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Short communication

Improved chiral stationary phase for β-blocker enantioseparations

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Abstract

The previously described α-Burke 1 chiral stationary phase (CSP) was designed for the chromatographic separation of the enantiomers of β-blockers. Difficulties with the reproducibility of the free radical addition reaction, used in the attachment of the chiral selector to the chromatographic support, have required the development of an alternative silane immobilization process (α-Burke 2 CSP). While the enantioselectivity afforded by this new CSP is generally equivalent to that of the original CSP, the α-Burke 2 CSP demonstrates longer analyte retention, necessitating the use of mobile phases of greater eluotropic strength. The increased retention of the new CSP presumably results from a greater surface density of functional selectors, an interpretation which is supported by the observation that the preparative capacity of the α-Burke 2 CSP is greater than that of the original. Some of the factors influencing the retention and separation of a group of 23 β-blockers on the α-Burke 2 CSP are discussed.

1. Introduction

Two fundamentally different immobilization strategies have been employed in the production of brush-type chiral stationary phases (CSPs). The most straightforward approach involves the preparation of a silica-reactive chiral selector (usually an alkoxysilane) followed by the "one-step" immobilization of this selector onto a silica surface (Fig. 1a). A more roundabout "two-step" bonding approach is also frequently employed. With this technique the silica surface is first functionalized with a heterobifunctional silane reagent to afford a reactive stationary phase which is then allowed to react with the chiral selector to afford the CSP (Fig. 1b). Many early CSPs, including the original DNB-phenylglycine "Pirkle column" [1] were made using a "two-step" bonding approach. It has long been realized that the two-step bonding approach, while often convenient, has a number of attendant problems [2].

Perhaps the best recognized of these problems is that CSPs produced via a two-step bonding approach almost always contain residual reactive groups which can function as sites for non-enantioselective retention [3] and lead to a decrease in the enantioselectivity of the column [4]. In addition, these residual reactive groups can be the sites for irreversible reaction with analytes, matrix components, or other entities injected onto the column, resulting in a column which changes properties over time.

Several years ago Pirkle and Burke [5,6]

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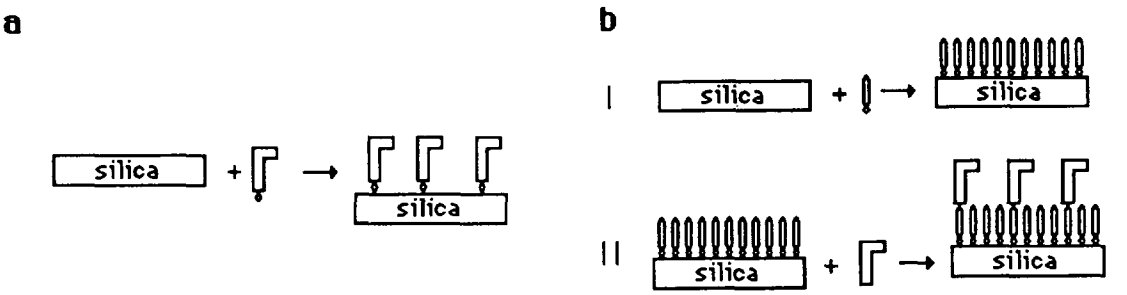

Fig. 1. Comparison of "one-step" and "two-step" approaches to CSP synthesis. (a) In the one-step approach a silica surface is allowed to react with a silica-reactive chiral selector to afford the CSP. (b) In the two-step approach, the silica surface is first allowed to react with a heterobifunctional reagent to afford a reactive stationary phase (step I). In step II, the reactive stationary phase is allowed to react with the chiral selector to afford the CSP.

reported the preparation of a new chiral stationary phase designed specifically for the chromatographic separation of the enantiomers of β-blockers. This column, subsequently commercialized by Regis as the α-Burke 1 [7], has proven to be very useful for the separation of the enantiomers of β-blockers and other analytes [8]. The two-step synthetic protocol used in the production of the α-Burke 1 CSP is illustrated in Fig. 2. Following preparation of mercaptopropyl silica, the enantiopure olefin is allowed to react with thiopropyl silica in the presence of the free radical initiator azobisisobutyronitrile (AIBN) to afford the thioether-linked CSP. This two-step free radical immobilization approach is widely used, being originally applied to the immobilization of alkaloids of the quinine family by Rosini et al. [9], and subsequently routinely used in the immobilization of 3,5-dinitrobenzoyl amino acid derivatives and other compounds by Tambuté and co-workers [10,11]. Pirkle and Burke opted to use this two-step bonding approach following failure of conventional hydrosilyation of the olefin with trichlorosilane [12].

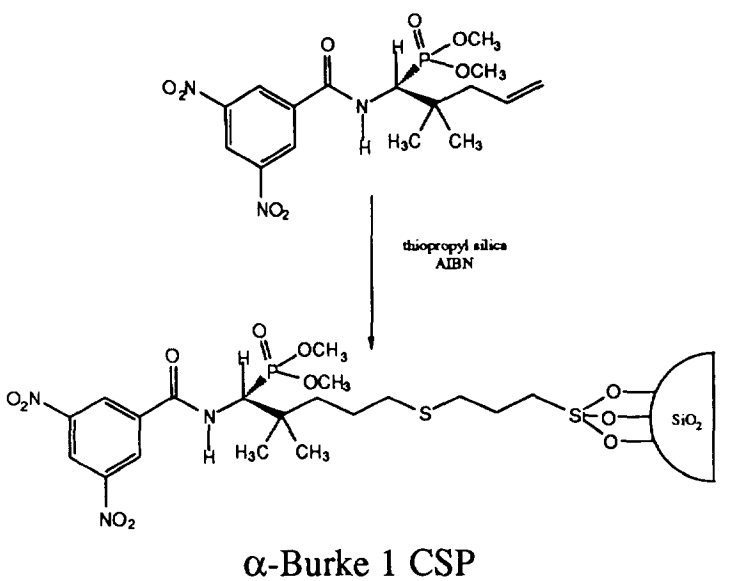

Fig. 2. Preparation of α-Burke 1 CSP. Reaction of enantiopure olefin with thiopropyl silica in the presence of the free radical initiator, AIBN, affords the thioether-linked α-Burke 1 CSP.

Apart from the general problems associated with two-step CSP production, the free radical thioether immobilization method has several additional disadvantages. First, the thiopropyl surface itself is redox active, can be oxidized to form disulfide linkages, and is potentially reactive in redox reactions with analytes, matrix components or metal ions. Second, the reaction of a thiol radical with an unactivated olefin is generally a poor reaction. For example, in the reaction of the olefin precursor with thiopropyltriethoxysilane, a complex mixture of products is obtained with a very low yield of the desired silane reagent [12].

Considering all of these disadvantages, it is perhaps not too surprising that we have experienced some difficulties in producing this CSP in a reproducible manner. After considerable expenditure of time and resources to develop conditions for reproducible preparation of the α-Burke 1 CSP, we chose to reinvestigate the hydrosilylation of the olefin precursor. Like Pirkle and Burke, we were also unable to effect hydrosilyation using trichlorosilane. However, hydrosilylation with dimethylchlorosilane gave no such problem, and the corresponding CSP (α-Burke 2) was produced using conventional one-step methods (Fig. 3).

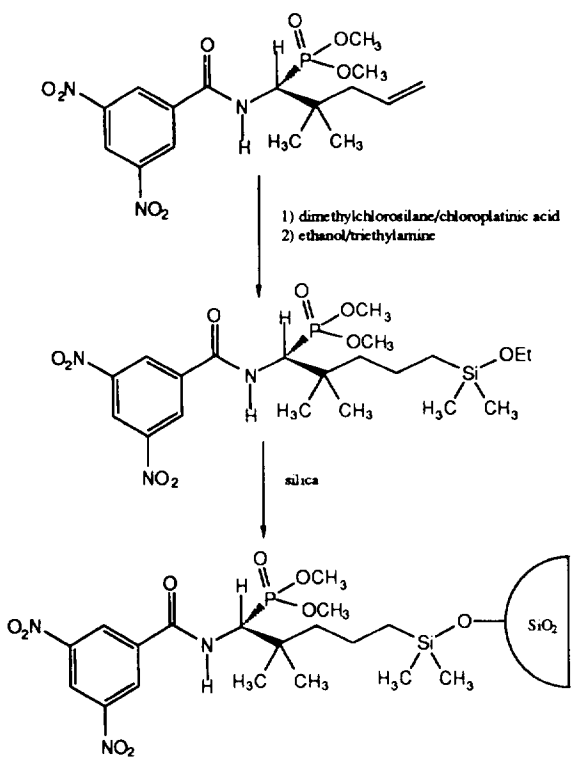

Fig. 3. Hydrosilyation of the enantiopure olefin with dimethylchlorosilane and chloroplatinic acid, followed by conventional immobilization of the resulting silane on silica, affords the silane-linked α-Burke 2 CSP.

A comparison of the separation of the enantiomers of the β-blockers metoprolol and bufuralol was undertaken on the two CSPs. In addition, factors influencing the separation of the enantiomers of a group of 23 β-blockers (Fig. 4) on the α-Burke 2 CSP was studied.

2. Experimental

2.1. Apparatus

Chromatographic analysis was performed using a Kratos Spectroflow 400 pump, a Rheodyne Model 7125 injector fitted with either a 2-ml or a 20-μl sample loop, a Kratos Spectroflow 757 variable-wavelength absorbance monitor, and a Hewlett-Packard HP 3394 integrating recorder.

2.2. Materials

The (*R*)-α-Burke 1 CSP was obtained from Regis Technologies, Morton Grove, IL, USA. The α-Burke 2 CSPs were prepared by conventional methods following the procedure outlined in Fig. 3. Ammonium acetate and ammonium formate were obtained from Aldrich, Milwaukee, WI, USA. HPLC solvents were HPLC grade. Variable-temperature column jacket was obtained from Aura Industries, Staten Island, NY, USA.

β-Blocker samples were available from previous studies, with many samples kindly donated by Dr. Joe Gal, University of Colorado School of Medicine, Denver, CO, USA. Column void time was measured by injection of 1,3,5-tri-*tert.*-butylbenzene.

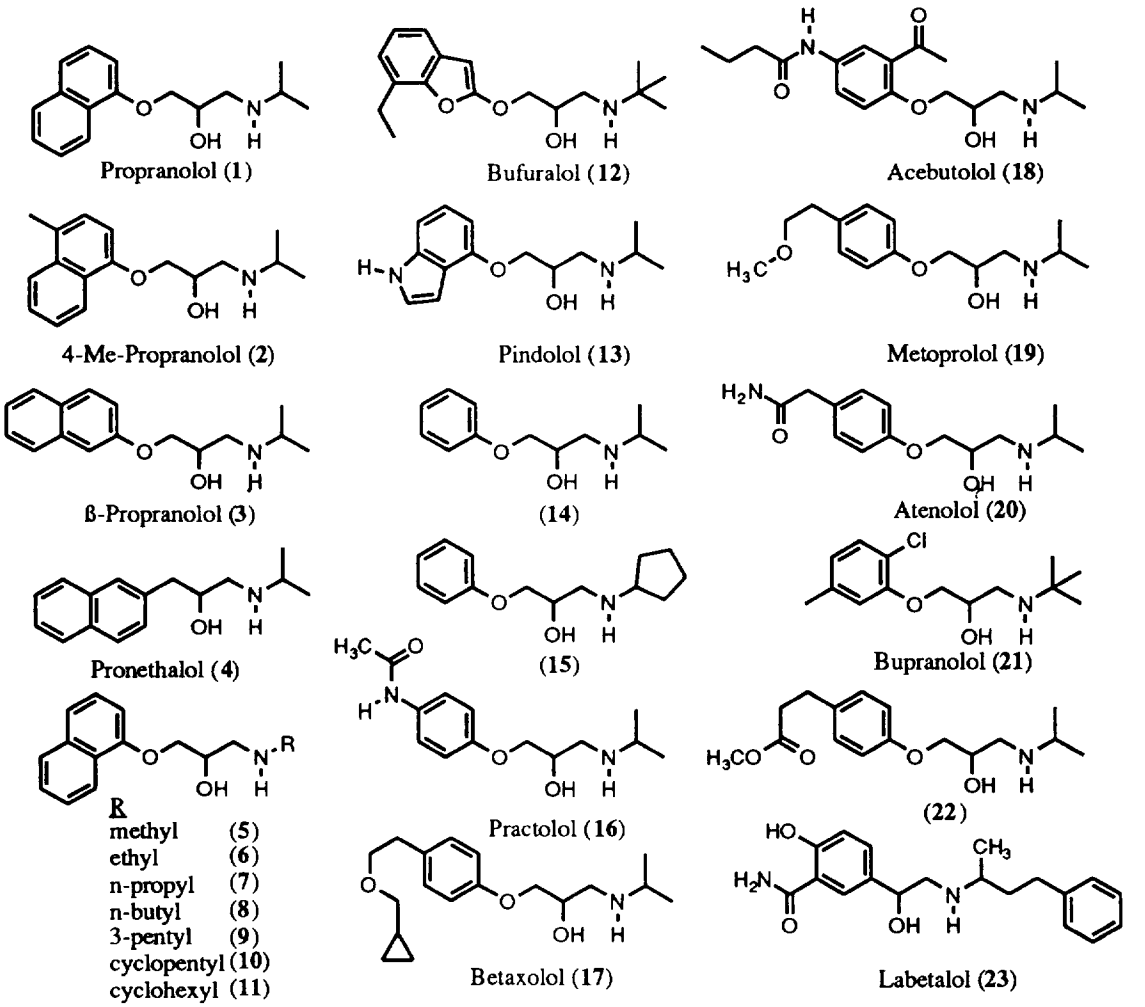

Fig. 4. β-Blocker analytes used in the study.

3. Results and discussion

A comparison of the separation of the enantiomers of the representative β-blockers, metoprolol and bufuralol, on the two CSPs reveals that the silane-linked α-Burke 2 CSP affords enantioselectivities which are more or less equivalent with those obtained with the original α-Burke 1 CSP (Table 1). The retention factors are appreciably greater with the α-Burke 2 CSP, an outcome which is initially somewhat surprising for a CSP in which sites for non-specific adsorption have presumably been eliminated. However, it has often been shown that changes in the length or chemical makeup of CSP tethers can influence both enantioselectivity and retention [2]. In addition, a simpler explanation may obtain in the present case: the greater retention of the α-Burke 2 CSP could also result from a denser population of functional chiral selectors (a higher phase ratio).

A comparison of the preparative capacity of the two CSPs in the separation of propranolol enantiomers (Fig. 5) shows that the (*R*)-α-Burke 2 column can indeed tolerate a significantly higher sample load than the thioether-linked CSP. This result is consistent with the (*R*)-α-Burke 2 having a denser coverage of functional chiral selectors. It must be noted that the preparative capacity of a stationary phase also

Table 1
Comparison of the separation of some β-blocker enantiomers on (*R*)-α-Burke 1 and (*R*)-α-Burke 2

Compound	(*R*)-α-Burke 1				(*R*)-α-Burke 2			
	k_1'	k_2'	α	R_s	k_1'	k_2'	α	R_s
Metoprolol	2.81	3.42	1.22	1.93	7.35	9.02	1.23	2.45
Bufuralol	3.21	6.59	2.05	7.37	7.36	14.86	2.02	8.32

Mobile phase, ethanol–dichloromethane (10:90) with 10 m*M* ammonium acetate; flow-rate, 2 ml/min; detection, UV at 280 nm. k_1' = Retention factor of first-eluted enantiomer; k_2' = retention factor of second-eluted enantiomer.

depends upon the retention. Nevertheless, changing to a mobile phase which affords greater retention on the (*R*)-α-Burke 1 CSP has only a marginal effect on improving the loading capacity.

The increased retention of the (*R*)-α-Burke 2 CSP requires the use of mobile phases of greater eluotropic strength to afford more convenient retention times. Therefore, a survey of the influence of mobile phase composition on the retention and enantioselectivity of the representative β-blockers, metoprolol and bufuralol, was undertaken. The results presented in Table 2 show a rather dramatic decrease in enantioselectivity with increasing concentrations of ethanol. Interestingly, the retention factor exhibits a striking non-linear dependence on ethanol concentration (Fig. 6). Substitution of methanol for ethanol results in only a marginal decrease in retention, which is accompanied by a marked

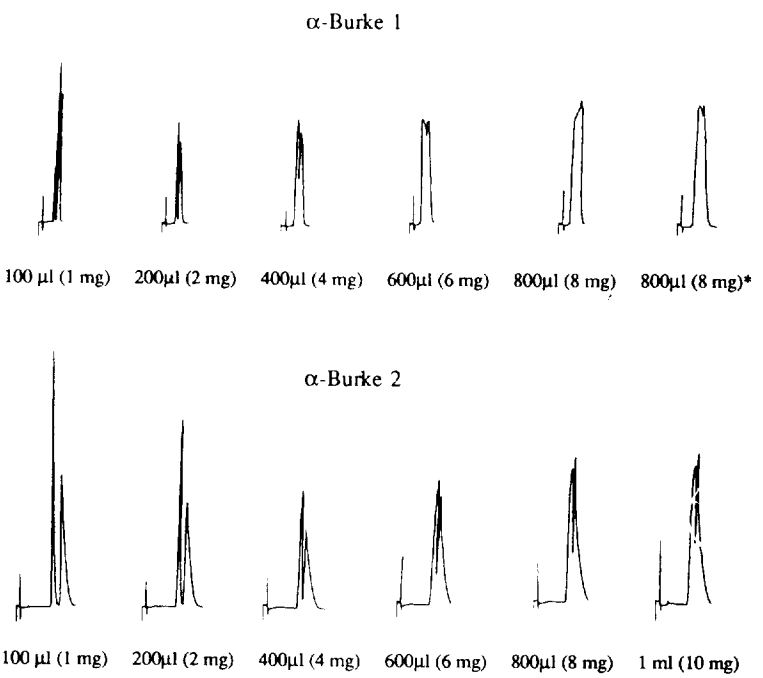

Fig. 5. Preparative separation of propranolol enantiomers on analytical (25 cm × 4.6 mm) (*R*)-α-Burke 1 and (*R*)-α-Burke 2 CSPs. Conditions: mobile phase, ethanol–dichloromethane (10:90) with 10 m*M* ammonium acetate; flow-rate, 2.00 ml/min; detection, UV at 325 nm; sample, propranolol, 10 mg/ml in ethanol. * Mobile phase, ethanol–dichloromethane (5:95) with 10 m*M* ammonium acetate.

Table 2
Effect of mobile phase composition on the separation of metoprolol and bufuralol enantiomers on (*S*)-α-Burke 2 CSP (2 ml/min; UV 280 nm)

Mobile phase[a]	Metoprolol				Bufuralol			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
Ethanol–dichloromethane (5:95)	7.14	8.95	1.25	2.91	6.84	15.89	2.32	9.35
Ethanol–dichloromethane (10:90)	4.98	6.15	1.23	2.45	5.12	11.01	2.15	8.63
Ethanol–dichloromethane (20:80)	4.66	5.61	1.20	2.08	4.98	9.56	1.94	7.92
Ethanol–dichloromethane (40:60)	5.25	6.18	1.18	2.02	5.63	10.14	1.80	7.05
Methanol	9.85	10.30	1.05	0.94	–	–	–	–
Methanol–dichloromethane (1:1)	4.30	4.67	1.09	1.36	4.82	6.23	1.29	4.23
Isopropanol–dichloromethane (1:1)	7.19	8.53	1.19	1.34	6.67	12.57	1.88	5.84
Ethanol–acetonitrile (1:1)	7.24	8.04	1.11	1.55	6.92	9.32	1.35	4.07

[a]All mobile phases contain 10 m*M* ammonium acetate.

decrease in enantioselectivity. Consistent with previous studies [7], the enantioselectivity afforded by isopropanol-based mobile phase is marginally greater than that afforded by ethanol-based mobile phases. However, the much greater efficiency provided by the ethanol-based mobile phase results in significantly greater resolution.

Increasing environmental concerns about the use of halogenated chromatographic solvents prompted the investigation of some non-halogenated co-solvents. Although the acetonitrile-based mobile phase affords significantly less enantioselectivity and resolution than the dichloromethane-based mobile phase, the resolution provided is sufficient for baseline resolution of many β-blockers. Methyl *tert.*-butyl ether (MTBE) was also evaluated as a co-solvent, but

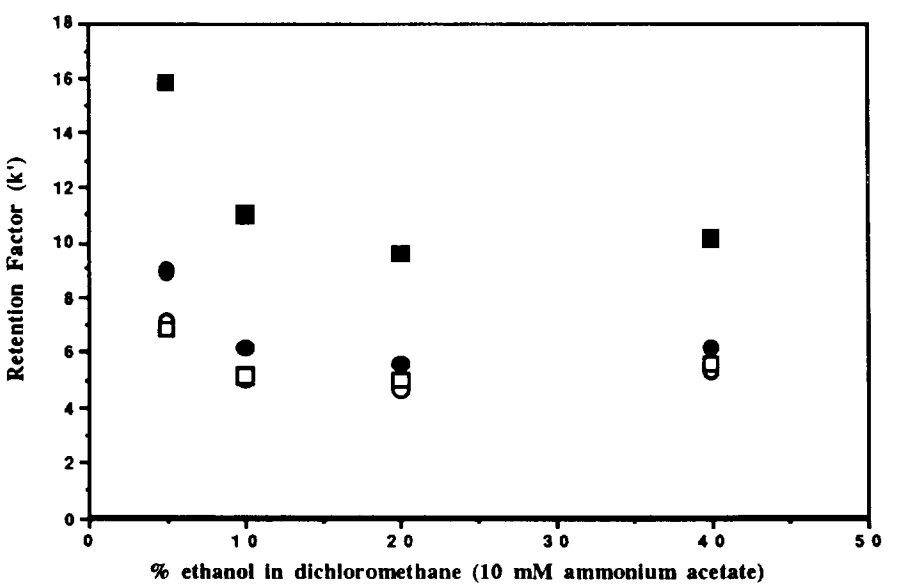

Fig. 6. Influence of ethanol content on the retention of metoprolol (○, ●) and bufuralol (□, ■) enantiomers on (*S*) α-Burke 2 CSP. ○, □ = k' of first-eluted enantiomer; ●, ■ = k' of second-eluted enantiomer.

found to afford an even greater decrease in enantioselectivity and resolution. In addition, MTBE has a poor ability to solubilize the required ammonium acetate modifier.

As noted by Pirkle and Burke, an effective way to control retention is to adjust the concentration of the polar modifier [6]. They found that increasing concentrations of ammonium acetate caused a decrease in retention factor, with little effect on separation factor. We also found that increasing salt concentration decreases retention. However, there is an accompanying decrease in separation factor, which is more evident for the analytes which are well resolved (Table 3). The use of ammonium acetate in these studies should not be taken as an indication that this is the optimum polar modifier. For example, Perrin found that the use of ammonium formate has a greater influence on decreasing retention, on a per mole basis, than does ammonium acetate [7]. In addition, a range of other salts have been used with good effect in a related system, sometimes affording significantly enhanced separations [13]. The separation of β-blocker enantiomers on the α-Burke 2 CSP appears to be dramatically influenced by both the nature and concentration of salt additives, and may be of importance to researchers trying to optimize individual separations.

The separation of the enantiomers of a group of 23 β-blockers (Fig. 4) was studied on (*R*)-α-Burke 2 CSP using both a dichloromethane-based and an acetonitrile-based mobile phase (Table 4). As with the work of Pirkle and Burke, enantioselectivity appears to be best for those analytes bearing the most π-basic aryl groups (compounds 1–13). Analytes bearing sites for strong non-selective interactions with the CSP (compounds 16, 18, 20, 23) are resolved most poorly, and are strongly retained, presumably owing to non-specific adsorption.

Most of the analytes shown in Table 4 are baseline resolved under the specified conditions. For those which are not, a change in mobile phase composition or column temperature may be helpful. The unusual influence of temperature on β-blocker enantioseparations found by Pirkle and Burke using the α-Burke 1 CSP is also observed with α-Burke 2 CSP. For β-blockers, a reduction in column temperature generally affords decreased retention and increased enantioselectivity on this column. For example, decreasing column temperature from room temperature to 0°C decreases the retention of analyte 22 by about one half, while the resolution improves from 1.35 to 1.60. Further decreases in column temperature oftentimes provide even greater improvements.

4. Conclusions

While the enantioselectivity afforded by the α-Burke 2 CSP is generally equivalent with that of the original α-Burke 1 CSP, the new CSP affords greater retention, necessitating the use of mobile phases of greater eluotropic strength. The increased retention of α-Burke 2 CSP is believed to result from a greater surface density of functional selectors, an interpretation supported by the observation that the preparative capacity of the new CSP is greater than that of

Table 3
Effect of ammonium acetate concentration on the separation of some β-blocker enantiomers on (*R*)-α-Burke 2

Compound	10 m*M* Ammonium acetate				20 m*M* Ammonium acetate			
	k_1'	k_2'	α	R_s	k_1'	k_2'	α	R_s
Metoprolol	7.35	9.02	1.23	2.45	2.40	2.94	1.23	2.02
Bufuralol	7.36	14.86	2.02	8.32	2.77	4.71	1.70	5.97

Mobile phase, ethanol–dichloromethane (10:90) with 10 or 20 m*M* ammonium acetate; flow-rate, 2 ml/min; detection, UV at 280 nm.

Table 4
Separation of some β-blocker enantiomers on (*R*)-α-Burke 2 CSP using two different mobile phases (2 ml/min; UV 280 nm)

Compound[a]	Ethanol–dichloromethane (10:90) with 20 m*M* ammonium acetate				Ethanol–acetonitrile (20:80) with 12 m*M* ammonium formate			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
1	4.09	5.59	1.37	3.27	8.60	10.45	1.22	3.31
2	4.36	6.26	1.44	3.73	10.74	13.48	1.26	3.93
3	3.63	4.22	1.16	1.60	7.28	7.94	1.09	1.46
4	4.30	4.98	1.16	1.59	8.24	8.50	1.03	0.58
5	7.77	9.94	1.28	1.95	11.83	13.97	1.18	1.91
6	5.48	7.40	1.35	3.03	9.91	11.99	1.21	2.94
7	3.68	4.97	1.35	2.95	8.42	10.08	1.20	2.92
8	3.01	4.05	1.35	2.40	7.55	9.06	1.20	2.88
9	11.90	2.29	1.21	1.82	5.63	6.41	1.14	2.13
10	1.89	2.28	1.21	1.82	5.63	6.41	1.14	2.10
11	2.81	3.77	1.34	3.07	8.67	10.45	1.21	2.94
12	2.77	4.71	1.70	5.97	5.52	7.32	1.33	4.34
13	1.66	16.11	1.38	3.30	9.04	11.00	1.22	3.30
14	3.05	3.49	1.14	1.56	5.09	5.52	1.08	1.18
15	2.45	2.96	1.21	2.08	5.43	5.92	1.09	1.37
16	8.02	8.89	1.11	0.91	7.83	8.45	1.08	1.12
17	2.04	2.41	1.18	1.48	4.63	5.10	1.10	1.57
18	5.81	6.34	1.09	0.96	6.50	7.03	1.08	1.07
19	2.40	2.94	1.23	2.02	4.99	5.50	1.10	1.56
20	12.43	13.77	1.11	1.11	8.86	9.54	1.08	1.19
21	2.55	3.16	1.24	2.03	5.42	6.23	1.15	2.28
22	2.48	2.97	1.20	1.35	4.65	5.13	1.10	1.68
23[b]	4.44	5.16	1.16	1.14	7.01	8.06	1.15	0.82

[a] See Fig. 4.
[b] Only two peaks were obtained for this separation (diastereomers were not resolved).

the original. Some factors influencing retention and enantioselectivity have been explored, and conditions for baseline resolution of a number of β-blockers have been provided.

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References

[1] W.H. Pirkle, D.W. House and J.M. Finn, *J. Chromatogr.*, 192 (1980) 143–158.
[2] C.J. Welch, *J. Chromatogr. A*, 666 (1994) 3–26.
[3] W.H. Pirkle and C.J. Welch, *J. Chromatogr.*, 589 (1992) 45–51.
[4] W.H. Pirkle, P.S. Spence, B. Lamm and C.J. Welch, *J. Chromatogr. A*, 659 (1994) 69–74.
[5] W.H. Pirkle and J.A. Burke, *Chirality*, 1 (1989) 57–62.
[6] W.H. Pirkle and J.A. Burke, *J. Chromatogr.*, 557 (1991) 173–185.
[7] S.R. Perrin, in C.M. Riley, W.J. Lough and I.W. Wainer (Editors), *Pharmaceutical and Biomedical Applications of Liquid Chromatography*, Vol. 1, Elsevier, Oxford, 1994, Ch. 4.
[8] S. Caccamese, G. Principato, U. Gruss, G. Hägele and S. Failla, *Tetrahedron Asymm.*, 5 (1994) 141–148.
[9] C. Rosini, C. Bertucci, D. Pini, P.Altemura and P. Salvadori, *Tetrahedron Lett.*, (1985) 3361–3364.
[10] A. Tambuté, A. Bégos, M. Lienne, P. Macaudière, M. Caude and R. Rosset, *New J. Chem.*, 13 (1989) 625–637.
[11] A. Tambuté, L. Siret, M. Caude, A. Bégos and R. Rosset, *Chirality*, 2 (1990) 106–119.
[12] W.H. Pirkle, personal communication.
[13] W.H. Pirkle and W.J. Lee, unpublished results, 1994.