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Separation of the Enantiomers of β-Blockers Using Brush Type Chiral Stationary Phase Derived from Conformationally Rigid α-Amino β-Lactam†

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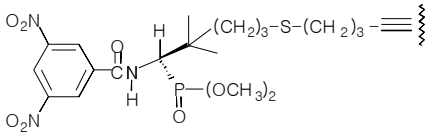
A brush type chiral stationary phase (CSP **2**) derived from α-amino β-lactam was prepared for the separation of the enantiomers of β-blockers. Compared to the CSP derived from α-amino phosphonate (CSP **1**), in general, the conformationally rigid CSP **2** showed greater scope and much enhanced enantioselectivity for the resolution of β-blockers. The effect of various salt additives on enantioseparation of β-blockers in the mobile phase was investigated. The unusual effect of temperature on the chromatographic behaviors was observed on CSP **2**. It also afforded appreciable increases in enantioselectivity without significantly affecting resolution, as the column temperature was reduced.

Key Words: Enantiomer separation, β-Blockers, Chiral stationary phase

Introduction

The β-blockers, widely used in the treatment of cardiac arrhythmias and hypertension as the adrenergic agonist, possess the arylpropanolamine structure containing a chiral center.[1] The (*S*)-enantiomers of β-blockers are 50 - 500 fold more biologically active than the (*R*)-enantiomers and may differ also in the nature of the physiological responses.[2] Owing to their importance, methods for separating β-blocker enantiomers and for determining their enantiomeric purity have been of considerable current interest.[3] Therefore, several liquid chromatographic chiral stationary phases (CSPs) derived from small molecules,[4,5] polysaccharides,[6] macrocyclic antibiotics[7] and chiral 18-crown-6 ethers[8,9] have been developed and applied to separate the enantiomers of β-blockers.[1,3] In terms of chiral recognition mechanism rationale, however, most of those have been developed empirically with no particular design being given to targeting β-blockers. Among several brush type CSPs, CSP **1** derived from an α-amino phosphate (Figure 1), is an exception, being developed with the aid of a mechanistic hypothesis specifically for β-blockers.[4,5] CSP **1** does indeed suffice to separate the enantiomers of a variety of β-blockers with performance adequate for both analytical and preparative applications.[4,5] Even so, it was suspected that a conformationally more rigid CSP using the same mechanistic principles might be even more selective. Most β-blockers of arylpropanolamine structure are conformationally somewhat "floppy" and it was reasoned that "floppiness" in the chiral selector ought to be avoided if the diastereomeric adsorbates are to differ significantly in their stabilities. In this study, we report a new conformationally rigid CSP derived from α-amino β-lactam (CSP **2**) for the separation of the enantiomers of β-blockers and compare the chromatographic efficiencies of CSP **2** with those of the conformationally less rigid CSP **1** derived from an α-amino phosphate.



(*R*) **CSP 1**

Figure 1. The structure of CSP **1** derived from α-amino phosphate.

†This paper is dedicated to Professor Sunggak Kim on the occasion of his honorable retirement.

Experimental

Apparatus. Chromatographic analysis was performed using an Anspec isocratic HPLC pump or a Rainin HPX solvent delivery system and pressure monitor, a Rheodyne 7125 injector with a 20 μL sample loop, a variable-wavelength UV detector, Linear UVIS 200 and an HP 3394A recording integrator. All ^{1}H NMR spectra were recorded on a Varian XL-200 relative to internal tetramethylsilane. Low resolution mass spectra were obtained on a Varian MAT CH-5 mass spectrometer with 70 eV electron impact ionization or a ZAB-SE mass spectrometer with fast atom bombardment. High resolution mass spectra were obtained on a Varian 731 or a 70 SE-4F mass spectrometer. Elemental analyses were performed by the University of Illinois microanalytical service.

Preparation of CSP 2. CSP **2** derived from α-amino-β-lactam was prepared according to conventional methods in Figure 2.[10]

***cis*-4-Phenyl-3-phthalimido-1-prop-2-enyl-2-azetidinone (3):** ^{1}H NMR ($CDCl_3$) δ 3.77 (dd, J = 15.2 and 7.0 Hz, 1H), 4.43 (dd, J = 15.4 and 5.2 Hz, 1H), 5.37 (d, J = 5.6 Hz, 1H), 5.21-5.30 (m, 2H), 5.54 (d, J = 5.6 Hz, 1H), 5.80-6.00 (m, 1H), 7.1-7.3 (m, 5H), 7.6-7.75 (m, 4H) ; IR (KBr, cm^{-1}): 1763, 1721, 1644, 1389, 990, 926.; mp 181.5-182.5 °C ; Mass spectrum: *m*/*z* (relative intensity) 332(2.8), 249 (58.0), 185 (99.5), 146 (100), 104 (67.6); Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.28: H, 4.85: N, 8.43 Found: C, 72.10: H, 4.82: N, 8.39; high-resolution mass spectrum: calculated for $C_{20}H_{16}N_2O_3$: 332.1161. Found : 332.1158.

***cis*-3-Amino-4-phenyl-1-prop-2-enyl-2-azetidinone:** ^{1}H NMR ($CDCl_3$) δ 1.16 (br. s, 2H), 3.47 (dd, J = 15.5 and 7.0 Hz, 1H),

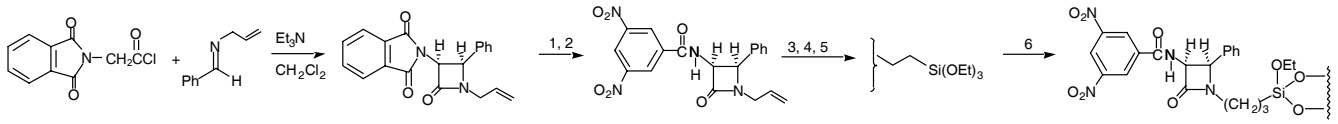


Figure 2. Synthetic procedure for the preparation of CSP **2**. (1) NH_2 NH_2, EtOH, reflux (2) 3,5-dinitrobenzoyl chloride, Et_3N in CH_2Cl_2 (3) resolution on (L)-*N*-(1-naphthyl)leucine(siloxyundecylester) column [11] (4) $HSiCl_3$, cat. H_2PtCl_6, reflux (5) Et_3N, EtOH (6) 5 μm silica gel in vacuum.

4.24 (dd, *J* = 15.3 and 5.1 Hz, 1H), 4.48 (d, *J* = 5.2 Hz, 1H), 4.84 (d, *J* = 5.0 Hz, 1H), 5.07-5.18 (m, 2H), 5.65-5.85 (m, 1H), 7.20-7.48 (m, 5H) ; IR (neat, cm^{-1}): 3389, 3326, 1750, 930, 739.

***cis-(N*-3,5-Dinitrobenzoyl)-3-amino-4-phenyl-1-prop-2-enyl-2-azetidinone (4):** ^{1}H NMR ($CDCl_3$) δ 3.68 (dd, *J* = 15.6 and 7.0 Hz, 1H), 4.28 (dd, *J* = 15.1 and 4.9 Hz, 1H), 5.16 (d, *J* = 4.2 Hz, 1H), 5.20-5.31 (m, 2H), 5.63 (dd, *J* = 7.2 and 4.4 Hz, 1H), 5.76-5.96 (m, 1H), 7.25-7.50 (m, 5H), 8.76 (d, *J* = 2.4 Hz, 2H), 9.03-9.11 (m, 2H); IR (KBr, cm^{-1}): 3312, 1763, 1644, 1028, 926.; mp 137.5-138.0 °C; Mass spectrum: *m/z* (relative intensity) 396 (0.2), 313 (49.9), 185 (63.4), 146 (100.0), 42 (43.8); Anal. Calcd for $C_{19}H_{16}N_4O_6$: C, 57.58: H, 4.07: N, 14.14 Found: C, 57.41: H, 4.02: N, 14.00; High-resolution mass spectrum: calculated for $C_{19}H_{16}N_4O_6$: 396.1070. Found : 396.1069.

(+)-(*3S,4R*)-(*N*-3,5-Dinitrobenzoyl)-3-amino-4-phenyl-1-prop-2-enyl-2-azetidinone (4): $[\alpha]_D$ = +33.57 (c 3.61 in CH_2Cl_2)

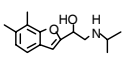
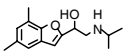
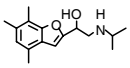
(+)-(*3S,4R*)-(*N*-3,5-Dinitrobenzoyl)-3-amino-4-phenyl-1-(3-triethoxysilylpropyl)-2-azetidinone (5): ^{1}H NMR ($CDCl_3$) δ 0.68 (t, *J* = 7.3 Hz, 2H), 1.21 (t, *J* = 7.4 Hz, 9H), 1.77 (t, *J* = 7.3 Hz, 2H), 3.09-3.25 (m, 1H), 3.62-3.78 (m, 1H), 3.82 (q, *J* = 7.0 Hz, 6H), 5.16 (d, *J* = 4.4 Hz, 1H), 5.58 (dd, *J* = 7.4 and 4.8 Hz, 1H), 7.28-7.40 (m, 5H), 8.77 (d, *J* = 1.8 Hz, 2H), 9.03-9.05 (m, 1H), 9.20 (d, *J* = 7.4 Hz, 1H) ; IR (neat, cm^{-1}) 3276, 3067, 1740, 1676, 1541, 1345, 1078 ; Mass spectrum (ZAB-SE): *m/z* (relative intensity) 561 (M+1, 5.2), 515 (42), 310 (100), 264 (24), 155 (25), 119 (54), 103 (29); High-resolution mass spectrum (70 SE-4F): calculated for $C_{25}H_{33}N_4O_9Si$ (M+H): 561.2017. Found : 561.1994. (MH^+)

CSP 2: Found : C, 3.98% : H, 0.58% : N, 0.59%, Calculated: 0.16 mmol/g (based on C) : 0.26 mmol/g (based on H) 0.11 mmol/g (based on N).

Results and Discussion

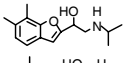
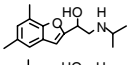
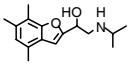
One way to achieve conformational rigidity is to employ a small ring as the scaffolding which holds the interaction sites essential for chiral recognition. The *N*-3,5-dinitrobenzoyl derivative of an α-amino-β-lactam was chosen as it contains a π-acidic group, a hydrogen bond donor and a hydrogen bond acceptor to interact with the complimentary π-basic, hydrogen bond acceptor and hydrogen bond donor sites present in β-blockers. Prior work with β-lactam synthesis[10] suggested a relatively straight-forward approach to the synthesis of the racemic selector which was resolved using a preparative π-basic CSP developed by Pirkle group.[11,12] The enantiomers of racemic **4** exhibited separation factor of 8.94 on an analytical column derived from (*S*)-*N*-(1-naphthyl)leucine using 20% 2-propanol in hexane. The synthetic strategy is well precedented and proceed-

Table 1. Separation of the enantiomers of several β-blockers and analogs on CSP **1** and CSP **2**

Analyte	(*3S,4R*) CSP **2**			(*R*) CSP **1***		
	α[a]	k_1[b]	Conf.[c]	α[a]	k_1[b]	Conf.[c]
Metoprolol	1.31	4.44		1.16	2.57	
Oxprenolol	1.40	5.32		1.00	2.28	
Pronethalol	1.04	6.97		1.13	5.14	
Propranolol	2.16	6.03	(+)R	1.39	4.36	(+)R
Pindolol	2.41	13.97		1.30	15.0	
Bufuralol	1.92	2.54	(+)R	1.93	2.79	(+)R
	2.30	4.27		2.15	3.43	
	2.28	4.05		2.23	3.28	
	3.09	4.79		2.58	4.43	

*The data were taken from reference 4. [a]Chromatographic separation factor. [b]The retention factor for the first eluted enantiomer using 5% ethanol in dichloromethane (v/v) containing 0.5 g/L of NH_4OAc as the mobile phase at a flow rate of 2 mL/min. The UV detector was operated at 254 nm. [c]The absolute configuration of the more strongly retained enantiomer.

Table 2. Effect of temperature on retention and enantioselectivity for several β-blockers and analogs on CSP **2**

Analyte	24 °C			0 °C			−10 °C		
	α[a]	k_1[b]	Rs[c]	α[a]	k_1[b]	Rs[c]	α[a]	k_1[b]	Rs[c]
Metoprolol	1.31	4.44	1.65	1.59	2.25	2.20	1.81	1.84	2.23
Oxprenolol	1.40	5.32	2.04	1.74	2.99	2.74	2.01	2.40	2.75
Pronethalol	1.04	6.97	-	1.00	4.89	-	1.00	4.42	-
Propranolol	2.16	6.03	4.24	3.19	3.91	4.88	3.78	3.23	4.94
Pindolol	2.41	13.97	3.91	3.94	10.07	4.85	4.73	8.91	4.72
Atenolol	1.15	31.13	0.63	1.20	19.10	0.81	1.24	15.89	0.84
Practolol	1.13	23.78	0.54	1.21	12.86	0.86	1.24	10.69	0.94
Acebutolol	1.11	16.36	0.24	1.12	9.48	0.54	1.12	7.94	0.45
Bufuralol	1.92	2.54	3.39	2.65	1.76	4.00	3.08	1.52	4.42
	2.30	4.27	4.68	3.51	3.41	5.91	4.13	3.13	5.28
	2.28	4.05	4.55	3.43	3.22	5.32	4.11	2.99	5.39
	3.09	4.79	5.79	4.80	4.28	6.42	5.82	4.20	5.88

[a]Chromatographic separation factor. [b]The retention factor for the first eluted enantiomer using 5% ethanol in dichloromethane (v/v) containing 0.5 g/L of NH_4OAc as the mobile phase at a flow rate of 2 mL/min. The UV detector was operated at 254 nm. [c]Resolution factor.

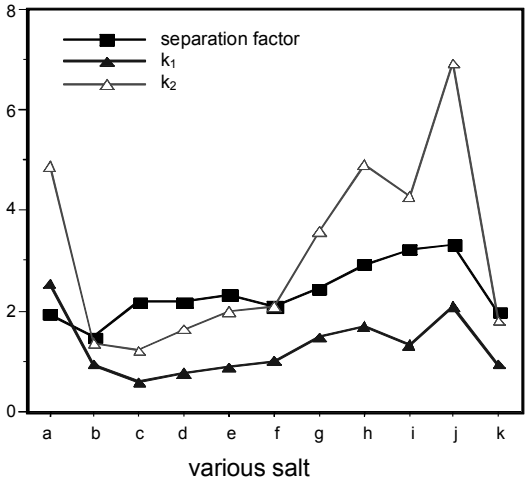


Figure 3. Effect of various salt additives on enantioseparation of bufuralol in 5% ethanol in dichloromethane (v/v). (a) 0.5 g/L NH_4OAc (b) 7.17 mM CF_3COOH/Et_3N (c) 7.17 mM AcOH/Et_3N (d) 7.17 mM AcOH/Diisopropylamine (e) 7.17 mM AcOH/Diisopropylethylamine (f) 7.17 mM AcOH/DABCO (g) 7.17 mM Formic acid/Et_3N (h) 7.17 mM Formic acid/Diisopropylethylamine (i) 7.17 mM 2-Hydroxymalonic acid/14.34 mM Et_3N (j) 7.17 mM 2-Hydroxymalonic acid/ 14.34 mM Diisopropylethylamine (k) 7.17mM Malonic acid/14.34 mM Et_3N.

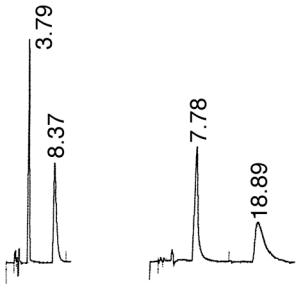


Figure 4. Enantioseparation of racemic bufuralol and propranolol on CSP **2** using 5% ethanol in dichloromethane (v/v) containing 7.17 mM formic acid/diisopropylethylamine as the mobile phase, respectively.

ed uneventfully (Figure 2). Once resolved, the enantiomer of the selector assigned the (*3S,4R*) configuration was hydrosilylated with trichlorosilane and immobilized on 5 μm spherical silica.[11,12] A 4.6 × 250 mm column was packed by conventional means (methanol slurry) and endcapped with hexamethyldisilazane.[13]

The column containing CSP **2** was evaluated initially using 5% ethanol in dichloromethane (v/v) containing 0.5 g/L of ammonium acetate as the mobile phase. Data relevant to the separation of the enantiomers of a variety of β-blockers is given in Tables 1 and 2, several temperatures being used. As noted with the earlier phosphonate CSP **1**, reduction of column temperature enhances enantioselectivity and unexpectedly reduces retention and improves band shapes.[4] A number of equilibria can be envisioned in this system, each being affected by temperature. The chiral recognition hypothesis used to rationalize the enantioselectivity observed in these systems does not take most of these equilibria into account. The ammonium acetate is considered a proton transfer agent but no other role was a priori ascribed to

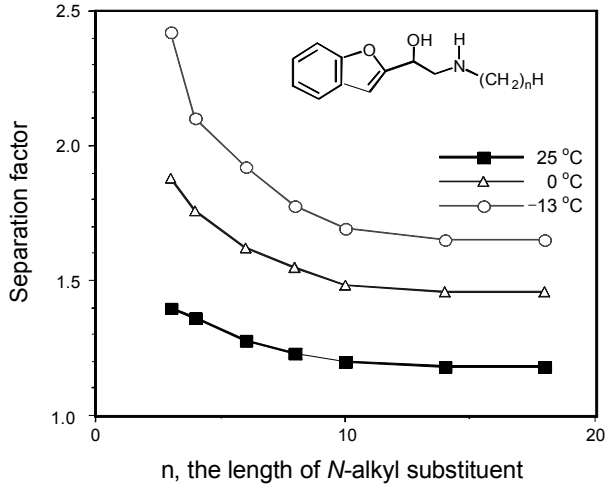


Figure 5. Relationship between enantioselectivity on CSP **2** and n, the number of methylenes in *N*-alkyl substituent of bufuralol-like analogs at three temperatures using 5% ethanol in dichloromethane (v/v) with 0.5 g/L of ammonium acetate as the mobile phase.

this salt. Other amine salts were used and these can substantially affected enantioselectivity, no rationalization for this being offered (Figure 3). Typical chromatograms using 5% ethanol in dichloromethane (v/v) containing 7.17 mM formic acid and diisopropylethylamine as the mobile phase are presented in Figure 4.

One will note that the aromatic substituent of the β-blockers influences retention and enantioselectivity in the manner expected provided that this group is a site for face to face π-π interaction. CSP **2** typically affords greater enantioselectivity than does CSP **1** for those β-blockers having a methyleneoxy unit between the stereogenic center and the aromatic substituent in Table 1.[4] However, for those analytes lacking this OCH_2 unit (pronethalol and bufuralol), the enantioselectivities are comparable or perhaps slightly poorer than those afforded by CSP **1**. Presumably, the analytes lacking the methyleneoxy unit are conformationally more rigid and less able to conform to the requirements of the more rigid CSP **2**.

A homologous series of linear *N*-alkyl substituted bufuralol-like racemates was chromatographed on CSP **2** at three temperatures (Figure 5). Enantioselectivity is reduced as the *N*-alkyl group becomes longer, the effect being greatest at the lower temperature. Such trends normally indicate differential extents of intercalation of the linear substituent between adjacent strands of bonded phase by the analyte enantiomers.[14] When enantioselectivity lessens as the *N*-alkyl substituent becomes longer, it is the more retained enantiomer which has the greater intercalation difficulty. It might well be that "reorientation" of the selector with respect to the tethers would cause enantioselectivity to increase, rather than decrease, as the *N*-alkyl substituent is lengthened.[15]

In summary, the brush type CSP **2** derived from α-amino β-lactam was prepared and evaluated for resolution of the enantiomers of several β-blockers with the use of various salt additives. The observed results showed that CSP **2** could be specially recommended for separation of β-blocker enantiomers. The conformationally rigid CSP **2** containing β-lactam afforded improv-

ed resolution of β-blockers enantiomers relative to the conformationally less rigid phosphonate derived CSP **1** and in terms of chiral recognition rationale further optimization of this type of CSP should be possible.

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