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Facile separation of the enantiomers of diethyl N-(aryl)-1-amino-1-arylmethanephosphonates on a rationally designed chiral stationary phase

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Abstract

Separation of the enantiomers of each member of a series of diethyl N-(aryl)-1-amino-1-arylmethanephosphonates is easily accomplished by HPLC using a WHELK-O column. This totally synthetic chiral stationary phase (CSP 1) is designed to utilize specifiable interactions to differentiate between enantiomers. The structural features of these phosphonates suggested that CSP 1 would be applicable to the separation of the enantiomers of this class of compounds even though there was an element of uncertainty owing to the presence of two π-basic interaction sites, one on either side of the stereogenic center. The structures of the π-basic N-aryl and C-aryl substituents have been varied, the structure of the latter being found to have the greatest effect on retention and enantioselectivity.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Diethyl N-(aryl)-1-amino-1-arylmethanephosphonates; N-Aryl-α-aminophosphonic acid esters

1. Introduction

N-Aryl-α-aminophosphonic acids and esters (Fig. 1) are useful as antifungal [1–3] and antibiotic agents [4–6] and, more recently, in the treatment of osteoporosis [7,8]. Because the bioactivity of compounds typically depends on their absolute configuration as well as structure, a general method for the optical resolution of phosphonates of this type would be significant. Some related α-aminophosphonates have been obtained in chiral nonracemic form either by "classical" separations of diastereomeric salts [9]

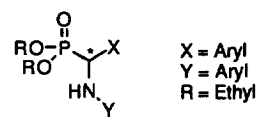

Fig. 1. General structure of the N-(aryl)-1-amino-1-arylmethanephosphonates used in this study.

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or peptide complexes [10] or by asymmetric synthesis [11–15]. The most direct approach for conveniently obtaining pure samples of each enantiomer of these phosphonates is to chromatographically resolve the racemate on a chiral stationary phase (CSP) which affords an appreciable level of enantioselectivity and can be used in both analytical and preparative applications.

These phosphonates are structurally similar to esters of N-aryl-α-amino acids, compounds previously resolved on several π-acidic CSPs. Indeed, separation of the enantiomers of a series of fluorinated N-(aryl)-1-amino-1-arylmethane phosphonates by high-performance liquid chromatography (HPLC) on covalently bonded brush-type α-Burke-1 and β-Gem CSPs as well as on polysaccharide-derived CSPs has been reported [7,16]. Because brush-type CSPs are typically "functional group selective" rather than size or shape selective, it was thought likely that these phosphonates would meet the requirements for stereodifferentiation by another π-acidic CSP developed in these laboratories [17–19]. This CSP, CSP 1, utilizes π-basic and hydrogen bond acceptor groups located on or near the stereogenic center(s) of the analytes in the stereodifferentiation process (Fig. 2). While these phosphonates contain π-basic aromatic rings and a phosphonyl group (to serve as a hydrogen bond acceptor) on the stereogenic center, prediction of the extent and sense of enantioselectivity was complicated by the presence of the two potential π-basic substituents (i.e. the C-aryl and N-aryl groups) on the stereogenic center. Which of these groups would afford the stronger $\pi-\pi$ interaction with the selector was not always evident a priori. Whichever affords the stronger interaction with the π-acid portion of CSP 1 was expected to determine both the elution order and extent of enantiodifferentiation. For example, if the best retained enantiomer utilized one of these π-basic groups as an interaction site and the least retained enantiomer similarly utilized the other, it would result in increased retention of the least retained enantiomer and reduced enantioselectivity, as judged by the magnitude of the separation factor of the enantiomers.

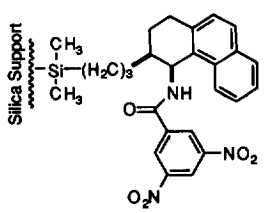

Fig. 2. CSP 1.

2. Experimental

2.1. Apparatus

Chromatographic analysis was performed using an Alcott Model 760 pump, a Rheodyne Model 7125 injector with a 20-μl sample loop, a Milton-Roy uvMonitor D fixed-wavelength detector (254 nm), and a Hewlett-Packard HP 3394A integrating recorder. Nuclear magnetic resonance (NMR) spectra were collected on a Varian Unity 400 spectrophotometer with C^2HCl_3 used as the solvent. Chemical shifts for ^1H are reported in ppm with $CHCl_3$ used as an internal standard. Elemental analysis was performed by the University of Illinois Microanalytical Facility. Melting points were obtained in open capillary tubes on a Buchi melting point apparatus and are uncorrected.

2.2. Materials

All chromatographic solvents were HPLC grade from EM Science. Diethyl phosphite, 2-*tert.*-butylaniline, and 3,5-dimethylaniline, purchased from Aldrich, were distilled prior to use. 1-Naphthaldehyde, 2-naphthaldehyde, 2-fluorenylaldehyde, and 4-(dimethylamino)benzaldehyde were similarly purchased and used without further purification. Tetrahydrofuran was distilled from sodium/benzophenone. Hexane and dichloromethane were distilled from calcium hydride. Analytical chromatography was performed on an (*S,S*)-Whelk-O-1 column (25 × 4.6 mm, 5-μm spherical silica particles with 100-Å pore size), the commercial version of CSP 1. Preparative chromatography was performed on a "semi-prep" (*S,S*)-Whelk-O-1 column (250 × 10

mm, 10-μm irregular silica particles with 100-Å pore size). Both columns were obtained from Regis Technologies (Morton Grove, IL, USA).

2.3. Methods

Analytical chromatography was carried out at a nominal flow-rate of 2 ml/min at ambient temperature. The void volume was determined by injection of 1,3,5-tri-*tert.*-butyl benzene. Preparative separations were carried out at a nominal flow-rate of 4 ml/min at ambient temperature. Phosphonates **2–5** (see Table 1 for structure of the different phosphonates) have been described previously [16], whereas characterization of compounds **1**, **6**, and **7**, prepared in a similar manner, will be reported elsewhere. A discussion of general synthetic methodology is given by Majer [20].

*Diethyl 1-(N-3″,5″-dimethylphenyl)-1-amino-1-(2′-naphthyl)-methanephosphonate (**8**)*

A flame-dried, one-necked, 100-ml flask equipped with a reflux condenser was charged with dry benzene (10 ml). 1-Naphthaldehyde (2 g, 12.81 mmol) and 3,5-dimethylaniline (1.55 g, 12.8 mmol) were added, along with 3-Å molecular sieves (ca. 1.5 g). The mixture was heated to reflux for 6 h, allowed to cool, and decanted into a one-necked, 100-ml flask. The reaction flask and molecular sieves were rinsed with benzene (20 ml), and the rinsings were added to the decantate. Boron trifluoride etherate (100 μl) and diethyl phosphite (1.9 g, 14.1 mmol.) were added, and a slight exotherm was observed. After the mixture had been stirred for 5 min, a white precipitate formed and was collected by suction filtration. After recrystallization from benzene, 2.41 g of **8** was obtained as a white powder; m.p.: 190–192°C; ^{1}H NMR: 8.26 (d, J = 8.4, 1H); 7.89 (d, J = 8.1, 1H); 7.78 (d, J = 7.5, 1H), 7.77 (d, J = 7.0, 1H); 7.61 (t, J = 6.8, 1H); 7.53 (t, J = 7.9, 1H); 7.44 (t, J = 7.7, 1H) 6.32 (s, 1H); 6.21 (s, 2H); 5.63 (d, J = 24, 1H); 5.25–5.20 (bs, 1H); 4.22–4.13 (m, 2H); 3.77-3.66 (m, 1H), 3.22–3.12 (m, 1H); 2.10 (s, 6H); 1.32 (t, J = 7.3, 3H); 0.71 (t, J = 7.3, 3H). Analysis ($C_{23}H_{28}NO_3P$): calc. for: C: 69.51, H: 7.10, N: 3.52, P: 7.79; found: C: 69.61, H: 7.10, N: 3.55, P: 7.72.

*Diethyl 1-(N-3″,5″-dimethylphenyl)-1-amino-1-(2′-naphthyl)-methanephosphonate (**9**)*

A flame-dried, one-necked, 100-ml flask equipped with a reflux condenser was charged with dry benzene (10 ml). 2-Naphthaldehyde (2 g, 12.81 mmol) and 3,5-dimethylaniline (1.55 g, 12.8 mmol) were added, along with 3-Å molecular sieves (ca. 1.5 g). The mixture was heated to reflux for 6 h, allowed to cool, and decanted into a one-necked, 100-ml flask. The reaction flask and molecular sieves were rinsed with benzene (20 ml), and the rinsings were added to the decantate. Boron trifluoride etherate (100 μl) and diethyl phosphite (1.9 g, 14.1 mmol.) were added, and a slight exotherm was observed. After being stirred 5 min, the white precipitate which had formed was collected by suction filtration. After recrystallization from benzene, 2.53 g of **9** was obtained as a white powder; m.p.: 148–149°C; ^{1}H NMR: 7.93 (t, J = 1.9, 1H), 7.83–7.80 (m, 3H), 7.60 (dt, J = 8.7, 1.7, 1H), 7.47–7.45 (m, 2H), 6.35 (s, 1H), 6.25 (s, 1H), 5.26–5.21 (bs (1H); 4.92 (d, J = 24.5, 1H), 4.04–4.12 (m, 1H), 3.87–3.97 (m, 1H), 3.65 (ddq, J = 1.8, 18.4, 7.2, 1H), 2.14 (s, 6H), 1.28 (t, J = 7.2, 3H), 1.08 (t, J = 7.2, 3H). Analysis ($C_{23}H_{28}NO_3P$): calc. for: C: 69.51, H: 7.10, N: 3.52, P: 7.79; found: C: 69.44, H: 7.12, N: 3.52, P: 7.72.

*Diethyl 1-[N-(2″-tert-butyl)-phenyl]-1-amino-1-(2′-fluorenyl)-methanephosphonate (**10**)*

A flame-dried, one-necked, 100-ml flask equipped with a reflux condenser was charged with 10 ml of dry benzene and 2-*tert.*-butylaniline (1 g, 6.67 mmol), 2-fluorenylaldehyde (1.3 g, 6.67 mmol), and 1.5 g of 3-Å molecular sieves. The mixture was heated to reflux for 8 h, cooled, and decanted into a one-necked, 100-ml flask. The reaction flask and molecular sieves were rinsed with 20 ml of benzene, which were added to the reaction mixture. The benzene was removed by rotary evaporation, and a magnetic stirring bar and diethyl phosphite (1.02 g, 7.44 mmol 1.1 equiv.) in 10 ml of dry benzene were added to the flask. After the mixture had been heated to

reflux for 12 h, the benzene was removed by rotary evaporation. Column chromatography (silica gel, 3:1, hexane–ethyl acetate) and recrystallization of the resulting yellow solid from hexane/tetrahydrofuran afforded 1.23 g of **10** as a slightly yellow solid; m.p.: 98.5–102°C; ^{1}H NMR: 7.53 (d, J = 5.9, 1H), 7.50 (d, J = 7.8, 1H), 7.66 (s, 1H), 7.53 (d, J = 6.6, 1H), 7.51 (d, J = 8.8, 1H), 7.36 (t, J = 7.1, 1H), 7.29 (t, J = 7.3, 1H), 7.27 (d, J = 8.3, 1H), 6.93 (t, J = 7.6, 1H), 6.66 (t, J = 7.8, 1H), 6.42 (d, J = 8.0, 1H), 5.28–5.23 (bs, 1H), 4.87 (d, J = 24.1, 1H), 4.14–4.01 (m, 2H), 4.00–3.92 (m, 1H), 3.89 (s, 1H), 3.87 (s, 1H), 3.77–3.68 (m, 1H), 1.57 (s, 9H), 1.28 (t, J = 7.1, 3H), 1.38 (t, J = 7.1, 3H). Analysis ($C_{28}H_{34}NO_3P$):

calc. for: C: 72.55, H: 7.39, N: 3.02, P: 6.68; found: C: 72.67, H: 7.38, N: 3.01, P: 6.64.

*Diethyl-1-(N-3″,5″-dimethylphenyl)amino-1-(4′-N,N-dimethylaminophenyl)-methanephosphonate (**11**)*

A flame-dried, one-necked, 100-ml flask equipped with a reflux condenser was charged with 10 ml of dry benzene, 4-(dimethylamino)benzaldehyde (1.6 g, 10.72 mmol), 3,5-dimethylaniline (1.30 g, 10.72 mmol), and 1.5 g of 3-Å molecular sieves. The mixture was heated to reflux for 16 h, cooled, and decanted into a one-necked, 100-ml flask. The reaction flask and molecular sieves were rinsed with 20 ml of benzene, these rinsings being added to the flask. The benzene was removed by rotary evaporation, and a magnetic stirring bar and diethylphosphite (1.63 g, 11.8 mmol) in 10 ml of dry benzene were added. The mixture was heated to reflux for 15 min, by which time the reaction mixture had solidified. Recrystallization of the slightly orange solid from hexane/tetrahydrofuran afforded 3.16 g of **11** as a white powder; m.p.: 166–117°C; ^{1}H NMR: 7.80 (d, J = 6.7, 2H), 6.68 (d, J = 7.5, 2H), 6.33 (s, 1H), 6.25 (s, 2H), 4.66 (d, J = 23.6, 1H), 5.29–5.25 (bs, 1H); 4.61–4.54 (bs, 1H), 4.17–4.02 (m, 2H), 3.98 (m, 1H), 3.71–3.61 (m, 1H), 2.92 (s, 6H), 2.16 (s, 6H), 1.27 (t, J = 7.11, 3H), 1.13 (t, J = 6.9, 3H). Analysis ($C_{21}H_{31}N_2O_3P$): calc. for: C: 64.60, H: 8.00, N: 7.17, P: 7.93; found: C: 64.53, H:8.03, N: 7.15, P: 7.85.

*Preparative scale resolution of phosphonate **11***

Fifty mg of phosphonate **11** was dissolved in THF (0.7 ml). The solution was introduced onto the preparative Whelk-O-1 column using a 1-ml injection loop. Elution with 5% 2-propanol in hexane completely separated the enantiomers of **11** as determined by the ultraviolet detector and by assay of each of the collected fractions on the analytical Whelk-O-1 column.

3. Results and discussion

Racemic phosphonates **1–7** were chromatographed on CSP 1 using hexane–2-propanol as a mobile phase, all being readily resolved. Data from this study are presented in Table 1. The separation and resolution factors observed are greater than those reported for the polysaccharide and GEM-1 CSPs. From these data, one can see that the degree of enantiomer separation is more heavily influenced by the C-aryl substituent than by the N-aryl substituent. As the

Table 1
Chromatographic data for the separation of compounds **1–7** on CSP 1

Compound	X	Y	k_1'	α	R_s
1	p-$CH_3OC_6H_4$	C_6H_5	2.26	1.5	4.95
2	C_6H_6	p-CF_3OC_6H4	4.72	1.26	2.14
3	C_6H_5	C_6H_5	4.26	1.26	2.73
4	C_6H_5	o-$CF_3OC_6H_4$	4.10	2.08	2.76
5	3,4-$(F)_2C_6H_3$	C_6H_5	4.09	1.31	2.25
6	1-Naphthyl	o-$CF_3OC_6H_5$	6.05	1.63	6.10
7	2-Naphthyl	Benzyl	9.61	1.75	7.77

Mobile phase: hexane–2-propanol (95:5, v/v); t_0 = 1.61 min.

π-basicity of the C-aryl substituent increases, the α values increase (compare entries **1** and **3**), whereas variation of the N-aryl groups does not necessarily alter enantioselectivity (compare entries **2** and **3**). Compound **4** contains the same C-aryl group as **2** and **3**, and it is not the most π-basic of the C-aryl substituents. However, the enantiomers of **4** show the largest of the separation factors given in Table 1. In addition to the electronic considerations, there are steric components in the chiral recognition process. The *ortho*-trifluoromethoxyl group of **4** undoubtedly alters the conformation of the N-aryl group relative to the remainder of the phosphonate so to increase the retention of the more retained enantiomer. If the increased enantioselectivity was electronic in origin, then phosphonate **2**, which has its trifluromethoxyl group in the *para*-position, would be expected to behave similarly. Phosphonate **6** contains the more π-basic α-naphthyl substituent and shows improved enantioselectivity although still less than **4**. This may seem to be at odds with the contention that enantioselectivity depends heavily on the π-basicity of the C-aryl group and, accordingly, will be discussed subsequently. Finally, compound **7** was prepared in order to gauge the effect of removing the N-aryl group from the phosphonate structure. As the data show, this has little effect on the enantioselectivity of **11**, further attesting that it is the C-aryl group which is most essential to the enantiodiscrimination process.

Motivated by these findings, several phosphonates bearing different C-aryl substituents were synthesized and fully characterized. As anticipated from the results in Table 1, phosphonates **8–11** (readily prepared through the addition of diethyl phosphite across the carbon–nitrogen double bond of imines formed by condensation of an aromatic aldehyde with an aromatic amine), all of which bear relatively π-basic C-aryl groups, show still larger separation factors for their enantiomers (Table 2). While the degree of enantioseparation depends predominantly on the C-aryl substituent, two interesting features emerge. In comparing phosphonates **8** and **9**, the greater separation factor of the enantiomers of the latter indicates that it is not the variation (if any) in π-basicity between the two naphthyl groups but the variation in their spatial orientations which causes the separation factors to differ significantly. Similar differences between 1-naphthyl and 2-naphthyl substituents have been noted previously [12] and may explain why the enantiomers of **6** show a smaller separation factor than do those of **4**. In compound **10**, as well as in other cases not presently described, use of the 2-fluorenyl group as the C-aryl substituent consistently gives large α values for the enantiomers of phosphonates of this type.

The above results stand in contrast to those previously obtained using the structurally related N-aryl-α-aminoesters. CSP **2**, derived from N-(3,5-dinitrobenzoyl)valine, is capable of separating the enantiomers of a variety of N-aryl-α-amino acid derivatives. The recognition model (Fig. 3) proposed by Pochapsky and supported by NMR and X-ray crystallographic studies [21–23] includes a face-to-face $\pi-\pi$ interaction involving the N-aryl rather than the C-aryl substituent as a π-donor. When a C-aryl substituent is also present, enantioselectivity is reduced owing to the ability of the C-aryl substituent of the least retained enantiomer to serve as a (less effective) π-basic site, thus increasing the retention of this antipode and reducing enantio-

Table 2
Chromatographic data for the separation of compounds **8–11** on CSP 1

Compound	X	Y	k_1'	α	R_s
8	1-Naphthyl	3,5-$(CH_3)_2C_6H_3$	4.58	1.23	3.62
9	2-Naphthyl	3,5-$(CH_3)_2C_6H_3$	7.35	2.54	2.14
10	2-Fluorenyl	2-$(CH_3)_3CC_6H_4$	5.87	5.12	14.09
11	4-(N,N)-Dimethyl aminophenyl	3,5-$(CH_3)_2C_6H_3$	12.30	2.00	6.87

Mobile phase: hexane–2-propanol (95:5, v/v); $t_0 = 1.61$ min.

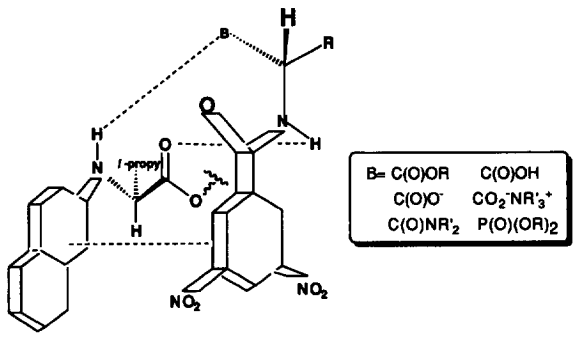

Fig. 3. Recognition of DNB amino acids by an N-(2-naphthyl)-valine-derived stationary phase (CSP 2).

selectivity. Our results suggest that this preference is reversed on CSP 1. While there are outward similarities between the two π-acidic CSPs, it is evident that they are not mechanistically equivalent. Indeed, CSP 2 does not separate the enantiomers of the present phosphonates very well, perhaps owing to the presence of the two π-basic substituents on the stereogenic center. The nature of the remaining interactions used by CSP 1 to differentiate between the enantiomers of these phosphonates is not yet fully understood.

From the data in Table 2, it is evident that the enantiomers of these phosphonates are easily differentiated by CSP 1, thus facilitating their separation on a preparative scale. Using approximately 50-mg samples of racemate per injection, the enantiomers of several of these phosphonates have been completely resolved on a 250 × 10 mm "semiprep" column containing CSP 1 and the mobile phase reported in the tables. Attempts to unambiguously establish the absolute configurations of these compounds and to better understand the details of the chiral recognition process are underway.

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