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A rational approach to the design of highly effective chiral stationary phases for the liquid chromatographic separation of enantiomers*

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Abstract: The design and rationale of some novel chiral stationary phases (CSPs) are discussed with respect to methods for determining enantiomeric purity, absolute configuration and for obtaining enantiomerically pure materials by liquid chromatography. The commercially-available dinitrobenzoylamino CSP type **1** is discussed with respect to the chiral recognition mechanisms which may operate in the resolution of some polycyclic and heterocyclic aromatic molecules and some benzodiazepines. *N*-Acyl α-arylalkylamines are also employed as models to formulate mechanisms for the chiral properties of type **1** CSPs in terms of enantiomeric stacking of the most stable conformations in solution. The properties of new types of 'reciprocal' CSPs are discussed and illustrated by enantiomeric separation of some amino acid and amino phosphonic acid derivatives, and by the separation of the following enantiomeric drugs as their 3,5-dinitrobenzoyl derivatives: metoprolol, oxoprenolol, ephedrine and alprenolol.

Keywords: *Chiral stationary phase; enantiomer separation; liquid chromatography.*

Introduction

Owing to the effect of absolute configuration on pharmacological response, there is widespread need for and interest in the ability to separate enantiomers directly by liquid chromatographic methods. When attainable, such separation affords a rapid, sensitive, convenient and accurate means of determining enantiomeric purity and absolute configuration. It also provides a preparative means for obtaining enantiomerically pure materials for a variety of purposes.

Chiral mobile or stationary phases must be used if enantiomers are to be chromatographically separated. However, this is a necessary but not sufficient condition, for additional requirements must be satisfied. Just what these requirements are is not

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always clear, so considerable effort has been expended by many laboratories seeking useful chiral mobile and/or stationary phases, devising analytical procedures for enantiomeric purity determination, and gaining mechanistic insight into chiral recognition requirements [1–3]. From geometric considerations, it has long been clear that two chiral molecules must undergo a minimum of three simultaneous interactions, one or more of which is stereodependent, if they are to 'recognize' each other's chirality.

As part of an ongoing study of chiral recognition mechanisms, the authors have developed a number of chiral stationary phases (CSPs), columns containing some of which are now commercially available.* Type **1** CSPs (Fig. 1), derived from *N*-3,5-dinitrobenzoylamino acids and anchored via three methylene groups to the silica, are capable of separating the enantiomers of tens of thousands of compounds, many of which are of medicinal and pharmacological interest. At present, approximately fifty classes of compound are known to be capable of resolution on type **1** CSPs [4]. Within each class, considerable structural variation is permissible, thus conferring upon one the ability to separate the enantiomers of tens of thousands of compounds. The authors' studies have contributed some understanding as to how these CSPs function and some insight into what types of analyte they will and will not resolve.

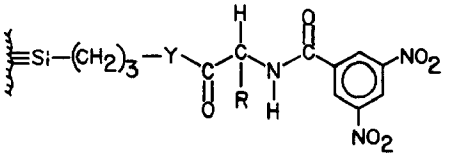


Figure 1
Basic structures of CSP type 1.

1 R = PHENYL, ISOPROPYL, ISOBUTYL, ETC.
Y = NH, $-O^{\ominus}NH_3^{\oplus}-$

Experimental

The type **1** CSP columns used in this study were commercial columns obtained from Regis Chemical Co. (Morton Grove, IL) or the J. T. Baker Chemical Co. (Phillipsburg, NJ). The preparation of the type **2** CSPs is to be reported elsewhere. The analytes used in this study were prepared from 3,5-dinitrobenzoyl chloride and the corresponding amines, most of which are well known or described elsewhere. Chromatography was performed isocratically using a Beckman System comprised of a model A-100 pump, model 210 injector, model 165 detector and a Kipp–Zonen model BD-41 recorder.

Results and Discussion

Type **1** CSPs utilize combinations of π–π-hydrogen bonding, electrostatic and steric interactions to generate chiral recognition. In the mechanism shown in Fig. 2 for the (*R*)-phenylglycine-derived type **1** CSP, the dinitrobenzoyl (DNB) group serves as a π-acid, the DNB NH-group serves as a hydrogen bond donor (acidic site), the carboxamide carbonyl oxygen serves as a hydrogen bond acceptor (basic site) and the bulky phenyl group serves to block access to the back face of the CSP. Six analytes having the requisite complementary functionality to be resolved by this mechanism are shown in Fig. 3, the

* Prepacked HPLC columns containing the CSPs derived from phenylglycine and from leucine are available both from Regis Chemical Co. (8210 Austin Ave, Morton Grove, IL 60053, USA) and from the J. T. Baker Chemical Co. (222 Red School Lane, Phillipsburg, NJ 08865, USA).

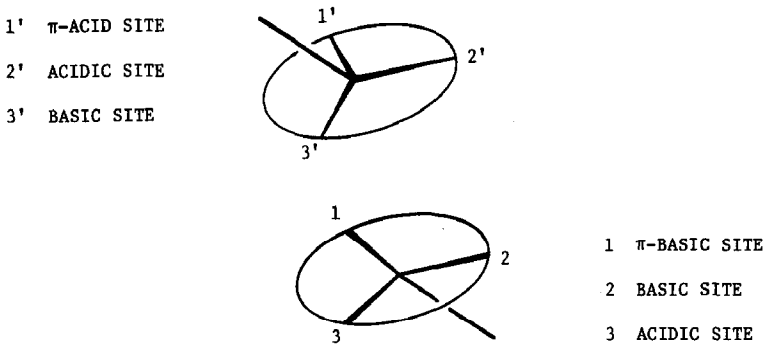


Figure 2
Depiction of the 'three-site' interaction model for chiral recognition on type **1** CSPs.

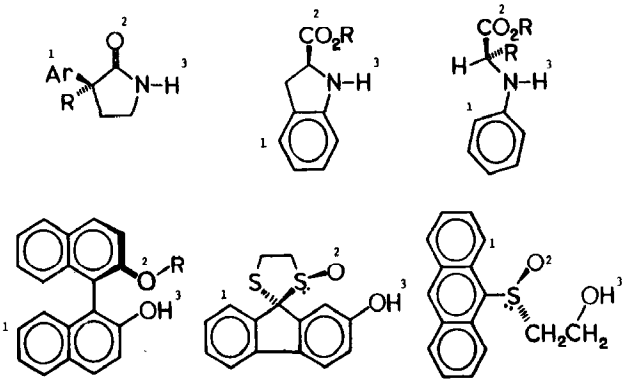


Figure 3
Compounds which can be resolved on phenylglycine type **1** CSPs.

configurations depicted being those most retained by the (*R*)-CSP. The sites of interaction are specified. A series of benzodiazepine analogues have been reported [5] to be readily resolvable on type **1** CSPs on both the analytical and preparative scale (cf. Fig. 4). These benzodiazepines can also be resolved by the mechanism shown in Fig. 3, for they contain π-basic, acidic and basic sites.

An alternative chiral recognition mechanism exhibited by this same CSP is shown in Fig. 5 for six representative analytes, again depicted in the most strongly retained configuration (Fig. 6).

Other mechanistic variations may also be displayed by type **1** CSPs. A study of the resolution of a series of *N*-acylated α-arylalkylamines led to the formulation of a mechanism in which electrostatic attraction between amide dipoles and π–π interaction between π-acidic and π-basic aryl groups leads to stacking of the analyte enantiomers on the most accessible (i.e. least hindered) face of the CSP [6, 7]. Chiral recognition then frequently involves steric differentiation between two analyte substituents. Figure 7 depicts such a stacked process, both components being shown in their most stable solution conformations.

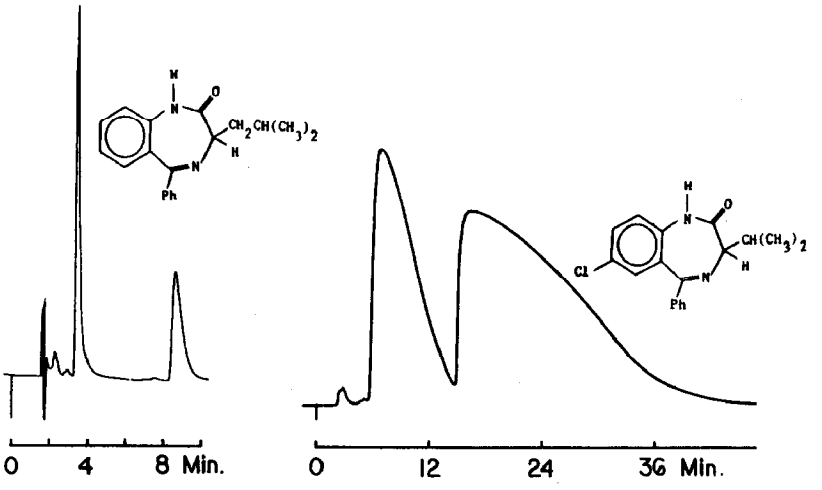


Figure 4
Analytical and preparative (100 mg) resolution of two racemic benzodiazepinones on a type 1 CSP derived from leucine. The columns were each 25 cm in length and 4.6 and 10 mm i.d. respectively. The mobile phases employed were 2-propanol–hexane (10:90, v/v) and (7.5:92.5, v/v), respectively. Flow rates were 2 and 5 ml/min.

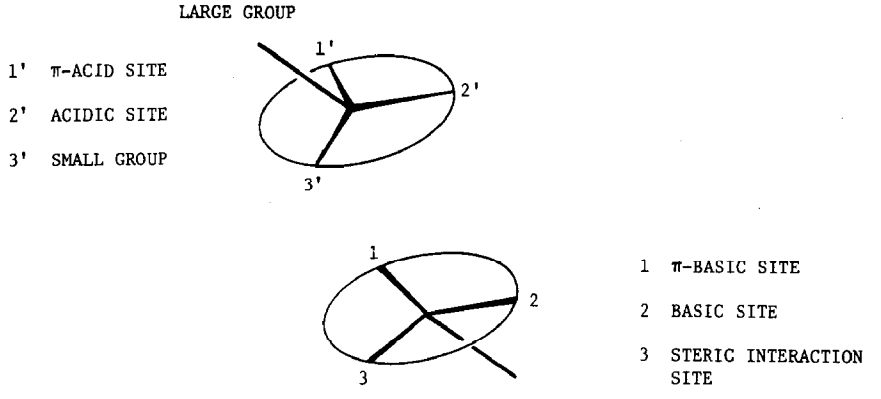


Figure 5
Alternative 'three-site' interaction pattern for type 1 CSPs.

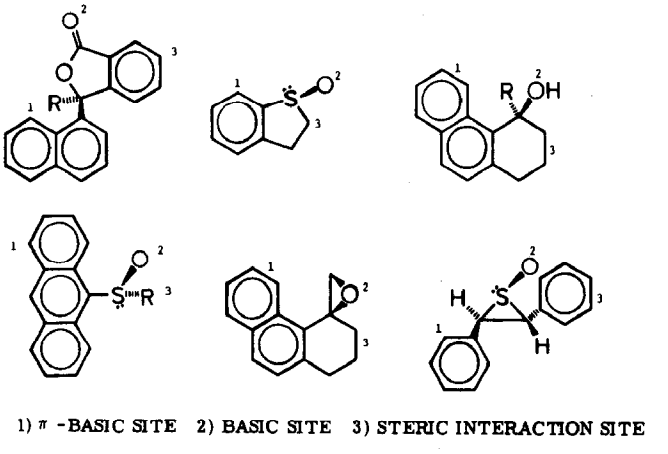


Figure 6
Compounds which can be resolved on phenylglycine type 1 CSPs.

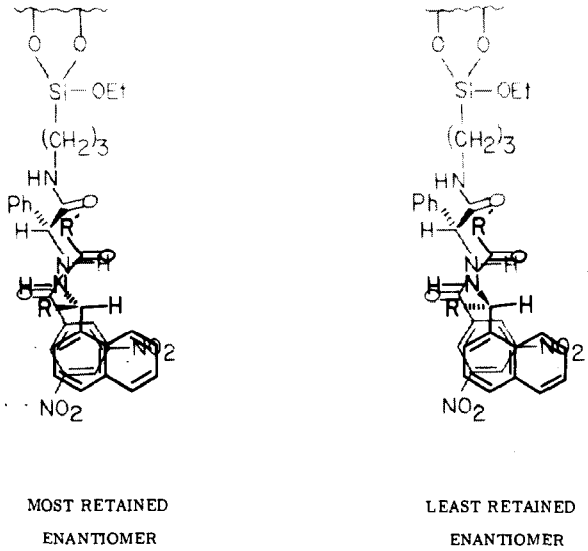


Figure 7
Stacked dipole chiral recognition mechanism (CSP type 1). The analyte is placed above the plane of the paper in which the rigid moiety of the CSP is located. Reprinted from [9] by permission of Elsevier Science Publishers B. V.

The mechanistic hypothesis formulated from the study of *N*-acyl α-arylalkylamines was instrumental in the design of 'reciprocal' CSPs intended to resolve analytes modelled after the type **1** CSPs [8, 9]. One such CSP, **2a**, shown in Fig. 8, is very effective for the resolution of amines, amino acids, aminoalcohols and aminophosphonic acids, all as their 3,5-dinitrobenzoyl (DNB) derivatives. Figure 9 shows the degree of enantioselectivity for several homologous series of amine DNB derivatives. The (*R*)-enantiomers are selectively retained. Table 1 contains data for the resolution of amino acid and aminophosphonic acid derivatives. As can be noted, the degree of enantioselectivity is quite high.

Two variations on the reciprocal theme, CSP types **2b** and **2c** (cf. Fig. 8), provide useful information concerning how the method of attaching a chiral molecule to the underlying support can influence the performance of the CSP. In the case of each of the CSP types **2a**, **2b** and **2c**, two competing 'opposite sense' chiral recognition mechanisms

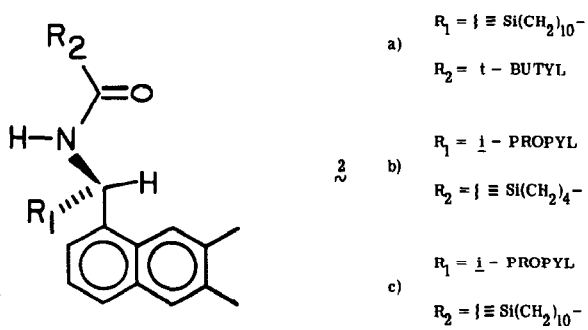


Figure 8
Structure of the 'reciprocal' type 2 CSPs.

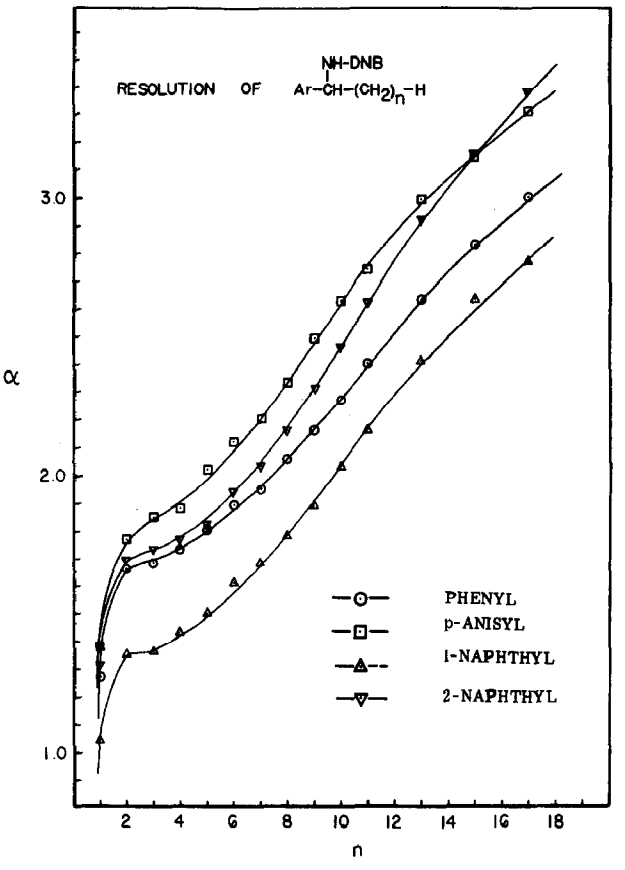
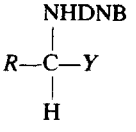


Figure 9
Resolution of α-arylalkylamine derivatives on (*R*)-CSP type **2a** using 2-propanol–hexane (20:80, v/v).

Table 1
Separation of the enantiomers of some amino acid and aminophosphonic acid derivatives* on CSP type **2a**.



R	*Y*	α†	k_1'‡	*R*	*Y*	α†	k_1'‡
Methyl	CO_2CH_3	4.16	21.7	Benzyl	CO_2CH_3	6.85	20.4
i-Propyl	CO_2CH_3	5.96	18.2	Phenyl	CO_2CH_3	2.93	38.4
i-Butyl	CO_2CH_3	6.45	17.7	Phenyl	$PO(OC_2H_5)_2$	1.58	10.5
$CH_3S(CH_2)_2$	CO_2CH_3	5.77	28.7	*p*-Anisyl	$PO(OC_2H_5)_2$	2.04	13.0
p-F-Benzyl	CO_2CH_3	8.29	19.4	*p*-Tolyl	$PO(OC_2H_5)_2$	1.93	8.6

* 3,5-Dinitrobenzoyl derivatives (DNB) were separated on CSP **2a** (cf. Fig. 8) with 2-propanol–hexane (20:80, v/v) as eluent.
† α = separation factor.
‡ k_1' = capacity ratio of the earlier eluting enantiomer.

are operative for the DNB derivatives used as analytes. When the dipole stacking process shown in Fig. 7 is applied to CSP types **2b** and **2c**, the $(CH_2)_nH$ alkyl 'tails' of the homologous series of analytes shown in Fig. 9 must intercalate between adjacent connecting 'arms' of the strands of bonded phase and are directed toward the underlying silica support. Long 'tails' or short connecting 'arms' raise the energy of the dipole stacking process and, by default, an alternative non-intercalative hydrogen-bonding process affording the opposite sense of enantioselection assumes dominance. Increased tail length eventually causes an inversion of elution order (Fig. 10). Because the non-intercalative process directs the alkyl tails outwards into the mobile phase, the use of an aqueous mobile phase tends to favor the intercalative process and requires a longer tail to cause inversion (Fig. 10). No inversion is noted on CSP type **2a** because the favoured dipole-stacking process is non-intercalative and is not affected adversely by long tails. In fact, on CSP type **2a**, the alternative hydrogen-bonding process is an intercalative process (see Fig. 11) and is suppressed by long tails. Thus, one or the other of the two

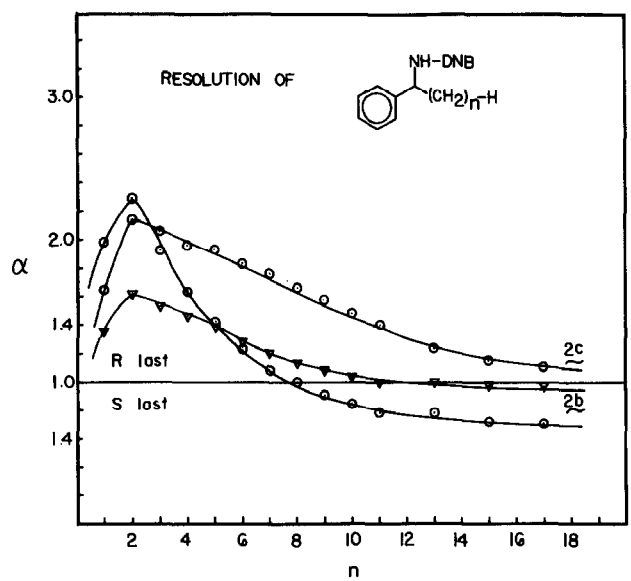


Figure 10
Chromatographic resolution of a series of DNB derivatives on CSP types **2b** and **2c**. —○—, data obtained using 2-propanol–hexane (20:80, v/v) as the mobile phase; —▽—, data obtained using methanol–water (90:10, v/v) as mobile phase.

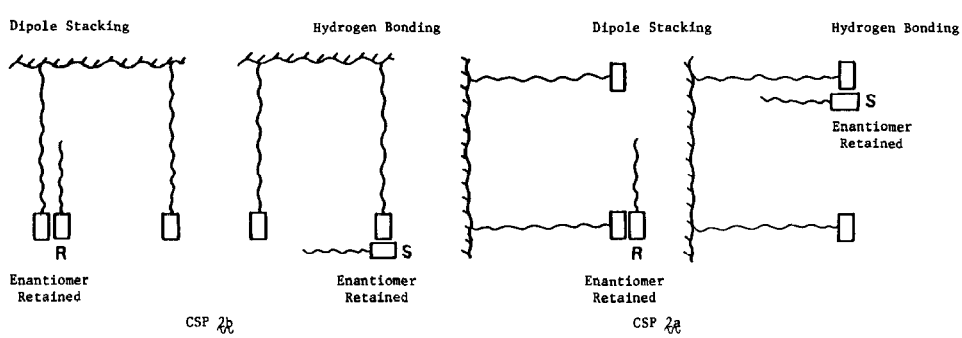


Figure 11
Schematic representation of the conflicting intercalative and non-intercalative processes on type 2 CSPs.

competing chiral recognition processes can be made to operate more effectively by changing the manner in which the chiral amide is anchored to the silica. As one might expect, there are systematic aspects to the mechanistic preferences of different categories of analyte. Hence, CSP type **2a** may perform better than CSP types **2b** and **2c** for some classes of analyte, whereas the reverse situation will hold for other analyte classes.

Figure 12 depicts several representative resolutions of derivatives of pharmacologically

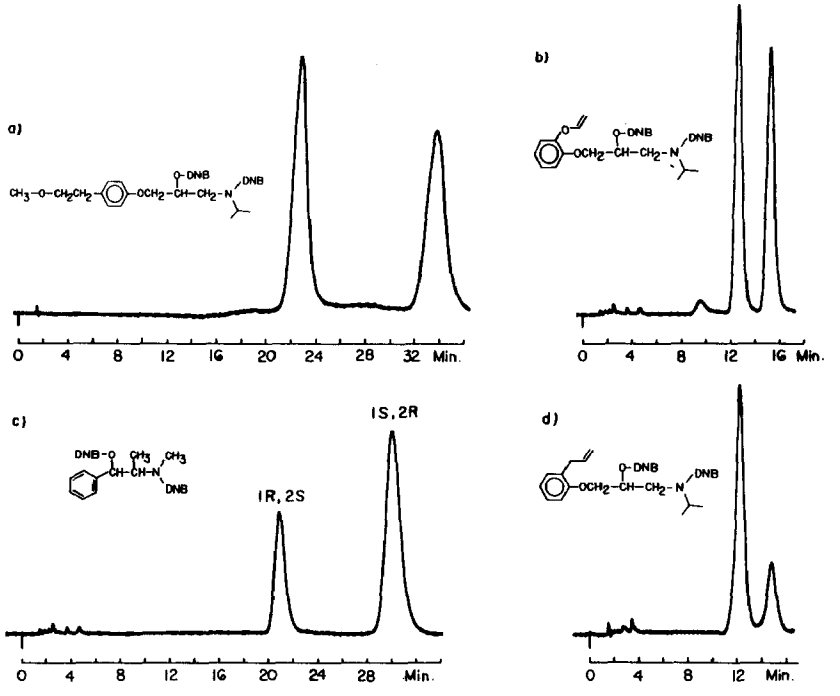


Figure 12
The separation of enantiomeric DNB derivatives on CSP type **2a** using 2-propanol–hexane (20:80, v/v): (a) metoprolol; (b) oxoprenolol; (c) ephedrine; (d) alprenolol.

interesting amines on CSP type **2a**. In view of the generality and the ease of use of type **2** CSPs, it is to be expected that they will find widespread use for the determination of enantiomeric purity, absolute configuration, and preparative separations of enantiomers. Although the authors have hitherto made no efforts to use these techniques to monitor the enantiomeric purity of drugs (e.g. the β-blockers) isolated from body fluids, it is apparent that such determinations can be made. The ability to use these CSPs in either normal- or reversed-phase mode is a particular advantage for such determinations, for one can readily shift the retention times of impurities relative to those of the enantiomers in order to avoid coelution. It may be anticipated that columns containing type **2** CSPs will become commercially available in the near future.

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