 nm.
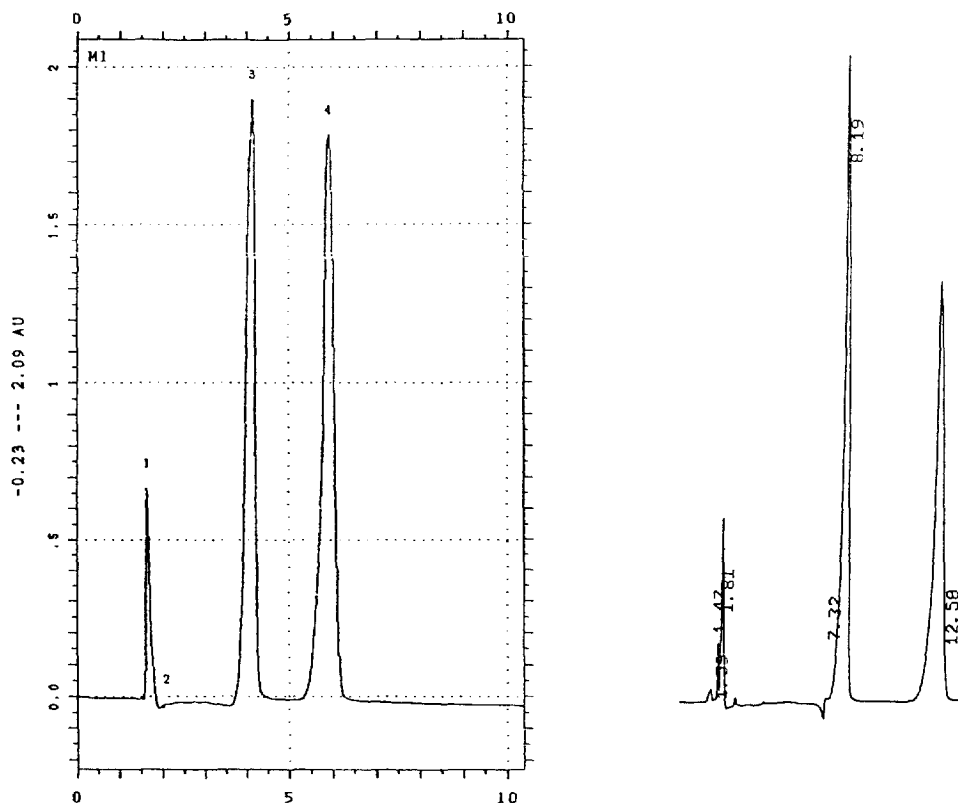
Fig. 4. Direct separation of the enantiomers of naproxen on columns having different densities of the chiral ligand bis(4-tert.-butylbenzoyl)-N,N'-diallyl-L-tartardiamide; (left) 0.44 μmole/m^2, (right) 0.83 μmole/m^2. Mobile phase: hexane–2-propanol (99:1) with 0.1% trifluoroacetic acid, flow-rate 2.0 ml/min; injected amount 50 μg; UV detection at 230 nm.

analyte, addition of a series of alkanols instead of ethers to the mobile phase gives lower retention times and in this case also lower α-values, Fig. 3. Further, k' increases with decreasing polarity of the alkanols used as mobile phase modifiers and a maximum in α-value is observed with 2-propanol in the mobile phase, mainly due to stronger retention of the last eluted enantiomer.

3.3. Influence of ligand density on retention and resolution

Chiral stationary phases based on the 4-*tert.*-butylbenzoate derivative of DATD (CSP III) having a ligand density of 0.44 and 0.83 μmol/m^2 were evaluated with respect to retention, enantioseparation and resolution using *rac*-naproxen (5) as a model compound. Retention decreases with decreasing surface density of the chiral ligand and the separation factor is constant; which means that the interaction existing between the enantiomers and the sorbent is principally chiral, Fig. 4. However, resolution decreases markedly due to a smaller number of theoretical plates on the column with low surface coverage (0.44 μmol/m^2, N=8200/5100; 0.83 μmol/m^2, N=12400/9300; analytes: 1,3-dinitrobenzene/binaphthol).

4. Conclusion

Substitution of the aromatic ring in the *para* position in the monomer unit gives sorbents that display interesting chiral discriminating properties. The most interesting CSP in terms of broad enantio-selectivity was prepared from the 4-*tert.*-butylben-zoate derivative of DATD molecule (CSP III) and showed good resolving power for both acidic, basic and neutral compounds. However, the acidic race-mates were best separated on CSP IV (4-$C_6H_5C_6H_4$). In general, changing the polar component of the mobile phase from an alkanol to an ether leads to higher k'- and α-values due to less competition for the hydrogen binding sites from the mobile phase additive.

Acknowledgments

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