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Separation of the enantiomers of the 3,5-dinitrobenzamide derivatives of α-amino phosphonates on four chiral stationary phases

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ABSTRACT

Four commercially available high-performance liquid chromatography columns containing chiral stationary phases (CSPs) have been evaluated for the separation of the enantiomers of a variety of α-amino phosphonates as the 3,5-dinitrobenzamide derivatives. The CSPs are divided into two mechanistic classes, the π-acidic N-(3,5-dinitrobenzoyl)phenylglycine / N-(3,5-dinitrobenzoyl)leucine class and the π-basic N-(2-naphthyl)alanine / N-(1-naphthyl)leucine class. As expected, the π-basic CSPs are found to be more effective for separating the enantiomers of these π-acidic analytes, separation factors being sufficiently large to make preparative or reversed-phase separations possible. A chiral recognition rationale consistent with the observed elution orders is postulated for the π-basic CSPs.

INTRODUCTION

The potential biological activity of the α-aminophosphonic acids, the phosphorus analogues of α-amino acids, has prompted the development of a variety of methods for their preparation [1]. While most of these approaches generate the racemic α-aminophosphonic acid, enantioselective procedures have been recently reported [2]. Interest in the preparation of chiral non-racemic α-aminophosphonic acids has led to a need for convenient and accurate methods for determining enantiomeric purity and absolute configuration for the members of this class of compounds. Methods reported for the assignment of absolute configuration of α-aminophosphonic acids include the relative retention of diastereomeric peptides [3] and use of the sign of the optical rotation [4]. These methods rely on having an α-aminophosphonic acid of known absolute configuration (this usually being determined by X-ray crystallography) for reference purposes.

Unlike α-amino acids and their derivatives [5], there are few data in the literature concerning separation of the enantiomers of α-aminophosphonic acids and their derivatives on chiral stationary phases (CSPs) [6]. Mechanistic rationales have been set forth for several of the CSPs developed in our laboratory and can be used to relate elution order to the absolute configurations of many analytes. From mechanistic considerations, it seemed likely that those CSPs so employed for α-amino acid derivatives might be similarly employed for α-aminophosphonic acids and their derivatives. This paper evaluates commercial versions of several of these CSPs in terms of their ability to separate the enantiomers of 3,5-dinitrobenzamides of α-aminophosphonic acid derivatives.

EXPERIMENTAL

Instrumentation

Chromatography was performed using an Anspec-Bischoff Model 2200 isocratic HPLC pump, a Rheodyne 7125 injector with 20-μl sample loop, a Milton Roy LDC UV Monitor D fixed-wavelength detector operating at 254 nm, and either an HP

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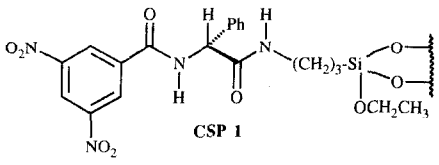
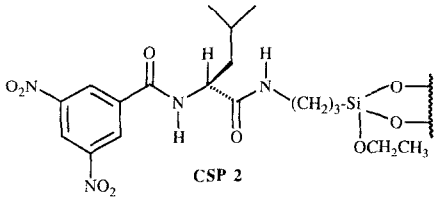
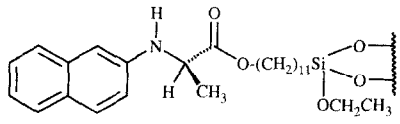






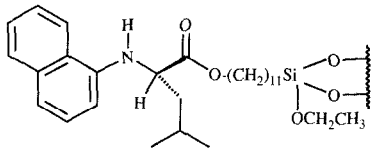


Fig. 1. Structures of CSPs.

3394A recording integrator or a Kipp & Zonen BD 41 dual-channel recorder. A Rudolph Autopol III with a 20-cm flow cell was used to monitor the sign of $[\alpha]_D$. The void volume was determined using tri-*tert*.-butylbenzene [7].

The ^1H NMR spectra were obtained using a Varian XL-200 (200 MHz) or GE GN-500 (500 MHz) spectrometers using tetramethylsilane (δ 0.00 ppm) as an internal reference. The ^{13}C NMR spectra were obtained on a GE GN-300NB (75 MHz) spectrometer using C^2HCl_3 (δ 77.0 ppm) as an internal reference. The ^{31}P NMR spectra were obtained using a GE GN-300NB (121 MHz) spectrometer with broad-band ^1H decoupling and referenced to external 85% H_3PO_4 (δ 0.00 ppm). The IR spectra were obtained on IBM IR-32 spectrometer. The mass spectra and microanalyses were obtained by the University of Illinois School of Chemical Sciences service laboratories.

Materials

· *Reagents.* The benzaldehyde, benzylamine, diphenyl phosphite and dimethyl phosphite were purchased from Aldrich and were distilled prior to use. All other reagents were used as received.

CSPs **1–4**. The CSPs **1–4** (Fig. 1) used in this study are available from Regis (Morton Grove, IL, USA).

*Diphenyl N-benzyl α-aminobenzyl phosphonate (**5**).* To a 100-ml round-bottomed flask equipped with Dean-Stark trap, reflux condenser, magnetic stir bar, and nitrogen inlet were added 3.00 g (28 mmol) of benzaldehyde, 40 ml of benzene, and 3.03 g (28 mmol) of benzylamine. The cloudy mixture was brought to reflux and, after 1 h, the benzene was removed by distillation. After the mixture cooled, the Dean-Stark trap was removed, 40 ml of dry tetrahydrofuran and 7.20 g (31 mmol) of diphenyl phosphite were added and the solution was brought to reflux under a nitrogen atmosphere. The progress of the reaction was monitored by ^{31}P NMR. After 36 h, the ratio of product (δ 16.70) to phosphite (δ 0.6) was 8:1. The solution was concentrated under reduced pressure and the residue was recrystallized from ethyl acetate–hexane (1:4, v/v) to give 7.90 g (65% yield) of colorless solid, m.p. 106–107°C. R_F (thin-layer chromatography) = 0.20 (silica/CH_2Cl_2). ^1H NMR (C^2HCl_3, 300 MHz) δ 2.50 br s 1H (exchanges with $2H_2O$) 1H; 3.75–4.0 two d (J = 54, 12.5 Hz) 2H; 4.37 d (J = 20.5 Hz) 1H; 6.80–7.6 m 20H. ^{31}P NMR (C^2HCl_3) δ 16.70. IR (KBr) 3279, 3028, 1591, 1489, 1454, 1265, 1215, 1192, 1161, 1093, 1070, 1024, 935 cm^{-1}. Mass spectrum (10 eV) *m/z* (relative intensity) 429 (0.7), 234 (100), 195 (74), 92 (52) Analysis, calculated for $C_{26}H_{24}NO_3P$: C, 72.71; H, 5.63; N, 3.23; P, 7.20; found: C, 72.71; H, 5.63; N, 3.25; P, 7.20.

*Diphenyl N-3,5-dinitrobenzoyl α-aminobenzyl phosphonate (**6**).* A solution of 0.5 g (1.16 mmol) of **5** in 10 ml of dry CH_2Cl_2 was poured into 30 ml of dry diethyl ether saturated with HCl (g) and allowed to stand at room temperature for 1 h. The cloudy suspension was then cooled to 0°C for 1 h and filtered. The collected solid, 0.494 g (m.p. 161–163 °C), was dissolved in 25 ml of dry methanol and poured over 0.1 g of 20% $Pd(OH)_2$ on carbon in a pressure bottle. The bottle was pressurized to 45 p.s.i. with hydrogen and rocked for 10 h on a Parr shaker. The catalyst was removed by filtration and

the filtrate was concentrated under reduced pressure. The ^{31}P NMR spectrum of the resulting oil has a peak at δ 11.4 ppm. The oil was dissolved in 50 ml of CH_2Cl_2 and 0.320 g (1.4 mmol) of 3,5-dinitrobenzoyl chloride was added, followed by 50 ml 1:1 water–satd. $NaHCO_3$ (1:1), and the two-phase mixture was magnetically stirred at room temperature for 1.5 h. The aqueous layer was removed and the organic layer was washed sequentially with 30-ml portions of 10% $NaHCO_3$, water, and saturated NaCl. The combined aqueous washings were extracted with two 10-ml portions of CH_2Cl_2. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica using CH_2Cl_2–diethyl ether (1:1), and the concentrated fractions were recrystallized from ethyl acetate–hexane (1:4, v/v) to give 420 mg (68% yield) of colorless solid, m.p. 209–210°C. R_F = 0.55 (silica/diethyl ether). ^1H NMR (C^2HCl_3, 200 MHz) δ 6.1–6.3 dd (J=21.5, 9.5 Hz) 1H; 6.6–6.7 m 2H; 7.0–7.4 m 11H; 7.6–7.7 m 2 H; 8.7–8.9 m 3H; 9.1–9.3 m 1H. ^{31}P (C^2HCl_3) δ 12.45. IR (KBr) 3279, 3080, 1643, 1542, 1489, 1344, 1210, 1183 cm^{-1}. Mass spectrum (70 eV) *m/z* (relative intensity) 440 (87), 300 (57), 195 (100), 149 (46), 140 (49), 94 (63). Analysis, calculated for $C_{26}H_{20}N_3O_8P$: C, 58.54; H, 3.78; N, 7.88; P, 5.81; found: C, 58.59; H, 3.74; N, 7.81; P, 5.72.

Diethyl N-3,5-dinitrobenzoyl α-aminobenzyl phosphonate. This compound was prepared following the procedure of Szewczyk *et al.* [8] using ethanol and **6**. Chromatographic purification and recrystallization from ethyl acetate–hexane (1:5, v/v) gives a colorless solid, m.p. 181–182°C. R_F = 0.33 (silica/diethyl ether). ^1H NMR (C^2HCl_3, 200 MHz) δ 1.1 t 3H; 1.32 t 3H; 3.6–3.8 m 1H; 3.85–4.05 m 1H; 4.1–4.35 m 2H; 5.7–5.9 two d (J=21.5, 9.5 Hz) 1H; 7.2–7.4 m 3H; 7.5–7.7 m 2H; 8.6-8.8 m 1H; 9.1–9.3 m 3H. ^{31}P NMR (C^2HCl_3) δ 20.64. IR (KBr) 3279, 3080, 2975, 1643, 1542, 1489, 1344, 1210, 1183 cm^{-1}. Mass spectrum (70 eV) *m/z* (relative intensity) 437 (1.8), 300 (73), 195 (100), 179 (20), 155 (28), 111 (38), 75 (57). Analysis, calculated for $C_{18}H_{20}N_3O_8P$: C, 49.42; H, 4.61; N, 9.61; P, 7.09; found: C, 49.66; H, 4.67; N, 9.46; P, 6.91.

Preparation of enantiomerically enriched samples

Dimethyl N-[-(S)-α-methylbenzyl]-α-aminobenzyl phosphonate. A 10:1 ratio of diasteromeric phosphonates was obtained from the procedure of Glowiak *et al.* [9] using benzaldehyde, (*S*)-α-methylbenzylamine and dimethyl phosphite. The stereochemistry of the major and minor diastereomers is known [9]. ^{31}P NMR major diastereomer (*S,R*) δ 27.28; minor diastereomer (*S,S*) δ 26.88; m.p. 83–84°C. ^1H NMR (C^2HCl_3, 200 MHz), δ 1.37 d (J=6.2 Hz) 3H; 2.12 broad s (exchanges with $2H_2O$) 1H; 3.50 d (J=10.6Hz) 3H; 3.80 m 4H; 4.12 d (J=20.7 Hz) 1H 7.25–7.5 m 10H. Analysis, calculated for $C_{17}H_{22}NO_3P$: C, 63.93; H, 6.95; N, 4.46; P, 9.70; found: C, 64.14; H, 7.15; N, 4.46; P, 9.51.

Dimethyl N-[-(S)-α-methylbenzyl]-α-aminoisobutyl phosphonate. Freshly distilled isobutyraldehyde, 5.15 g (71 mmol), was placed in a 100 ml flask with 10 g of anhydrous $MgSO_4$, 30 ml of dry CH_2Cl_2 and cooled to 0°C. The *S*-(−)-α-methylbenzylamine 8.65 g (72 mmol) was added dropwise and the mixture was stirred overnight at room temperature. Distilled dimethyl phosphite (86 mmol) was added and the resulting mixture was stirred for 72 h. The reaction mixture was filtered and the filtrate was diluted with 100 ml of CH_2Cl_2. The CH_2Cl_2 was washed with 50 ml of water and the organic layer was dried with anhydrous $MgSO_4$. After removal of the drying agent by filtration, the filtrate was concentrated under reduced pressure using a rotary evaporator to afford 19.0 g of pale yellow oil (95% yield). The diastereomeric ratio of 3:1 was determined by ^{31}P NMR analysis: major diastereomer (*S,R*) δ 33.0, minor diastereomer (*S,S*) δ 31.4. The stereochemistry of the major and minor diastereomers is known [9]. R_F = 0.28 (silica/diethyl ether). ^1H NMR (C^2HCl_3, 500 MHz), major diastereomer δ 0.84 m 3H; 0.94 m 3H; 1.32 m 3H; 1.6 broad s 1H; 2.00 m 1H; 2.57 dd (J=18.6, 6.8 Hz) 1H; 3.78 m 6H; 4.1 m 1H; 7.2–7.5 m 10H; minor diastereomer δ 1.00 d (J=7.0 Hz) 3H; 1.10 d (J=6.4 Hz) 3H; 1.6 broad s 1H; 2.2 m 1H; 2.80 dd (J=18.6, 2.7 Hz) 1H; 3.6–3.8 m 6H; 4.02 m 1H; 7.2–7.5 m 10 H. ^{13}C NMR (C^2HCl_3, 75 MHz) major diastereomer δ 17.13, 20.21, 20.41, 24.58, 28.73, 28.79, 51.70, 51.8, 56.0, 127.8, 127.12, 144.42; minor diastereomer δ 17.95, 19.99, 20.26, 23.49, 28.24, 28.30, 52.3, 52.4, 57.17, 126.0, 126.83, 144.80. IR (neat 3316, 3026, 2957, 2874, 1454, 1246, 1180, 1122, 1099, 1057 cm^{-1}. Mass spectrum 70 eV *m/z* (relative intensity) 285 (1.8), 176 (72), 120 (29), 105

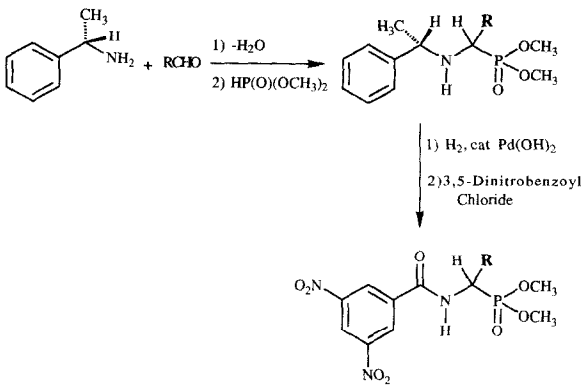


Fig. 2. Preparation of configurationally known samples. R = phenyl; isopropyl.

(93), 72 (100). Analysis, calculated for $C_{14}H_{24}NO_3P$: C, 58.93; H, 8.48; N, 4.91; P, 10.86. Found: C, 58.79; H, 8.55; N, 4.88; P, 10.73.

Preparation of (R)-enriched dimethyl N-3,5-dinitrobenzoyl α-amino phosphonates samples of known absolute configuration

The (*R*)-enriched samples were prepared from each of the preceding diastereomeric mixtures. If one uses (*S*)-α-methylbenzylamine, the major diastereomer formed has the (*R*) configuration at the stereocenter adjacent to phosphorus [9]. The ratio of diastereomers produced when (*S*)-α-methylbenzylamine was used to form the imine was monitored using ^{31}P NMR and ^{1}H NMR (Fig. 2). After hydrogenolysis of the α-methylbenzyl group using Pd $(OH)_2$ as a catalyst, N-acylation of the resulting amines with 3,5-dinitrobenzoyl chloride and analysis of the amide derivatives on a CSP, the observed enantiomeric excess was the same as the starting diastereomeric excess. These materials show the same chromatographic behavior on the CSPs and silica thin-layer plates as the corresponding racemates.

RESULTS AND DISCUSSION

A series of dimethyl esters of alkyl and aryl substituted N-(3,5-dinitrobenzoyl)-α-amino phosphonates were available from prior studies. In order to investigate the role played by the alkoxyl portion of these phosphonates in chiral recognition, a homologous series of diesters of the 3,5-dinitrobenzamide of α-aminobenzyl phosphonate was prepared by transesterification of the diphenyl phosphonate **6**. Addition of diphenyl phosphite to the imine derived from benzaldehyde and benzylamine gives N-benzyl protected diphenyl α-aminobenzyl phosphonate **5** (Fig. 3). Hydrogenolysis of **5** using $Pd(OH)_2$ on carbon gives the α-amino phosphonate which can be N-acylated with 3,5-dinitrobenzoyl chloride to give the 3,5-dinitrobenzamide of diphenyl α-aminobenzyl phosphonate **6**. The various diesters **7** were obtained from **6** following a reported procedure [8]. The elution orders of the enantiomers of α-phenyl- and α-isopropyl-substituted dimethyl α-amino phosphonates were rigorously established using the (*R*)-enriched samples.

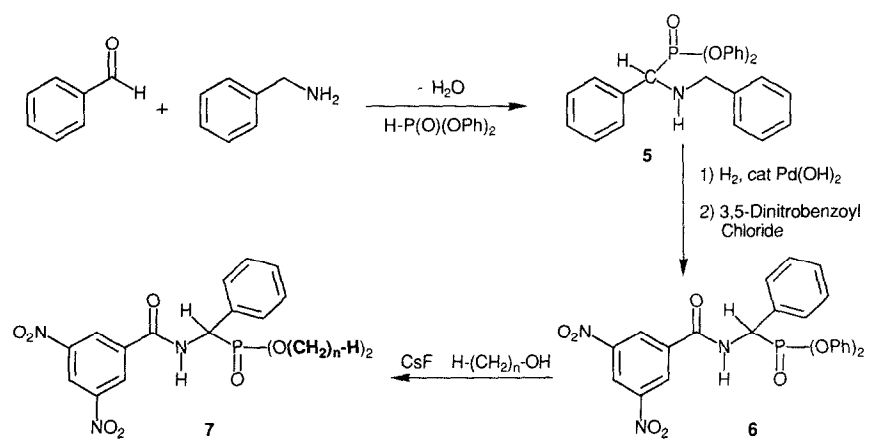
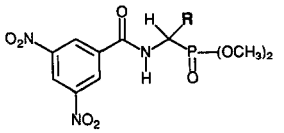


Fig. 3. Preparation of the dialkyl esters of N-(3,5-dinitrobenzoyl)-α-aminobenzyl phosphonic acid. ph = phenyl.

TABLE I

COMPARISON OF THE ABILITY OF CSPs **1** AND **2** TO SEPARATE THE ENANTIOMERS OF DIMETHYL α-AMINO-PHOSPHONATES AS THE 3,5-DINITROBENZAMIDE DERIVATIVES



α = Chromatographic separation factor; k'_1 = capacity factor for the first eluted enantiomer using 20% (v/v) isopropyl alcohol in hexane as the mobile phase, flow-rate 2 ml/min; the detector was operating at 254 nm. The $[\alpha]_D$ column gives the sign of $[\alpha]_D$ of the second eluted enantiomer using a polarimetric detector; the letter refers to the absolute configuration of the second eluted enantiomer.

R	(*R*)-**1**			(*S*)-**2**		
	α	k'_1	$[\alpha]_D$	α	k'_1	$[\alpha]_D$
Phenyl	1.67	12.44	(−) *R*	1.40	5.14	(+) *S*
2-Tolyl	1.50	8.57	(−)	1.18	3.55	(+)
3-Tolyl	1.63	10.07	(−)	1.44	4.45	(+)
4-Tolyl	1.63	11.27	(−)	1.52	4.47	(+)
Mesityl	1.00	9.86		1.00	3.43	
p-Cymyl	1.47	7.88	(−)	1.31	3.18	(+)
4-Chlorophenyl	1.70	12.13	(−)	1.62	4.63	(+)
4-Allyloxyphenyl	1.66	16.8	(−)	1.40	6.85	(+)
α-Naphthyl	1.39	22.7	(−)	1.49	6.23	(+)
β-Naphthyl	1.88	31.4	(−)	2.61	8.82	(+)
4-Nitrophenyl	1.62	37.7	(−)	1.42	10.9	(+)
Isopropyl	1.44	6.86	(+) *R*	1.00	3.65	
Isobutyl	1.67	5.43	(+)	1.00	2.78	
tert.-Butyl	1.37	5.94	(+)	1.00	3.13	
1,1-Dimethyl-3-butenyl	1.39	5.34	(+)	1.00	2.72	

π-Acidic CSPs

The ability of π-acidic CSPs (*e.g.* **1** and **2**) to separate the enantiomers of a wide clientele of analytes has been extensively documented [10]. While these CSPs were designed to separate the enantiomers of π-basic analytes, they often suffice to separate the enantiomers of π-acidic analytes, typically with reduced levels of enantioselectivity [11]. Although not yet studied in detail, the interactions employed by these CSPs to "recognize" the stereochemistry of π-acidic analytes are not, in totality, those employed for π-basic analytes. The separation of the enantiomers of π-acidic analytes on π-acidic CSPs is frequently observed and has also been noted by Caude *et al.* [12].

After chromatographing the 3,5-dinitrobenzamides of a series of α-aryl- and α-alkyl-substituted dimethyl α-amino phosphonates on **1** and **3**, certain structure–activity relationships become apparent (Table I and Fig. 4). Perusal of the data reveals that

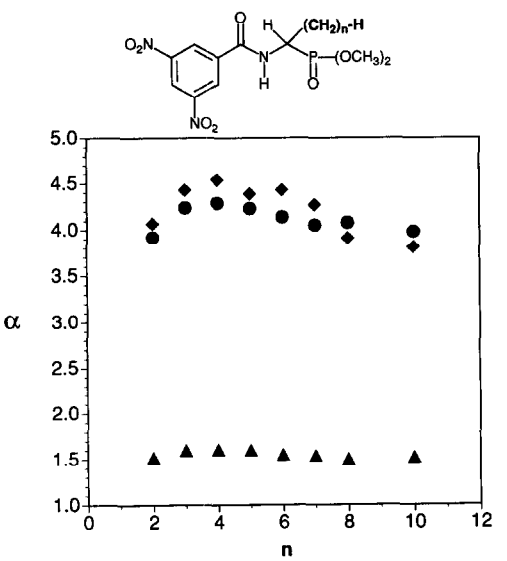


Fig. 4. The relationship between α and the number of methylene groups, *n*, in the alkyl group of dimethyl N-(3,5-dinitrobenzoyl)-α-aminoalkyl phosphonates using 20% isopropyl alcohol in hexane as the mobile phase. ◆ = CSP **3**; ● = CSP **4**; ▲ = CSP **1**.

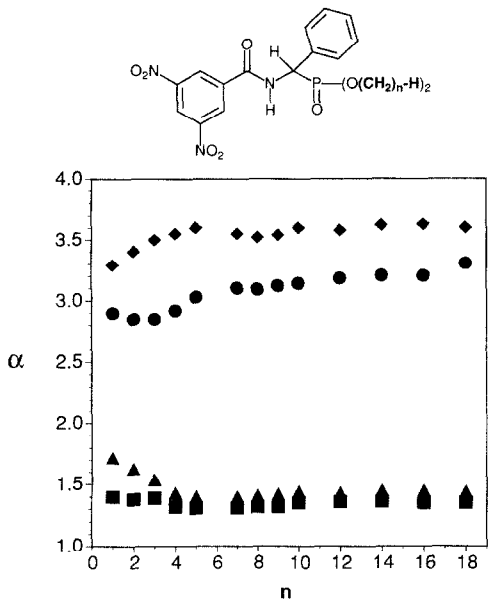


Fig. 5. The dependence of the separation factors upon the number of methylene groups, *n*, in the alkoxyl portion of N-(3,5-dinitrobenzoyl)-α-aminobenzyl phosphonates using 20% isopropyl alcohol in hexane as the mobile phase. ◆ = CSP **3**; ● = CSP **4**; ▲ = CSP **1**; ■ = CSP **2**.

1 more effectively separates these enantiomers than does **2**. Moreover, **2** is not effective for the separation of the enantiomers of 3,5-dinitrobenzamides of dimethyl α-amino-α-alkyl phosphonates. The enriched samples were used to relate the sign of the specific rotation to the absolute configuration of the dimethyl N-(3,5-dinitrobenzoyl)-α-amino phosphonates. Note from Table I that the more retained enantiomers within each of the series of 3,5-dinitrobenzamides of dimethyl α-amino phosphonates have the same sign of rotation. The more retained enantiomers in the alkyl series differ in their sign of specific rotation from their α-aryl counterparts even though they have the same absolute configuration. Using **1**, the more retained enantiomer of each member of the linear alkyl series of analytes included in Fig. 4 is dextrorotatory. The relative lack of dependence of the separation factor upon the length of alkoxyl groups of the phosphonate ester suggests that this phase has considerable generality for most esters of these analytes (Fig. 5).

Note, this CSP–analyte combination contains several possible interaction sites which may or may not be involved in the chiral recognition process. Although there are a number of plausible absorbates which may be responsible for the observed enantioselectivity, no single chiral recognition mechanism presently seems capable of rationalizing all of the structure–activity relationships observed with CSPs **1** and **2**. Even so, these CSPs are useful in determining the enantiomeric purities of members of this class of analytes.

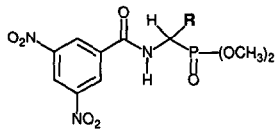
π-Basic CSPs

The π-basic CSPs, **3** and **4**, prepared from the undecenyl esters of (*R*)-N-2-(naphthyl)alanine and (*S*)-N-1-(naphthyl)leucine respectively, effectively distinguish the enantiomers of suitably functionalized analytes [13]. For example, the separation factor for the enantiomers of the 1-adamantyl amide of N-(3,5-dinitrobenzoyl)leucine is almost 60 on **4** [14]. The chiral recognition mechanisms utilized by this class of CSPs have been extensively investigated for the N-3,5-dinitrobenzoyl α-amino amides and esters using various solution techniques such as ^{1}H NMR chemical shift non-equivalence–nuclear Overhauser effects and UV–VIS absorption studies using mixtures of the analyte and a soluble analogue of the CSP **3** [15]. X-ray crystallographic analysis of a 1:1 co-crystal of the chiral selector and an analyte shows that the interactions proposed for the more stable diastereomeric complex are also present in the solid state [16]. In the model proposed to account for the observed enantioselectivity, one of the essential interactions is a hydrogen bond between the C-terminal carbonyl oxygen of the analyte and the amino N–**H** of the selector. Since, in the phosphorus analogues of α-amino acids, one has simply replaced the C-terminal carboxyl group with the phosphonate group, one expects essentially the same chiral recognition process to operate. Since the carboxamide and phosphonate groups differ in basicity and possibly conformational disposition, the levels of enantioselectivity may differ somewhat for the two analyte classes.

The data in Table II allow one to compare the abilities of **3** and **4** to separate the enantiomers of a variety of dimethyl α-amino phosphonates as the 3,5-dinitrobenzamide derivatives and show the order of elution of the configurationally known samples. The π-basic CSPs used in this study are of opposite configurations and the elution orders observ-

TABLE II

COMPARISON OF THE ABILITY OF CSPs 3 AND 4 TO SEPARATE THE ENANTIOMERS OF DIMETHYL α-AMINOPHOSPHONATES AS THE 3,5-DINITROBENZAMIDE DERIVATIVES



Symbols as in Table I.

R	(*R*)-3			(*S*)-4		
	α	k'_1	$[\alpha]_D$	α	k'_1	$[\alpha]_D$
Phenyl	3.29	4.34	(+) *S*	2.90	5.11	(−) *R*
2-Tolyl	3.08	3.71	(+)	3.04	3.68	(−)
3-Tolyl	3.37	3.87	(+)	3.26	4.07	(−)
4-Tolyl	3.35	3.93	(+)	2.95	4.38	(−)
Mesityl	2.03	3.16	(+)	1.92	3.28	(−)
p-Cymyl	3.04	3.14	(+)	2.28	3.52	(−)
4-Chlorophenyl	3.35	4.07	(+)	3.07	4.77	(−)
4-Allyloxyphenyl	3.11	5.00	(+)	2.87	5.25	(−)
α-Naphthyl	3.05	5.07	(+)	2.81	5.53	(−)
β-Naphthyl	3.55	5.18	(+)	3.49	5.72	(−)
4-Nitrophenyl	3.41	8.27	(+)	2.93	11.3	(−)
Isopropyl	3.33	2.94	(−) *S*	2.90	3.64	(+) *R*
Isobutyl	4.89	2.57	(−)	4.66	2.98	(+)
tert.-Butyl	2.72	2.57	(−)	3.60	2.55	(+)
1,1-Dimethyl-3-butenyl	2.82	2.43	(−)	3.83	2.52	(+)

ed are those expected from the chiral recognition mechanism. This mechanism accounts for the observation that (*R*)-**3** or **4** preferentially retains the (*R*)-enantiomer of the α-amino acid derivative [15]. Owing to Cahn–Ingold–Prelog priority sequence, the (*S*)-α-amino phosphonate derivative is mechanistically equivalent to the (*R*)-α-amino acid derivative [*i.e.*, the (*S*)-enantiomer of the α-amino phosphonate derivative would be more strongly retained on (*R*)-**3** or **4**]. This is what is observed.

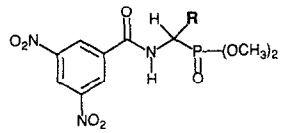
Even those sterically congested analytes which contain mesityl, 2-tolyl, *tert.*-butyl, or 1,1-dimethyl-3-butenyl [17] substituents elute in the predicted order, thus providing additional evidence of the mechanistic generality of these phases. The ability of **3** and **4** to separate the enantiomers of the homologous series of alkyl substituted N-(3,5-dinitrobenzoyl)-α-amino phosphonates is shown in Fig. 4. The magnitudes of the separation factors depend only slightly upon the lengths of the α-alkyl substituents, the effect being greater for the early members of the series. Data for the series of the various alkyl diesters of N-(3,5-dinitrobenzoyl)-α-aminobenzyl phosphonate are shown in Fig. 5. Again, the separation factors depend but slightly upon the lengths of the alkoxyl groups. The relative insensitivity of the separation factors to structural changes in "non-essential" (*i.e.* those not used in chiral recognition) portions of the analyte attests to the generality of the chiral recognition process.

The recognition process utilized by these CSPs in the present instances in believed to be essentially that proposed for the 3,5-dinitrobenzamides of α-amino acid derivatives [15]. Consistent with the proposed face to face π–π interaction between the π-acidic 3,5-dinitrobenzoyl portion of the analyte and the π-basic naphthyl portion of **3** or **4** is the observation that the enantioselectivities shown by the less π-acidic *p*-nitrobenzamide derivatives are greatly diminished.

In the case of the 3,5-dinitrobenzamide derivatives, reversed-phase separation on **3** and **4** are pos-

TABLE III

COMPARISON OF THE ABILITY OF CSPs 3 AND 4 TO SEPARATE THE ENANTIOMERS OF DIMETHYL α-AMINOPHOSPHONATES AS THE 3,5-DINITROBENZAMIDE DERIVATIVES USING METHANOL–WATER (4:1) AS THE MOBILE PHASE



α = Chromatographic separation factor; k'_1 = capacity factor for the first eluted enantiomer using 20% water in methanol (v/v) as the mobile phase, flow-rate 2 ml/min; the detector was operating at 254 nm. In the sense column, the letter refers to the absolute configuration of the second eluted enantiomer.

R	(*R*)-3			(*S*)-4		
	α	k'_1	Sense	α	k'_1	Sense
Phenyl	1.62	1.77	*S*	1.57	2.82	*R*
2-Tolyl	1.68	1.14		1.66	3.53	
3-Tolyl	1.75	1.39		1.73	3.51	
4-Tolyl	1.68	1.41		1.60	3.56	
Mesityl	1.33	1.94		1.31	5.35	
p-Cymyl	1.75	1.89		1.59	5.48	
4-Chlorophenyl	1.71	1.37		1.64	3.76	
4-Allyloxyphenyl	1.71	1.72		1.60	4.69	
α-Naphthyl	1.73	2.37		1.70	6.16	
β-Naphthyl	1.83	2.10		1.80	5.59	
4-Nitrophenyl	1.56	1.22		1.46	3.34	
Isopropyl	1.51	0.63	*S*	1.60	1.33	*R*
Isobutyl	1.95	0.77		2.22	1.79	
tert.-Butyl	1.36	0.64		1.62	1.37	
1,1-Dimethyl-3-butenyl	1.40	0.77		1.75	1.89	

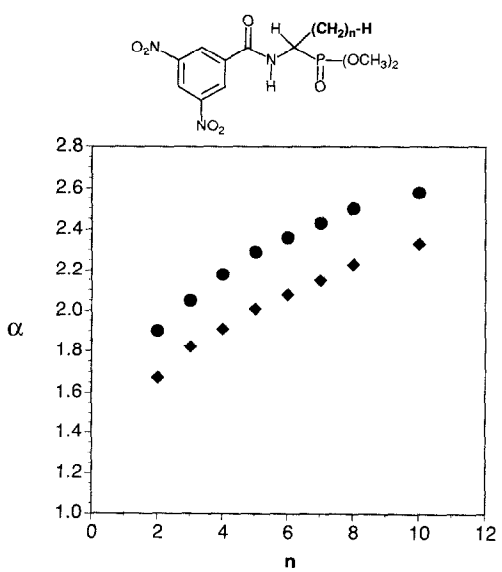


Fig. 6. The dependence of the separation factors upon the number of methylene groups, *n*, in the alkyl portion of dimethyl N-(3,5-dinitrobenzoyl)-α-aminoalkyl phosphonates using 20% water in methanol as mobile phase. ● = CSP 4; ◆ = CSP 3.

sible. Using a mobile phase of methanol–water (4:1), both retention and enantioselectivity are reduced although separation factors of the enantiomers still typically exceed 1.5 (Table III and Fig. 6). Elution orders are the same for both reversed and normal mobile phases. Notice that, for the α-alkyl series of analytes, enantioselectivity increases as the lengths of the alkyl groups on the stereogenic centers of the analytes increase. This is also observed for the corresponding phosphonic acid analytes using these phases and ion-pairing reagents. [18].

CONCLUSION

The use of four commercially available CPSs to determine enantiomeric excess and to relate elution order to the absolute configuration of the enantiomers of a variety of N-(3,5-dinitrobenzoyl)-α-amino phosphonates has been described. These phases show broad scope and levels of enantioselectivity adequate for preparative separations as well as analytical separations.

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