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Doubly tethered tertiary amide selectors

Modified version of Doyle *et al.*'s naproxen chiral stationary phase

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ABSTRACT

The synthesis of an (*S*)-naproxen-derived chiral stationary phase (CSP) that differs from those of Doyle *et al.* [T.D. Doyle, C.A. Brunner and E. Smith, *US Pat.*, 4919 803 (1990) and T.D. Doyle, in W.J. Lough (Editor), *Chiral Liquid Chromatography*, Chapman & Hall, New York, 1989, pp. 102–128] and of Oliveros *et al.* [L. Oliveros, C. Minguillón, B. Desmazières and P. Desbène, *J. Chromatogr.*, 589 (1992) 53 and 606 (1992) 9] is reported. Instead of linking naproxen to a primary amino group in the tether, linkage is to a secondary amino group. This avoids the presence of an amide hydrogen which often serves to increase retention, attenuate enantioselectivity and diminish the efficiency of the CSP. The presently described CSP 2 typically shows improved performance relative to those described by the Doyle *et al.* and Oliveros *et al.*

INTRODUCTION

A naproxen-derived chiral stationary phase, CSP 1 (Fig. 1), initially developed by Doyle *et al.* [1,2], was later described [3] and then modified by Oliveros *et al.* [4]. Like other π-basic CSPs, CSP 1 typically requires that analytes contain π-acidic functionality if it is to afford more than trivial levels of enantioselectivity. Because we often have occasion to separate the enantiomers of π-acidic compounds on a preparative scale, the ease of preparation and the commercial availability of (*S*)-naproxen made CSP 1 attractive even though it typically affords less enantioselectivity than several other π-basic CSPs [5,6].

From our studies of chiral recognition mechanisms, we suspected that several structural features of Doyle *et al.*'s CSP were potentially detrimental to its performance, although they contribute to its ease of preparation. Doyle *et al.* passed (*S*)-carboxyl-activated naproxen through an aminopropyl column to perform an *in situ* modification, a procedure which fails to functionalize all of the amino groups. The presence of residual amino groups was early recognized as undesirable [7]. By joining the chiral selector to the silane tether prior to bonding to silica, one avoids the presence of free amino groups in the CSP. This approach has been used by at least one manufacturer of CSPs for some time, and while less convenient, affords a better CSP. This "pre-assembly" technique was applied by Oliveros *et al.* [4] to Doyle *et al.*'s CSP and found to improve its performance. We had independently prepared a "pre-assembled" naproxen CSP with similar results.

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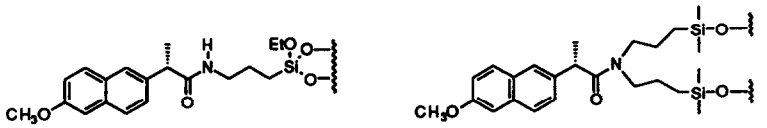


CSP 1 CSP 2

Fig. 1. Structures of the CSPs used in this study.

Experience gained from the chromatography of various naproxen derivatives led us to suspect that the amide N–H in this CSP is unnecessary for chiral recognition and likely to be deleterious to the performance of the CSP. Accordingly, we prepared CSP 2 in which the selector is a tertiary amide of (*S*)-naproxen, thus avoiding the supposedly superfluous N–H. This paper describes the preparation of this CSP and provides some comparative data concerning its performance relative to the naproxen-derived CSPs previously described. To better enable comparisons to be made, all of the CSPs described herein are bonded to the same spherical silica, 5 μm, 100 Å Rexchrom. The comparisons are of mechanistic interest and relevant to the design of other CSPs.

EXPERIMENTAL

General

The analytes used in this comparative study were either available from prior studies or were prepared in the manner described by the original workers [1–3].

^{1}H NMR spectrum was recorded on a Varian XL-200 Fourier transform NMR instrument operating at 200 MHz in the ^{2}H lock mode. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as the internal standard. Elemental analyses were performed by T. McCarthy and associates at the University of Illinois Microanalytical Service Facility. High- and low-resolution mass spectrum analyses were performed by R. Milberg and associates at the University of Illinois Mass Spectrometry Laboratory using fast atom bombardment on either a VG ZAB-SE (low resolution) or a VG 70-SE-4F (high resolution). HPLC analyses were performed using either a Rheodyne injector, Rainin Rabbit Model HPX pump and a Linear UVIS 200 variable-wavelength detector or a Rheodyne injector, Anspec pump and a Milton-Roy UV detector system (254, 280 nm)]. Rotations were measured on a Rudolph Autopol III digital polarimeter, equipped with a 1 dm flow cell operating at 589 nm.

Preparation of CSP 1A

The procedure used was that reported by Doyle *et al.* [1,2], without modifications.

Preparation of CSP 1B

(*S*)-Naproxen (2.30 g, 10 mmol) was suspended in 50 ml of CH_2Cl_2 and 2 ml of oxalyl chloride were added. After 3 h, the solution was evaporated to dryness and the remaining crystalline solid was dried under vacuum for 3 h. The solid was dissolved in dry diethyl ether–CH_2Cl_2 (1:1) and added dropwise to a solution of 1.2 g of triethylamine and 2 g of triethoxyaminopropylsilane in CH_2Cl_2. After 15 min, the reaction mixture was filtered through a sintered glass funnel and the filtrate was added to 5 g of Rexchrom 5 μm 100 Å silica which had been azetropically dried over benzene. The solvents were carefully evaporated, the residue was slurried in CH_2Cl_2, and 1 ml of dry N,N-dimethylformamide was added. The silane–silica mixture was then placed in an evacuated Kugelrohr apparatus at 45–50°C overnight. The resulting bonded phase was slurry packed into a 250 × 4.6 mm stainless-steel column. Elemental analysis of the dried silica remaining after packing showed a loading of 0.20 mmol selector per gram silica (by carbon) and 0.16 mmol selector per gram silica (by nitrogen). Chiral silane recovered from the bonding process had undergone no loss of enantiomeric purity (HPLC analysis on a π-acidic CSP).

PREPARATION OF CSP 2

(S)-Naproxen diallyl amide

(*S*)-Naproxen acid chloride (2.7 g, 10.86 mmol), prepared in a manner similar to that used by Doyle *et al.* [1,2], was placed in a 250-ml round-bottom flask with 100 ml of dry CH_2Cl_2 and cooled in an ice–water bath. Diallylamine (10.55 g, 108.6 mmol) was added slowly with stirring, and the reaction mixture was allowed to stand at room temperature for 2 h. The reaction mixture was washed twice with 100-ml portions of 1 *M* HCl, dried over $MgSO_4$, and the solvent removed by vacuum to afford 3.4 g (100%) of (*S*)-naproxen diallyl amide. The enantiomeric purity of the amide [evaluated using an (*S,S*)-Whelk-O 1 CSP (Regis)] was greater than 98%. ^{1}H NMR: δ 1.5 (d, 3H), 3.55–3.75 (m, 2H), 3.9–4.0 (m, 5H), 4.3–4.4 (dd, 1H), 4.9–5.2 (m, 4H), 5.5–7.5 (m, 2H), 7.1–7.2 (m, 2H), 7.4 (dd, 1H), 7.6–7.7 (m, 3H). Elemental analysis: theory: 77.64% C, 7.49% H, 4.53% N; found: 77.50% C, 7.52% H, 4.49% N.

Hydrosilylation of (S)-naproxen diallyl amide. (*S*)-Naproxen diallyl amide (2.9 g, 9.4 mmol) was placed in a 50-ml round-bottom flask with 15 ml of dry CH_2Cl_2. Dimethylchlorosilane (8.5 g, 89.9 mmol) was added to the reaction mixture in one portion, then followed by addition of *ca.* 5 mg of H_2PtCl_6. The reaction was heated to reflux and checked periodically by TLC. After 8 h, the solvent was evaporated and 15 ml of a solution of diethyl ether–ethanol–triethylamine (1:1:1) was added. The precipitated amine hydrochloride was removed by filtration and the filter cake was washed with diethyl ether. The combined filtrates were evaporated to near dryness and purified by flash chromatography on silica to afford 1.6 g (33%) of the bis-ethoxysilane. Low-resolution mass spectrum (M^+ = 518.4 a.m.u.); high-resolution mass spectrum; [$M^+_{exp.}$] was within 0.0002 a.m.u. of the calculated value.

Bonding the selector to silica to afford CSP 2. The bis-ethoxysilane (1.6 g, 3.09 mmol) was dissolved in 15 ml of dry CH_2Cl_2 and added to 5.0 g of azeotropically dried silica in a 50-ml round-bottom flask. The slurry was then evaporated to near dryness, another 15 ml of dry CH_2Cl_2 were added, and the slurry was again evaporated to near dryness. This was repeated once more with 1.0 ml of dry N,N-dimethylformamide added to the CH_2Cl_2. Following evaporation to near dryness, the silane–silica mixture was placed in a Kugelrohr apparatus and heated to 90–95°C for 24 h under reduced pressure (2 mmHg; 1 mmHg = 133.322 Pa) with gentle mixing. The bonded phase was then slurry packed into a 250 × 4.6 mm stainless-steel HPLC column. Elemental analysis of the thoroughly washed and dried silica packing material showed a loading of 0.22 mmol selector per gram silica (by carbon) and 0.21 mmol selector per gram silica (by nitrogen).

RESULTS AND DISCUSSION

Doyle *et al.* [1,2] prepared CSP 1 by recirculating a mixture of (*S*)-naproxen and a carboxyl activating agent through a commercial aminopropyl column. Any attempt to derivatize γ-aminopropyl silanized silica with a carboxyl-activated carboxylic acid will leave many aminopropyl strands underivatized. Since amino groups interact strongly with many π-acidic groups, any residual amino groups would be expected to increase retention, diminish enantioselectivity, and adversely affect the resolution afforded by any CSP so prepared. The presence of residual amino groups might well account for the addition of acetonitrile to the mobile phase by the original workers [1,2].

From chromatography of the tertiary amide derivatives of chiral acids on CSPs, we knew that they often exhibit greater enantioselectivity than do the corresponding secondary amide derivatives. Derivatives of naproxen and other α-arylpropionic acids fall into this category. Additionally, we have made several tertiary amide CSPs which afford greater enantioselectivities than do the corresponding secondary amide CSPs. Because one need not invoke the carboxamide N–H as an interaction site essential to the chiral recognition *of* naproxen, we suspected that the carboxamide N–H in CSP 1 is not essential for chiral recognition *by* naproxen. Hence, this N–H might lead to interactions with

analytes which increase their retention without distinguishing between enantiomers, thereby reducing enantioselectivity. By coupling carboxyl-activated naproxen to a secondary amine prior to immobilizing this selector on silica, several undesirable features of the original design can be eliminated. Additionally, use of diallylamine as the secondary amine potentially allows the selector to be doubly tethered to silica, thus increasing the robustness of the CSP.

CSP 1A was prepared by pumping an excess of carboxyl-activated (*S*)-naproxen through a commercial (Regis) aminopropyl column as described [1,2]. This column should be similar, but not necessarily identical, to those used by Doyle *et al.* and Oliveros *et al.* owing to differences in the silicas used and in the aminopropyl surface coverage. CSP 1B was prepared by acylation of γ-aminopropyltriethoxysilane with (*S*)-naproxen acid chloride *prior* to bonding the chiral silane to silica. This was done to avoid the presence of residual aminopropyl strands. Again, this CSP should be similar, but not necessarily identical, to the modified CSP of Oliveros *et al.* [4] for the reasons just stated. CSP 2 was made by acylating diallylamine with naproxen acid chloride, followed by hydrosilylation of the resulting olefinic amide precursor of CSP 2 with dimethylchlorosilane. Conversion of the enantiomerically pure bis-chlorosilane to the bis-ethoxysilane (to facilitate purification and characterization) led to the selector which was bonded to silica. The variously modified silicas were each slurry packed into 250 × 4.6 mm stainless-steel columns. The performance of these CSPs (see Table I) was compared with that reported by Doyle *et al.* [1,2] and by Oliveros *et al.* [3] for CSP 1 (first column, Table I).

The data reported (Table I) for CSP 1 by Oliveros *et al.* [3] (the first three entries), and by Doyle *et al.* [1,2] (the fourth entry) make it evident that the prior versions of CSP 1 generally afford longer retention and less enantioselectivity than is presently observed for CSP 1A. This possibly results from differences in the three types of aminopropyl silica used (J.T. Baker [1,2], Nucleosil [3] and Rexchrom). Consistent with the observations of the Oliveros *et al.* [4], CSP 1B affords less retention and greater enantioselectivity than does CSP 1A. The retention and enantioselectivities afforded by our CSP 1B correspond almost exactly to these reported by Oliveros *et al.* for their CSP 1B. The expectation that elimination of the amide N–H would be beneficial is borne out by the reduced retention, the increased enantioselectivity, and the greater resolution usually afforded by CSP 2 relative to those afforded by the other CSPs (see Table II).

For the last two entries in Table II, the levels of enantioselectivity afforded by CSP 2 are substantially greater than those afforded by the other CSPs, thus making it an attractive phase for the preparative resolution of these and related analytes. These greater enantioselectivities arise, to some extent, from the elimination of interactions which increase retention without distinguishing between enantiomers. Moreover, the extent of solvation of the selectors and their conformational preferences will be influenced by the manner of tethering. These factors also affect enantioselectivity and will influence the kinetics of adsorption and desorption in ways neither yet fully understood nor utilized to best advantage.

Oliveros *et al.* note that endcapping a CSP sometimes significantly improves enantioselectivity and sometimes has little effect. They speculate that this may be related to differences in the silicas used [4]. In our experience, endcapping leads to the greatest gains in enantioselectivity for those CSPs having low surface coverages by the chiral silane. High surface coverages reduce the number of silanol groups which remain to be endcapped. Consequently, endcapping causes only a modest change in the performance of a CSP having a high initial level of surface coverage.

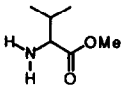
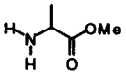
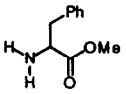
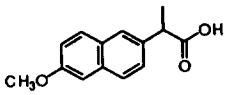
CONCLUSIONS

There are doubtless other π-basic carboxylic acids which, when immobilized, will afford CSPs of greater enantioselectivity than does naproxen. Even so, naproxen is conveniently available and can be incorporated into a CSP which affords levels of enantioselectivity adequate for many applications, including preparative separations. By omitting an unnecessary polar site from the selector, one can often improve the performance

TABLE I

COMPARISON OF NAPROXEN-DERIVED CSPs

The same chromatographic conditions were used for all CSPs. For the first three analytes, the conditions were: chloroform–(0.5% methanol in heptane) (70:30) at 1.0 ml/min flow-rate, as reported [3]. For the last analyte, the conditions were: hexane–2-propanol (60:40) at 0.5 ml/min flow-rate [1,2] ($T = 20 \pm 2$°C). Void volumes were measured using 1,3,5-tri-*tert.*-butylbenzene (TTBB).

	CSP 1		CSP 1A		CSP 1B[a]		CSP 2		Ref.
	k_1' [b]	α	k_1' [b]	α	k_1' [b]	α	k_1' [b]	α	
3,5-Dinitrobenzamide of:									
	1.37 (*S*)	1.51	1.09 (*S*)	1.66	0.91 [1.00] (*S*)	1.84 [1.84]	0.56 (*S*)	3.11	3
	5.53 (*S*)	1.34	3.84 (*S*)	1.50	2.61 [3.00] (*S*)	1.69 [1.76]	1.60 (*S*)	2.47	3
	2.37 (*S*)	1.51	1.65 (*S*)	1.67	1.21 [1.29] (*S*)	1.83 [1.89]	0.74 (*S*)	2.30	3
3,5-Dinitroanilide of:									
	5.4 (*R*)	1.21	3.44 (*R*)	1.14	2.23 (*R*)	1.24	2.88 (*R*)	1.19	1, 2

[a] Values in brackets correspond to data reported in ref. 4.
[b] Absolute configuration of the least retained enantiomer.

of a CSP. This is the case for the present naproxen-derived CSP 2. Additional differences in the behavior of CSPs 1B and 2 and the mechanistic origins of these differences will be addressed subsequently.

In response to one reviewer's question regarding the origin of the much increased enantioselectivity afforded by CSP 2 for the last two entries in Table II, we can but *speculate*. Similar but less dramatic reductions of retention and enhancement of enantioselectivity are observed when these analytes are chromatographed on another naproxen-derived tertiary amide CSP (*i.e.* N–CH_3 instead of N–H). NMR studies of analyte–selector combinations are underway in the hope that a better understanding of the details of these recognition processes can be so attained.

It is known that the mode of attachment to silica can play an important role in determining retention and enantioselectivity. It is not unreasonable to think that, by virtue of the fact that the selector is doubly tethered, the spacing between the selector strands are altered. This could affect enantioselectivity in a manner expected to be rather analyte dependent.

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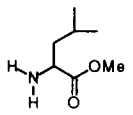
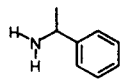
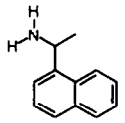
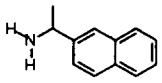
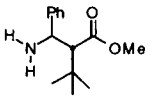
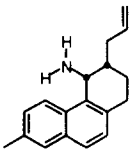
TABLE II

SEPARATION OF SOME π-ACIDIC ENANTIOMERS ON NAPROXEN-DERIVED CSPs

Conditions: 2-propanol–hexane (20:80) at 2.0 ml/min flow-rate. Void volumes measured with TTBB.

3,5-Dinitrobenzamide of:	CSP 1A			CSP 1B			CSP 2		
	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
	5.57	9.93	1.78	5.17	10.78	2.09	4.06	7.26	1.79
	9.50 (*S*)	10.83	1.14	8.44 (*S*)	10.51	1.25	8.99 (*S*)	11.09	1.23
	14.20 (*S*)	16.90	1.19	11.20 (*S*)	15.31	1.37	10.67 (*S*)	19.87	1.86
	11.77 (*S*)	13.97	1.19	9.58 (*S*)	12.73	1.33	11.83 (*S*)	14.81	1.25
	9.87	20.93	2.12	8.68	21.27	2.45	4.12	27.49	6.67
	15.53	25.40	1.64	14.46	24.03	1.66	7.95	46.55	5.86

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