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Chiral stationary phase designed for β-blockers

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ABSTRACT

A chiral stationary phase (CSP) derived from an N-3,5-dinitrobenzoyl-α-amino phosphonate was prepared for the direct separation of the enantiomers of underivatized β-blockers. Structure–chromatographic activity relationships for β-blockers and closely related analogues are reported for this CSP and are found to be consistent with the model used to design this CSP. The effect of temperature on the chromatographic behavior of β-blocker enantiomers is unusual. A reduction in temperature reduces the retention of the less retained enantiomer and increases the retention of the more retained enantiomer without appreciable band broadening.

INTRODUCTION

It is widely recognized that stereoisomers of pharmaceutical agents may have drastically different pharmacological potencies or actions [1,2]. For example, the so-called β-blockers, widely used in the treatment of angina pectoris and hypertension, differ considerably in the physiological responses that they elicit. Typically, the *S* enantiomers are 50-500 fold more active than their antipodes and may differ also in the nature of the elicited responses [3].

Owing to their importance, many potential β-blockers have been developed and tested and a number are now marketed. In the present scientific climate, all stereoisomers of a potential pharmaceutical must be evaluated individually. Consequently, methods for preparatively separating β-blocker stereoisomers and for ascertaining their stereochemical purity are of considerable current interest. Moreover, much effort continues to be expended by pharmacologists on the study of how β-blocker stereochemistry influences the extent and mode of their action. There are now a variety of liquid chromatographic methods which facilitate determinations of the stereochemical purity of β-blockers, studies of differences in the rate of metabolism of their enantiomers and studies of the stereochemical pathways of metabolism [4]. Although it is possible and often practical to derivatize enantiomers with a chiral reagent so as to obtain diastereomers which are separable on an achiral column, there are potential disadvantages to this approach. In some instances, the enantiomers of β-blockers have been separated on achiral columns through the use of chiral mobile phase additives [5,6]. However, the scope of this method remains undetermined and it too is disadvantageous in some applications. Instances of derivatization with an

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achiral reagent prior to enantiomer separation on a column containing chiral stationary phases (CSPs) have been reported [7,8]. Such derivatization, while requiring additional effort for sample preparation and validation, can aid in detection and help meet chiral recognition requitements. However, the need for derivatization and, in the case of preparative separations, de-derivatization, is an obstacle to be avoided if possible. The direct separation of underivatized enantiomers on a CSP is to be preferred but is neither always possible nor feasible.

Certain CSPs, notably those derived from chiral polymers, either natural or synthetic in origin, have shown the ability to separate the enantiomers of some underivatized β-blockers [9–13]. Such reports are typically fragmentary in that no indication is given of the ability of the phase to separate the enantiomers of β-blockers in general nor is any indication given of the mechanism(s) of chiral recognition employed by these CSPs. In the case of the protein and cellulosic CSPs, column lifetimes can be uncertain and band shapes are often unsatisfactory. A notable exception to this generalization is the recently reported cellulase-derived CSPs [14]. Protein CSPs, even though useful for analytical separations, are of limited utility for preparative separations owing to their extremely low sample capacities. To a great extent, the chiral recognition ability of polymeric CSPs depends on their secondary and tertiary structure, something difficult to understand and consequently harder to control. Extremely useful in certain applications, these polymeric CSPs must currently be regarded as empirical in their origin and application.

Several years ago, we undertook the design, based on mechanistic understanding, of a brush-type CSP intended to separate the enantiomers of underivatized β-blockers (Fig. 1). This paper reports the progress to date in this endeavor.

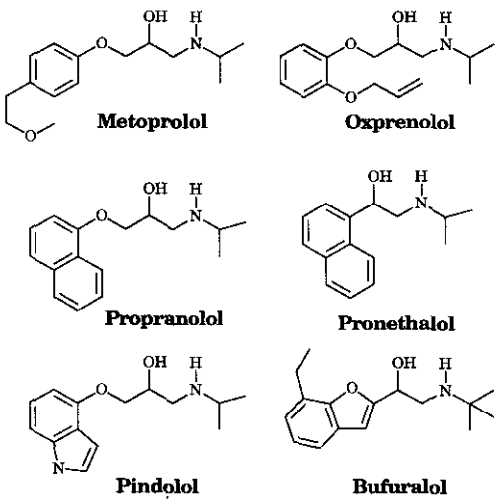


Fig. 1. Structures of β-blockers.

EXPERIMENTAL

Apparatus

Chromatography was performed using either of two systems. System one consisted of an Anspec–Bischoff Model 2200 isocratic high-performance liquid chromatographic (HPLC) pump, a Beckman Model 210 injector with a 20-μl sample loop, a Milton Roy LDC UV Monitor D fixed-wavelength detector operating at 254 nm and a Kipp and Zonen BD 41 dual-channel recorder. A Rudolph (Flanders, NJ, USA) Autopol III with a 20-cm flow cell was used to monitor the sign of $[\alpha]_D$. System two consisted of an Anspec–Bischoff Model 2200 isocratic HPLC pump, a Rheodyne Model 7125 injector with a 20-μl sample loop, two Milton Roy LDC UV Monitor D fixed-wavelength detectors connected in series operating at 254 and 280 nm and a Kipp and Zonen BD 41 dual-channel recorder. All HPLC equipment was purchased from Anspec (Ann Arbor, MI, USA). For variable-temperature experiments, *ca.* 1 m of 1/16-in. O.D. stainless-steel tubing was used between the injector and the column, the bulk of this tubing being coiled about the column as a heat exchanger. The entire column and heat exchanger were immersed in a Dewar flask containing the coolant. This experimental arrangement has given rise to linear Van 't Hoff plots for other columns and analytes.

Allyl alcohol, isobutyraldehyde and dimethyl phosphite were purchased from Aldrich (Milwaukee, WI, USA) and distilled prior to use. 2-Acetylbenzofuran was used as received from Aldrich. The N-(3,5-dinitrobenzoyl)phenylglycine column and the N-(2-naphthyl)alanine undecyl ester CSP were obtained from Regis Chemical (Morton Grove, IL, USA).

*Dimethyl N-(3,5-dinitrobenzoyl)-α-amino-2,2-dimethyl-4-pentenylphosphonate (**8**)*

A 100-ml oven-dried flask was charged with 2.20 g (12 mmol) of sodium hexamethyldisilamide and 50 ml of dry tetrahydrofuran (THF) followed by 1.75 g (12 mmol) of aldehyde **7** [15] and magnetically stirred under a nitrogen atmosphere at room temperature. After 1 h, 2.50 g (22.7 mmol) of dimethyl phosphite were added and the cloudy mixture was refluxed for 24 h. After cooling, the reaction mixture was diluted with 200 ml of diethyl ether followed by 100 ml of saturated sodium hydrogencarbonate solution and the resulting mixture was stirred for 1 h, then the phases were separated and the organic layer was washed with 50 ml of water then 50 ml of saturated sodium chloride solution. The combined aqueous layers were back-extracted with three 50-ml portions of dichloromethane. The combined organic layers are dried over sodium carbonate. After filtration, the solution of crude amino phosphonate was treated with 3.51 g (15 mmol) of 3,5-dinitrobenzoyl chloride and 100 ml of water–saturated sodium hydrogencarbonate solution (1:1). After strirring for 1 h, the aqueous layer was removed and replaced with 100 ml of the latter mixture. After stirring for a further 1 h, the layers were separated and the organic layer was washed with 50 ml of saturated sodium chloride solution, dried over magnesium sulphate and concentrated under reduced pressure. After column chromatography on silica using dichloromethane–diethyl ether (2:1) as eluent, (±)-**8** was obtained as a colorless oil (1.35 g, 25% yield). After recrystallization from methyl-*tert.*-butyl ether–hexane, ±-**8** melts at 128–129°C. Satisfactory analytical values for C, H, N and P were obtained. Thin-layer chromatography: R_F = 0.30 [silica gel plates with

dichloromethane–diethyl ether (1:1) as eluent]. ^{1}H NMR (C^2HCl_3): δ 1.15, 2 × s, 6H; 2.24, m, 1H; 2.32, m, 1H; 3.75, d (J = 16 Hz), 3H; 3.80, d (J = 16 Hz), 3H; 4.6–4.92, dd (J = 20, 10 Hz), 1H; 5.20, m, 2H; 5.90, m, 1H; 7.4, d (J = 10 Hz), 1H; 9.02, m, 2H; 9.19, m, 1H. $^{31}P\{^1H\}$ NMR (C^2HCl_3): δ 25.78 (ref. 85% H_3PO_4). IR (KBr, neat): 3248, 3098, 2961, 1734, 1670, 1630, 1541, 1344, 1284, 1234, 1035 cm^{-1}. Mass spectrum (70 eV): m/z 414 (0.8); 238 (18.0); 195 (100); 149 (81.5); 75 (76.7). High-resolution mass spectrum: m/z calculated for $C_{16}H_{22}N_3O_8P$, 415.1144; found, 415.1137.

Resolution of racemic ***8***

Enantiomer separation was accomplished by medium-pressure liquid chromatography on a 30 × 1 in. I.D. column packed with (+)-(*R*)-N-(2-naphthyl)alanine undecyl ester CSP bonded to 60-μm irregular silica. The mobile phase was isopropanol–hexane (2:98). Two chromatographic fractions were collected. The first was (+)-(*R*)-**8** of 98% enantiomeric purity, as judged by HPLC assay on a Regis (*R*)-N-(2-naphthyl)alanine column. The subsequently collected (−)-(*S*)-**8** was found to be of 99% enantiomeric purity. Each enantiomer was obtained as a colorless foam after drying *in vacuo*. The NMR spectrum of each enantiomer was identical with that of the racemate.

*Chiral stationary phase (R)-****6***

Mercaptopropylsilica (2.75 g), 0.60 g of enantiomerically pure (*R*)-**8** and 0.10 g of 2,2′-azobis(2-methylpropionitrile) were slurried in 30 ml of chloroform and heated to reflux [16]. After 36 h, the light-red mixture was cooled and the derivatized silica was collected by filtration. The silica was washed sequentially with 100 ml of methanol, 50 ml of ethyl acetate and 50 ml of diethyl ether. The modified silica was packed as a methanol slurry into a 120 × 4.6 mm I.D. column using conventional methods. Found: C 5.80, H 1.03, N 0.69%; calculated, 0.15 mmol/g (based on C); 0.16 mmol/g (based on N).

Analytes

The β-blocker samples were obtained as follows: pindolol from Sandoz, metoprolol from Ayerst Labs., proenthalol and propranolol from Imperial Chemical Industries, oxprenolol from Ciba-Giegy and bufuralol and its methylated analogues from Roche Products.

RESULTS AND DISCUSSION

Very early in the development of the CSPs, it became evident that basic amines are strongly retained by the π-acidic N-(3,5-dinitrobenzoyl)amino acid-derived phases. Consequently, basic primary and secondary amines are typically N-acylated prior to chromatography on these CSPs. The enantiomers of propranolol (as variously N-acylated derivatives, *e.g.*, the laurylamide) are modestly separated on an N-(3,5-dinitrobenzoyl)phenylglycine-derived CSP [17]. It is also known that the enantiomers of oxazolidones derived from β-blockers by treatment with phosgene are also separable on this CSP [18]. The need for derivatization arises from the basicity of the secondary amines. One way to reduce the basicity of an amine is through protonation, an easily reversed (and hence not undesirable) derivatization. Perhaps the ammonium

ion portion of a protonated β-blocker could be used as one of the essential interactions for chiral recognition.

During a sabbatical leave from Sumitomo Chemical, K. Moriguchi prepared several N-(3,5-dinitrobenzoyl)amino acid CSPs (**1–3**) in which the carboxylic acid group was left free (Fig. 2). By using a mobile phase containing ammonium acetate, it was expected that the carboxylic acid group of the CSP would be converted to a carboxylate group, which was intended to interact ionically with the ammonium ion portion of the protonated β-blocker. Propranolol, having a π-basic aromatic group, was expected additionally to undergo a strong π–π interaction with the CSP. Finally, the dinitrobenzamide N–H was expected to hydrogen bond to the hydroxy group of the β-blocker, affording a third bonding interaction. This *a priori* chiral recognition model predicts that CSPs **1–3**, if of the *R* configuration, should retain the *S* enantiomer of propranolol. NMR studies of mixtures of propranolol and a precursor to CSP **1** suggested that the expected interactions occur, although one cannot tell whether they occur simultaneously owing to time averaging.

When tested, CSP **1** was found to separate the enantiomers of propranolol with the expected elution order. Several other β-blockers are similarly resolved [19]. Satisfaction at having designed a CSP from mechanistic considerations which performs as expected was tempered by the observation that it led to long retention of the β-blockers and afforded undesirably broad bands and modest selectivity.

Chiral recognition typically stems from the weaker, not the stronger, of the essential interactions. The electrostatic interaction between the ammonium and carboxylate ions is sufficiently strong that, in order to keep retention at a reasonable level, a strong eluent has to be used. This interferes with the remaining weaker but

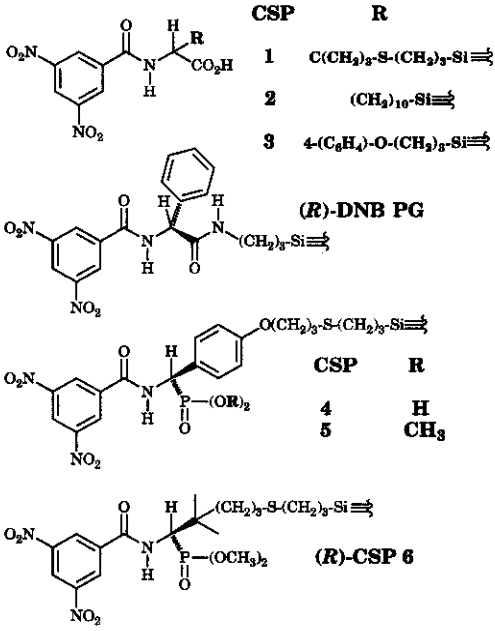


Fig. 2. Structures of chiral stationary phases.

essential interactions. To remedy this, phosphonic acid CSP **4** was prepared, the idea being that the charge on the phosphonate anion would be more diffuse and the ionic interaction would be weakened. Also considered was the possibility that the phosphonic acid group might be differently oriented (conformationally) and differently solvated, factors which might influence the strength of the electrostatic interaction. Indeed, CSP **4** does show improved enantioselectivity towards propranolol relative to CSPs **1–3**, but still shows undesirably strong retention.

Abandoning the use of a formal ionic interaction, phosphonate ester CSP **5** was prepared. Here, the intention is to have the amine (or ammonium ion) interact with the phosphonate ester through hydrogen bonding to the phosphinyl oxygen. The previously mentioned π–π and hydrogen bonding interactions were still expected to occur. Indeed, CSP **5**, the preparation of which has been described [20], does separate the enantiomers of propranolol and affords improved performance relative to its phosphonic acid analogue, **4**.

Being aware from other studies that phenyl substituents may serve as hydrogen bond sites [21], CSP **6** was prepared so as to avoid having a hydrogen bonding site on each face of the semi-rigid backbone of the CSP. In CSP **6**, the objectionable phenyl group has been replaced with a geminal dimethyl group which was expected to exert some conformational control and to provide a steric barrier projecting from one face of the chiral selector, thus controlling the preferred direction of approach by an analyte.

The synthetic sequence used to prepare CSP **6** is similar to that employed to prepare CSP **5** (Fig. 3). The route to CSP **6** begins with aldehyde **7**, readily available from the reaction of allyl alcohol and isobutyraldehyde [15]. This aldehyde has a terminal double bond (ultimately to be used to for attachment to silica) and is non-enolizable. Treatment of **7** with sodium hexamethyldisilamide affords the N-trimethylsilylimine, which adds dimethyl phosphite to give, after work-up, the α-amino phosphonate. The crude α-amino phosphonate was acylated with 3,5-dinitrobenzoyl chloride to afford racemic precursor **8**, resolvable on a variety of π-basic CSPs [22]. Preparative resolution of **8** was accomplished using a large column containing the N-2-(naphthyl)alanine-based CSP. The enantiomerically pure phosphonate was covalently bonded to 3-mercaptopropylsilanized silica using 2,2′-azobis(2-methylpropionitrile) as an initiator. The modified silica gel was slurry packed into a 120 ×

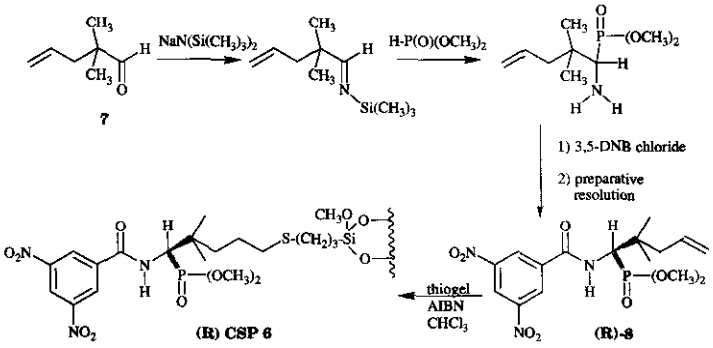


Fig. 3. Synthetic sequence for the preparation of CSP **6**.

4.6 mm I.D. stainless-steel column, end-capped with hexamethyldisilizane and evaluated for its ability to separate the enantiomers of an assortment of β-blockers and β-blocker analogues.

As mentioned earlier, the presence of a basic amino group in an analyte typically leads to long retention and peak tailing on silica-based π-acidic CSPs. Control of mobile phase pH and/or addition of amines to the mobile phase are frequently used cures for such peak tailing. Previous experience had shown that mobile phases consisting of halocarbons and lower molecular weight molecular alcohols and containing a low concentration of ammonium acetate allow the separation of enantiomers of propranolol on CSPs **1**–**3**. The ammonium acetate provides a means of protonating the amino group of the β-blockers and reduces peak tailing. In halocarbon–alcohol solvent mixtures, extensive formation of aggregated tight ion pairs is thought to occur. Increasing the concentration of the ammonium acetate in the mobile phase diminishes the retention of propranolol on CSP **6**, but does not drastically alter the enantioselectivity, thus suggesting that the ammonium acetate competes with the protonated β-blockers for absorption sites. This behavior is shown in Fig. 4, obtained using a chloroform–methanol mobile phase. In preparative separations, the volatility of the mobile phase components including ammonium acetate makes it possible to retrieve the β-blocker simply by evaporation of the mobile phase under vacuum.

For comparison of CSPs **5** and **6**, a mobile phase of dichloromethane–ethanol (19:1) containing 0.5 g/l (6.5 m*M*) of ammonium acetate was used. To improve the

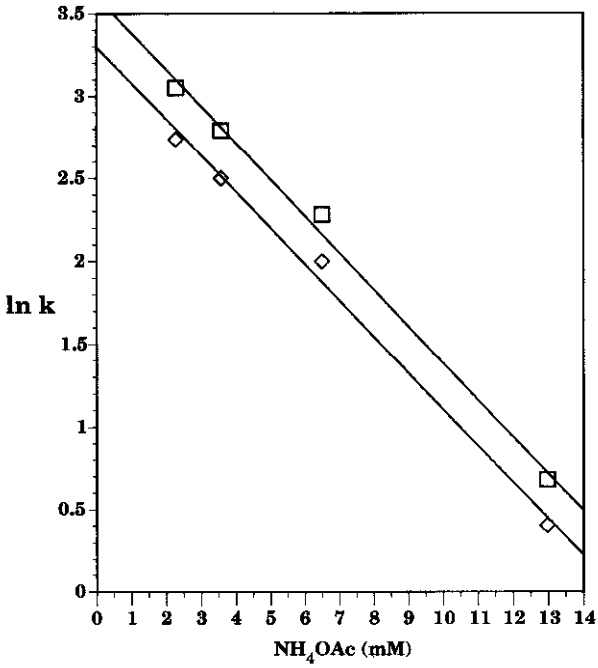


Fig. 4. Relationship between retention of propranolol enantiomers on CSP **6** and the amount of ammonium acetate (NH_4OAc) in chloroform–methanol (9:1) mobile phase. ◇ = ln k'_1; □ = ln k'_2.

TABLE I

SEPARATION OF THE ENANTIOMERS OF SOME β-BLOCKERS

Analyte	(*R*)-DNB PG			(*R*)-CSP **5**			(*R*)-CSP **6**		
	α^a	$k'_1{}^b$	$[\alpha]_D{}^c$	α^a	$k'_1{}^b$	$[\alpha]_D{}^c$	α^a	$k'_1{}^b$	$[\alpha]_D{}^c$
Metoprolol	1.05	9.86		1.15	6.57		1.16	2.57	
Oxprenolol	1.00	16.1		1.00	6.14		1.00	2.28	
Pronethalol	1.03	11.2		1.06	12.36		1.13	5.14	
Propranolol	1.00	12.8		1.34	13.4,	(+)-(*R*)-	1.39	4.36,	(+)-(*R*)-
Pindolol	1.12	45.1		1.12	50.1		1.30	15.0	
Bufuralol	1.16	4.94,	(+)-(*R*)-	1.22	6.67,	(+)-(*R*)-	1.93	2.79,	(+)-(*R*)-

[a] Chromatographic separation factor.

[b] The capacity factor for the first-eluted enantiomer using dichloromethane–ethanol (19:1) containing 0.5 g/l of ammonium acetate as the mobile phase at a flow-rate of 2 ml/min. The detector was operated at 254 nm.

[c] Sign of $[\alpha]_D$ of the more strongly retained enantiomer as determined using a polarimetric HPLC detector. The letter refers to the absolute configuration of the more strongly retained enantiomer.

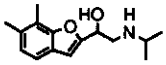
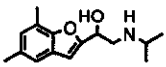
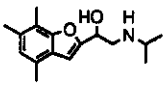
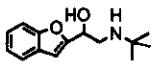
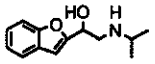
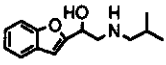
reproducibility, a stock solution of ammonium acetate in absolute ethanol was prepared and diluted with dichloromethane as required. Comparative chromatographic data for six β-blockers obtained using a commercial covalent (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine-derived phase (DNB PG), the (*R*)-phosphonate ester phase **5** and (*R*)-phosphonate ester phase **6** are presented in Table I. From these data, it is evident that the more π-basic β-blockers are the more strongly retained. However, enantioselectivity does not necessarily parallel retention. Note that bufuralol is one of the more weakly retained, judged by k'_1, of the β-blockers on CSP **6**, yet affords the largest separation factor in Table I.

The elution orders on CSP **5** and **6** can be explained using the aforementioned model, although this model is not in accord with the elution order noted on (*R*)-DNB PG. Owing to the Cahn–Ingold–Prelog priority sequence, (*R*)-CSPs **5** and **6** are stereochemically equivalent to (*S*)-DNB PG. To evaluate further the chiral recognition process, the effect of temperature on β-blocker retention by CSPs **5** and **6** was investigated. One generally expects a linear Van 't Hoff response (*i.e.*, a linear ln k' *versus* $1/T$ plot) with increases in retention, enantioselectivity and peak width as the column temperature is reduced. Using the CSP–mobile phase combination described, non-linear Van 't Hoff behavior is observed for an extended series of β-blockers and their analogues. As can be seen from the data in Table II, there are dramatic increases in enantioselectivity with comparatively little accompanying peak broadening (see Fig. 5). A reduction in temperature always decreases the retention of the less retained enantiomer and often slightly decreases that of the more retained enantiomer. For some analytes, the retention of the more retained enantiomer initially decreases then increases as the temperature is lowered further. In view of the number of equilibria possible in these complex systems, equilibria which may respond differently to temperature change, no rationalization of these observations is offered here.

It is not surprising that the enantiomers of bufuralol are better resolved than those of propranolol and pindolol. Unlike the later two, bufuralol lacks the methylene group between the π-basic aromatic group and the stereogenic center. Consequently, it

TABLE II

EFFECT OF TEMPERATURE ON RETENTION AND ENANTIOSELECTIVITY FOR SOME β-BLOCKERS AND ANALOGUES USING CSP **6**

Analyte	21°C			0°C			−24°C		
	α^a	$k'_1{}^b$	$k'_2{}^b$	α^a	$k'_1{}^b$	$k'_2{}^b$	α^a	$k'_1{}^b$	$k'_2{}^b$
Metoprolol	1.16	2.57	2.98	1.21	1.05	1.27	1.48	0.64	0.95
Oxprenolol	1.00	2.28	2.28	1.00	0.75	0.75	1.03	0.50	0.52
Pronethalol	1.13	5.14	5.81	1.21	2.21	2.67	1.31	1.50	1.97
Propranolol	1.39	4.36	6.06	1.63	1.86	3.03	2.11	1.28	2.70
Pindolol	1.30	15.0	19.5	1.43	7.29	10.4	1.72	6.71	11.5
Bufuralol	1.93	2.79	5.38	2.50	1.43	3.58	4.08	0.73	2.98
	2.15	3.43	7.37	2.83	2.07	5.86	4.18	1.57	6.56
	2.23	3.28	7.31	3.04	1.86	5.65	4.44	1.46	6.48
	2.58	4.43	11.43	3.44	2.57	8.84	5.03	2.21	11.1
	1.75	4.14	7.25	2.38	1.86	4.43	3.76	1.13	4.25
	1.64	4.01	6.58	2.08	1.80	3.74	3.08	1.08	3.33
	1.63	1.71	2.79	1.94	1.19	2.31	3.02	0.73	2.20

[a] Chromatographic separation factor.

[b] Capacity factors for the two enantiomers using dichloromethane–ethanol (19:1) containing 0.5 g/l of ammonium acetate as the mobile phase at a flow-rate of 2 ml/min. The detector was operated at 254 nm.

is more restricted conformationally, a circumstance often associated with appreciable degrees of enantioselectivity. Note that replacing the 7-ethyl substituent of bufuralol with two or, better, three methyl substituents on the benzofuran ring enhances enantioselectivity by increasing the π-basicity without adding polar sites for additional bonding interactions with the stationary phase (see Fig. 6) which increase retention but may possibly reduce enantioselectivity. The methyl substituents enhance enantioselectivity relative to bufuralol even though the analogues have N-isopropyl substituents, shown here to be inferior to N-*tert.*-butyl substituents in engendering enantioselectivity in bufuralol-like systems. For example, a series of bufuralol-like racemates were prepared by a synthetic route which allows variation of the N-alkyl substituent (Fig. 7). This sequence, similar to that reported for bufuralol [23], entails α-bromination of 2-acetylbenzofuran, reduction of the bromo ketone to the bromo alcohol with sodium tetrahydroborate and substitution of the desired *n*-alkylamine for the bromine. Fig. 8 shows the effect of the length of the N-alkyl substituent on α at 21, 0 and −24°C. As can be seen, alkyl groups longer than propyl have a negligible effect

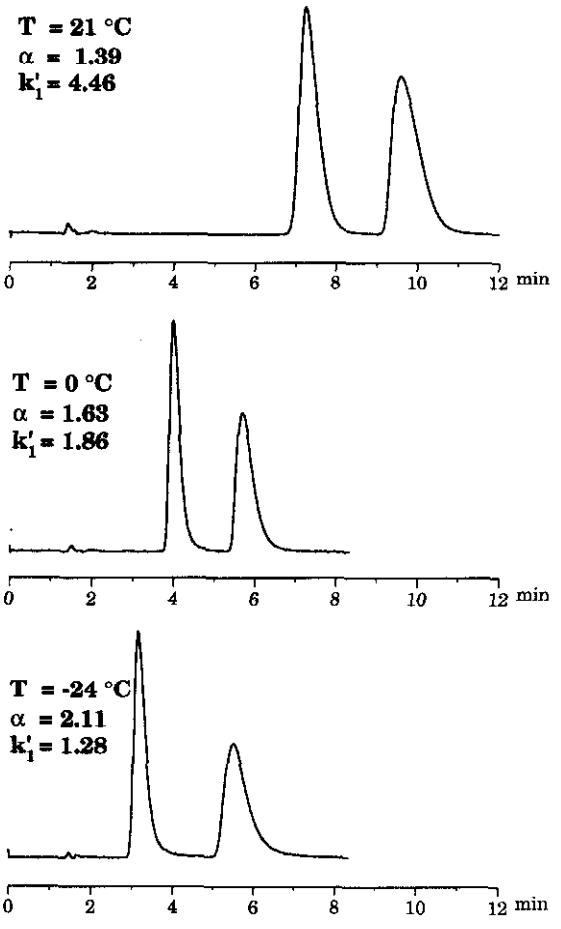


Fig. 5. Effect of temperature on retention and enantioselectivity of propranolol on CSP 6. Mobile phase: chloroform–ethanol (19:1) containing 0.5 g/l of ammonium acetate.

on the magnitude of α, suggesting that the enantiomers show either no or little differential intercalation of the N-alkyl groups between strands of bonded phase [24,25].

In all instances, α increases dramatically as the temperature is diminished. The chromatographic response to temperature change of the bufuralol analogues having N-isopropyl, N-isobutyl and N-*tert.*-butyl substituents is shown in Table II. The N-isopropyl and N-isobutyl analogues show comparable levels of enantioselectivity at ambient temperature and are exceeded in this respect by the N-*tert.*-butyl analogue. This difference is accentuated at lower temperatures. All three analogues show lower selectivities than bufuralol, doubtless owing to the absence of a π-basicity-enhancing alkyl substituent on the benzofuran system.

Elution orders were rigorously established using β-blocker samples of known absolute configuration. In some instances, the signs of the rotation of the enantiomers were related to elution orders using a polarimetric detector in series with the ultraviolet

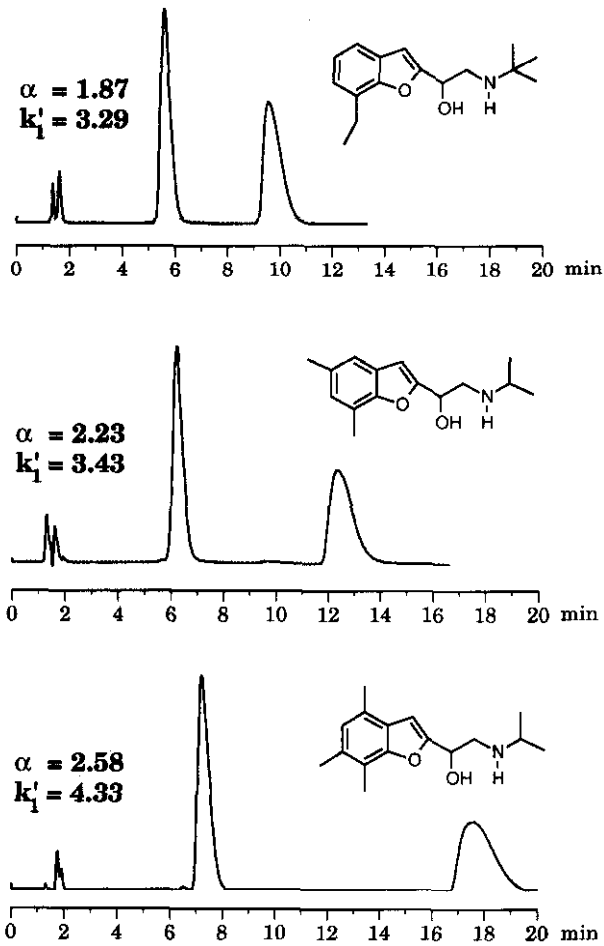


Fig. 6. Influence of ring methylation on the retention and enantioselectivity shown by several bufuralol analogues when chromatographed on CSP **6** using dichloromethane–ethanol (19:1) containing 0.5 g/l of ammonium acetate as the mobile phase.

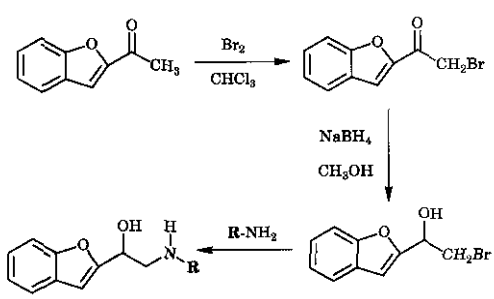


Fig. 7. Preparation of bufuralol-like racemates.

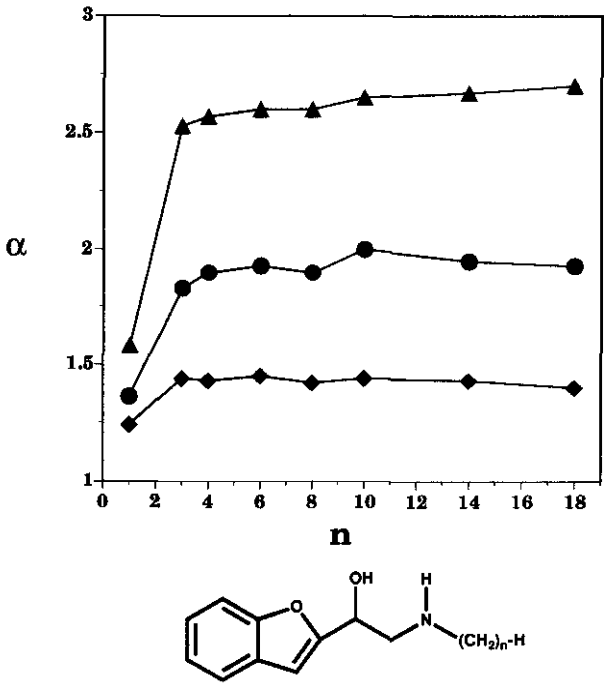


Fig. 8. Relationship between enantioselectivity on CSP **6** and *n*, the number of methylenes in the N-alkyl substituent, at three temperatures (◆ = 21; ● = 0; ▲ = −24°C) using dichloromethane–ethanol (19:1) containing 0.5 g/l of ammonium acetate as the mobile phase.

detectors. The observed elution orders on CSP **5** and **6** are consistent with the *a priori* formulated chiral recognition model, as are the structure–activity relationships noted. Although it is likely that further design changes will lead to CSPs that show greater scope and selectivity, it is evident that CSP **6** is useful for both analytical- and preparative-scale separations of a variety of β-blockers, no derivatization being required.

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Since submission of this paper, a comprehensive review ("Chromatography of β-adrenergic blocking agents") has appeared [26].

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