# Enantioseparation of α-Hydroxyallylphosphonates and Phosphonoallylic Carbonate Derivatives on Chiral Stationary Phases Using Sequential UV, Polarimetric, and Refractive Index Detection

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*ABSTRACT* Chromatographic separation of the enantiomers of parent compounds dimethyl α-hydroxyallyl phosphonate **1a** and 1-(dimethoxyphosphoryl) allyl methyl carbonate **1b** was demonstrated by high-performance liquid chromatography (HPLC) using Chiralpak AS-H and AD-H chiral stationary phases (CSP), respectively, using a combination of UV, polarimetric, and refractive index detectors. A comparison was made of the separation efficiency and elution order of enantiomeric α-hydroxyallyl phosphonates and their carbonate derivatives on commercially available polysaccharide AS, AD, OD, IC-3, and Whelk-O 1 CSPs. In general, the α-hydroxyallyl phosphonates were resolved on the AS-H CSP, whereas the carbonate derivatives **1b** and **2b** were preferentially resolved on the AD-H CSP. The impact of aryl substitution on the resolution of analytes **1d–f** and **2–8** was evaluated. Thermodynamic parameters determined for enantioselective adsorption hydroxyphosphonates **1a** and **4** on the AS-H CSP and carbonate **1b** on the AD-H CSP demonstrated enthalpic control for separation of the enantiomers. *Chirality 00:000–000, 2016.* © 2016 Wiley Periodicals, Inc.

*KEY WORDS:* α-hydroxy phosphonates; chiral stationary phases; enantiomer separation; HPLC; multiple detectors; phosphonoallylic carbonates; polarimetric detection

Direct chromatographic analysis of the synthetically valuable enantiomers of dimethyl α-hydroxyallyl phosphonate **1a** and 1-(dimethoxyphosphoryl) allyl methyl carbonate **1b** has been a significant challenge (Fig. 1). These particular compounds do not contain strong UV-absorbing chromophores and coupled with limited retention on a number of chiral stationary phases (CSPs) under both reverse phase and normal phase conditions pose difficulties for chromatographic analysis. Recently, we reported in collaboration with the Welch-Regalado group at Merck the moderate resolution of phosphono allylic carbonate and acetate derivatives **1b** and **1c**, respectively, using technologies developed in the pharmaceutical industry.[1] Supercritical fluid chromatography (SFC) screening using various Chiralcel and Chiralpak CSPs coupled with mass spectrometry (MS) detection afforded detection and resolution of the enantiomers of carbonate **1b** and acetate **1c**. Optimized separation used a Chiralpak IC column with isocratic elution at 4% methanol in $CO_2$ and detection of the 2 M + 1 extracted ion. The resolution of the parent compound **1a** was not achieved using this approach. While this represents the first observed analytical separation of the enantiomers of **1b** and **1c**, it does require specialized equipment that is not readily available in academic organic synthesis laboratories.

We are interested in evaluating the enantiomeric purity of α-hydroxyallylphosphonates **1a** and **1b** due to their utility as valuable chiral synthetic intermediates.[2] They have been employed for the preparation of a wide variety of biologically active compounds including both organophosphorus and, by chirality transfer, nonphosphorus-containing target molecules.[3] The ability to selectively prepare either enantiomer of **1b** has led to the synthesis of nematocidal oxylipids[4] and antimicrobial cyclophostins.[5] Palladium coupled cross-metathesis reactions have been employed for transfer of chirality of α-hydroxyallylphosphonates to the gamma position of the carbon chain,[6,7] resulting in enantioselective synthesis of cyclopentanone substituted phosphonate,[8] cyclic ethers,[9] and (S)-tumerone.[10] Enantiomerically pure dimethoxyphosphorylallyl methyl carbonates **1b** can be prepared on a multigram scale from readily available precursors by coupling an asymmetric Pudovik reaction with enzyme catalyzed kinetic resolution.[2] This three-step process initially provides phosphonate **1a** in 60–70% enantiomeric excess (ee), which after conversion to a carbonate derivative can be kinetically resolved to yield carbonate **1b** in high ee. The degree of enantiomeric purity is dependent on the specific conditions for the kinetic resolution and can be facilitated by direct monitoring of the enantiomeric purity of the product **1b** in the reaction mixture.

Determination of the enantiomeric purity of aryl substituted α-hydroxyphosphonates such as **2a** and **3** by high-performance liquid chromatography (HPLC) using either Chiralpak AS or Whelk-O 1 CSPs was first reported in 1995 by Spilling and colleagues.[11,12] In these initial studies, it was shown that the aromatic (R = aryl) phosphonates **2a** and **3** were baseline resolved with the R enantiomer eluted first on an AS CSP; however, resolution of the parent compounds **1a–c** were not addressed. Recently, the analytical resolution of reaction mixtures by HPLC using CSPs has proven to be critical for determination of the effectiveness of catalysts used for asymmetric hydrophosphonylation. Chiral aluminum complexes have been reported as catalysts for highly enantioselective hydrophosphonylation of aldehydes.[13,14]

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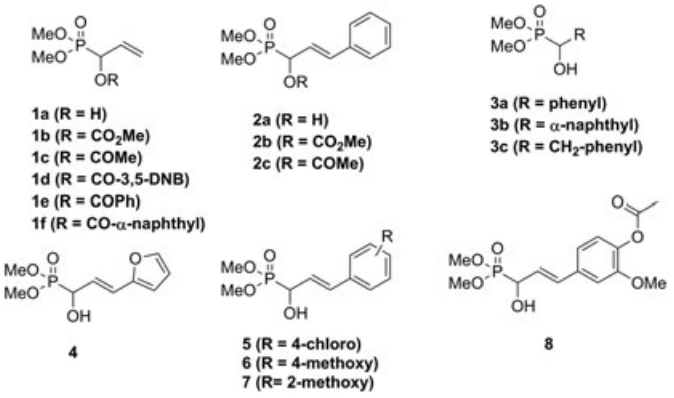


**Fig. 1.** Chemical structure of the chiral analytes **1–8**.

Self-assembled bifunctional catalysts have also been shown to afford exceptionally efficient chiral catalysts for synthesis of **2a** and **3**.[15] Ooi and colleagues used an alternative approach for chiral hydrophosphonylation using a phosphonium dialkyl phosphite.[16] Typically, the analytical details are relegated to the supplementary material and the relative merits of the chromatographic separations are not addressed.

In this report we describe the resolution of substituted α-hydroxyallylphosphonates **1–8** and their derivatives on commercially available chiral stationary phases using sequential UV, polarimetric, and refractive index detectors. The analysis was carried out using an HPLC with normal phase solvents and allows direct determination of the enantiomeric purity of synthetically valuable carbonate **1b** from gram-scale reaction mixtures. A comparison is made of the enantioselectivity of various CSPs for separation of the target analytes. A temperature study of the remarkably selective separation of furan derivative **4** allowed determination of the thermodynamic parameters leading to enantioselectivity on the AS-H CSP.

## MATERIALS AND METHODS

All reactions were carried out in oven-dried glassware under an atmosphere of argon unless otherwise noted. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ at 300 or 500 ($^1$H), 75 or 125 ($^{13}$C), and 121 ($^{31}$P) MHz, respectively. $^1$H NMR spectra were referenced to residual $CHCl_3$ (7.27 ppm) and collected phosphorus-31 decoupled, $^{13}$C NMR spectra were referenced to the center line of $CDCl_3$ (77.23 ppm), and $^{31}$P NMR spectra were referenced to external 85% $H_3PO_4$ (0 ppm). Reverse phase HPLC analysis was conducted using an Agilent (Palo Alto, CA) 1100 system equipped with UV diode array and evaporative light scattering (ELS) detectors. A Zorbax XBD-C18 column was employed using a gradient of 95% $H_2O$/5% $CH_3CN$ to 95% $CH_3CN$ over 6 min followed by a hold of 95% $CH_3CN$ for 3 min. Mobile phase solvents were prepared from HPLC-grade $CH_3CN$ and $H_2O$ containing 0.1% TFA. General procedures for the preparation of racemic and enantiomerically enriched dimethyl (1-hydroxyallyl)phosphonates **1a–c** and (*E*)-dimethyl (1-hydroxyl-3-phenylallyl)phosphonates **2a-c** have been previously reported.[7,17–19] Compounds **3** were prepared by hydrophosphonylation from the appropriate aldehyde and dimethyl phosphite and were obtained from our in-house collection.[20] Enantiomerically enriched samples of allylic phosphonates **1b–f** and cinnamylic phosphonates **4b–c** were prepared from enriched (*R*)-hydroxyphosphonates **1a** and **2a**, respectively. The (*R*) enantiomers of **1a–f** gave rise to a negative rotation, whereas the (*R*) enantiomers of **2a–c** resulted in a positive rotation at 670 nm using the polarimetric ALP detector.

Analytical normal phase chiral HPLC was performed using a ThermoSeparations P4000 pump or Rainin HPX, Rheodyne 7125 injector equipped with a 20-uL sample loop and a set of sequential Waters (Milford, MA) UV 484 variable wavelength, Advanced Laser Polarimeter (ALP, PDR-Chiral, LLC), and ERC-7512 (ERC, Huntsville, AL) refractive index detectors. Data collection of the multiple detectors was achieved using PeakSimple SRI Model 302 data collection system (SRI Instruments, Menlo Park, CA) for simultaneous monitoring of all three data outputs. HPLC columns employed were 250 x 4.6 mm I.D., containing 5 μm particle size chiral stationary phases Chiralpak AD-H, AS-H, OD, OC, OJ, IC (Chiral Technologies, West Chester, PA) or (*S,S*)-Whelk-O 1 (Regis Chemical, Morton Grove, IL). The IC-3 HPLC column was 100 x 4.6 mm I.D., containing 3 μm particle size CSP. Chromatographic runs were performed at a flow rate of 1.0 mL/min and at a temperature of 25 °C, unless otherwise stated. Temperature studies were carried out using a 100 x 4.6 mm I.D. column containing 3 μm particle size AS-H or AD-H CSP equipped with a Cera Column Cooler 250 digital Peltier temperature controller (Cobert Associates, St. Louis, MO). Resolution (Rs) and theoretical plate count (N) for the enantiomers was determined by the method of measurement of mid-height of the peaks.[21]

### *(Dimethyloxyphosphoryl)allyl 3,5-dinitrobenzoate (1d)*

To allylic hydroxyphosphonate **1a** (0.17 g, 1.0 mmol) in 4 mL of $CH_2Cl_2$ was added trimethylamine (0.22 g, 2.1 mmol), DMAP (12 mg, 0.1 mmol), and 3,5-dinitrobenzoyl chloride (0.23 g, 1.0 mmol). After stirring at room temperature (rt) overnight, the mixture was quenched with 1 M $KHSO_4$ and extracted three times with ethyl acetate. The combined extracts were concentrated and purified by chromatography (10 g $SiO_2$, 50% ethyl acetate/hexanes) to afford 90 mg (25%) of a clear, colorless oil: HPLC (reverse phase) 2.63 min (>98% by UV 254 and ELS); TLC (50% EtOAc/hexanes) $R_f$ = 0.22; IR (ATR) 3096, 2957, 1736 (C = O), 1627, 1539, 1021; $^1$H NMR ($CDCl_3$, 300 MHz) δ 3.85 (d, 3H, 3.2 Hz), 3.87 (d, 3H, 3.2 Hz), 5.50 (d, 1H, 9.5 Hz), 5.58 (dd, 1H, 19.8 Hz, 4.2 Hz), 5.99 (dd, 1H, 13.3 Hz, 8.0 Hz), 6.07 (m, 1H), 9.17 (s, 2H), 9.23 (s, 1H); $^{31}$P NMR ($CDCl_3$) δ 18.8; $^{13}$C NMR ($CDCl_3$) δ 53.1, 71.4 (d, 170 Hz), 121.2 (d, 11.6 Hz), 123.0, 128.4 (d, 4 Hz), 129.7, 133.1, 149.0, 161.3 (d, 7.1 Hz); MS(FAB+) 361 (MH+, 66), 149 (100); HRMS (FAB, NaI, M+) calcd for $C_{12}H_{13}N_2O_9PNa$: 383.0247; found: 383.0256.

### *(E)*-dimethyl(3-(furan-2-yl)-1-hydroxyallyl)phosphonate 4 and enantiomerically enriched 4(*S*)

To a solution of *trans*-3-(2-furyl)acrolein (1.22 g, 10 mmol) in 35 mL of anhydrous toluene cooled in a wet ice-acetone bath was added a catalytic amount of quinine (42 mg, 0.13 mmol) and dimethyl phosphite (2.28 mL, 2.74 g, 25 mmol). After stirring for 72 h at rt., the mixture was concentrated, purified by chromatography (50 g $SiO_2$, ethyl acetate), and concentrated in vacuo to give 2.0 g of a reddish oil. Enantiomeric composition of the product was determined by 31P NMR in the presence of 5 equivalents of quinine as a chiral solvating agent.[22] $^{31}$P NMR (CDCl3 + quinine) δ 23.7 (R enantiomer, 40%), 23.97 (S enantiomer, 60%). Repeated trituration with CCl4 gave 1.08 g (46.5%) of a tan solid which was nearly racemic **4**. Concentration of the mother liquors gave an orange solid which was 40% enriched in the S enantiomer **4(*S*)**; mp 55–60 °C (dec). HPLC (reverse phase) 1.64 min (>98% by UV 254 and ELS); TLC (EtOAc) $R_f$ = 0.23; IR (ATR) 3270 (br, OH), 2953, 2848, 1457, 1021; $^1$H NMR ($CDCl_3$, 300 MHz) δ 3.82 (s, 6H), 4.71 (dd, 1H, 5.8 Hz, 1.5 Hz), 6.22–6.37 (m, 3H), 6.63 (d, 1H, 15.8 Hz), 7.34 (s, 1H); $^{31}$P NMR ($CDCl_3$) δ 23.5; $^{13}$C NMR ($CDCl_3$) δ 53.7 (d, $J_{CP}$ = 7.5 Hz), 54.0 (d, $J_{CP}$ = 7.5 Hz),68.7 (d, $J_{CP}$ = 162 Hz),108.8, 111.4, 120.4 (d, $J_{CP}$ = 15.8 Hz),122.2 (d, $J_{CP}$ = 4.5 Hz), 142.2 (d, $J_{CP}$ = 1.5 Hz), 152.2 (d, $J_{CP}$ = 4.5 Hz); HRMS (FAB, NaI, M+) calcd for $C_9H_{13}O_5PNa$: 255.0398; found: 255.0405.

### *(E)*-dimethyl(3-(4-chlorophenyl)-1-hydroxyallyl) phosphonate 5

A stirred solution of 4-chlorocinnamaldehyde (0.5 g, 3.6 mmol) dissolved in 1 mL of CH2Cl2 was cooled in a wet ice-acetone bath and treated sequentially with trimethylamine (0.25 ml, 3.4 mmol) and dimethyl phosphite (0.35 ml, 3.63 mmol). After stirring for 3 days, the

mixture was concentrated and purified by chromatography ($SiO_2$, ethyl acetate) to afford 0.88 g (88%) of a yellow crystalline solid; mp 94–95.5 °C. TLC (ethyl acetate) $R_f$ = 0.24; HPLC (reverse phase) 2.37 min (>98% by UV 254 and ELS); IR(ATR) 3252, 2957, 2846, 1688, 1488, 1024; $^1$H NMR ($CDCl_3$, 300 MHz) δ 3.75 (s, 6H), 3.89 (bs, 1H), 4.65 (dd, 1H, 6.0 Hz, 1.3 Hz), 6.22 (dd, 1H, 15.9 Hz, 6.0 Hz), 6.68 (d, 1H, 15.9 Hz), 7.22 (m 4H); $^{31}$P NMR ($CDCl_3$) δ 23.7; $^{13}$C NMR ($CDCl_3$) δ 53.8 (d, 7.4 Hz), 54.0 (d, 7.1 Hz), 69.1 (d, 161 Hz), 124.3 (d, 4.3 Hz), 127.9 (d, 1.8 Hz), 128.8, 131.2 (d, 13.0 Hz), 133.7, 134.8 (d, 3.0 Hz); HRMS (FAB, NaI, M+) calcd for $C_{11}H_{14}O_4PNa$ 299.0216; found: 299.0221.

## RESULTS AND DISCUSSION

### *Chromatographic Resolution of Parent Compounds 1a–c*

Our initial goal was development of a suitable HPLC method for evaluation of the enantiomeric purity of the parent α-hydroxyallylphosphonate **1a** and its carbonate derivative **1b** for use in monitoring scale-up synthesis of these useful chiral building blocks. Analysis of **1a** and **1b** by reverse-phase HPLC equipped with UV and ELS detection showed moderate response for **1a** at 210 nm; however, the carbonate derivate was not visible even at lower limits of UV detection. Neither compound exhibited a response by ELS. Even in the case of UV detection of **1a**, minor impurities can dominate the chromatogram and obscure measurements of purity. To circumvent these difficulties, we assembled in sequence multiwavelength UV, laser polarimeter (ALP), and refractive index (RI) detectors. The ability to detect **1a-c** by ALP and RI was confirmed by analysis of racemic and enantiomerically enriched samples using an achiral column. The laser polarimeter uses 670 nm plane polarized laser light source which can have a different specific rotation and occasionally sign of rotation compared to the standard sodium D line. For the known phosphonates **1–3** and derivatives, we found that the sign of rotation from the ALP was identical to the reported sign of rotation at the sodium D line (598 nm). The **(R)-1a** and its derivatives **(R)-1b-f** show negative rotation, while the aromatic substituted **(R)-2a-c** and **(R)-4** exhibit a positive rotation at both 598 and 670 nm.

Analysis of **1a** using a Chiralpak AS-H CSP and sequential detectors shows nearly baseline resolution and, as expected, the R-(−) enantiomer elutes first (Fig. 2A). Determination of the enantiomeric purity can be made using either the UV at 210 nm or polarimetric detectors. Significantly higher concentrations of the analytes were required for RI detection of **1a**. However, the presence of all three detection modes allows for easy identification of the two enantiomers even in more complex mixtures. Attempts to resolve carbonate **1b** on the AS-H or WhelkO-1 CSPs were unsuccessful. However, baseline resolution and determination of the enantiomeric purity of **1b** was achieved using the Chiralpak AD-H CSP with 10% isopropanol in hexanes as the mobile phase (Fig. 2B). For carbonate **1b,** no UV signal was observed. The **(S)-(+)-1b** eluted first, as indicated by the ALP, and enantiomeric purity can be easily determined using either the ALP or RI detectors. It is interesting to note that the carbonate functionality of **1b** resulted in improved efficiency and column performance for the AD-H CSP (first peak $N$ = 1700) compared to the resolution of hydroxyl bearing **1a** on AS-H (first peak $N$ = 460). Since both columns were of similar dimensions and particle size, this is likely due to the hydrogen bonding character of the OH group of **1a** resulting in nonideal chromatographic performance.

The previously reported SFC-MS study[1] showed separation of the carbonate **1b** (α = 1.14) and acetate **1c** (α = 1.38) on IC

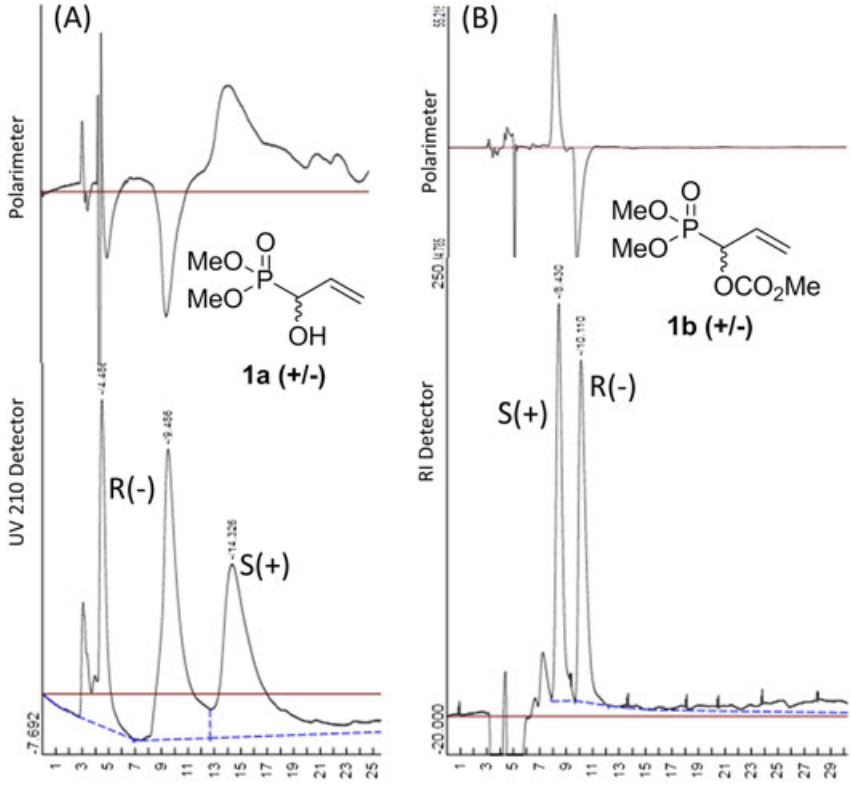


**Fig. 2.** Chromatograms showing the retention and separation of the enantiomers of compounds **1a** and **1b**. (**A**) Resolution of **1a** using polarimetric and UV (210 nm) detectors. Column: AS-H CSP; mobile phase: 10% isopropyl alcohol in hexanes. (**B**) Resolution of **1b** using polarimetric and refractive index detectors. Column: AD-H CSP; mobile phase: 10% isopropyl alcohol in hexanes.

amylose derived CSP with baseline resolution, but no separation of the parent compound **1a** (Table 1). Having shown the utility of the sequential polarimetric and RI detectors for analyte **1b**, we decided to reevaluate use of the IC column using standard normal phase conditions. Surprisingly, when the IC column was investigated using hexane-isopropanol as a mobile phase, no resolution of the phosphonate derivatives **1b** or **1c** was observed. After evaluation of eight different CSPs (Chiralpak AS-H, AD-H, OD, OC, OJ, IC-3, IA-3, and Whelk-O 1) we found that the AS-H CSP was best for resolution of **1a** and AD-H for the carbonate **1b**. Acetate **1c** was not successfully resolved on the Whelk-O 1 or AS-H CSP and showed only moderate separation on AD-H. This was somewhat surprising, since **1c** had previously performed well in SFC mode with an IC-3 stationary phase.

Benzoate derivatives of simple aliphatic α-hydroxyphosphonates have been previously prepared to enhance resolution by CSP-HPLC and facilitate detection by UV.[23] Since this approach had not been reported for allylic phosphonates, we prepared three ester derivatives, DNB (3,5-dinitrobenzoyl) **1d**, benzoate **1e,** and naphthoate **1f** from either racemic **1a** or enantiomerically enriched **1a** (Fig. 3). The DNB **1d** was successfully resolved on the amylose based CSPs, AS-H, AD-H, and IC-3, with the largest separation (α = 1.50; Rs = 4.9) observed on the AD-H CSP. Benzoate **1e** was not resolved on AD-H, but did give modest resolution on the AS-H and IC-3 CSPs. Overall, the esters **1d–f** were consistently resolved on the AS-H CSP with the R enantiomer eluted first. Resolution factors (Rs) were not optimized since we were typically introducing a slight column overload to enhance polarimetric detection and injection concentration could be reduced to improve measurement of enantiomeric composition by UV. The IC-3 CSP consistently resolved analytes **1d–f** and, although the separation factors were smaller, superior performance of the column allowed nearly baseline resolutions. The elution order of the enantiomers, however, was not consistent on the IC-3 CSP.

Behavior of the phenyl allyl phosphonates **2a-c** was significantly different than the simple allyl phosphonates **1a–c**. While the Regis Whelk-O 1 CSP did not resolve allyl phosphonates **1a-1e**, all three compounds **2a–c** were baseline-resolved. Interestingly, the S(−) enantiomer of the hydroxyphosphonate **2a** eluted first, while the R(+) enantiomer of **2b** and **2c** eluted first on Whelk-O 1 CSP. The behavior of the hydroxyl **2a** and carbonate **2b** on the amylose-based CSPs reflected what we previously observed for allylphosphonates **1a** and **1b**. The free hydroxyl group of **2a** afforded the best resolution on AS-H (α = 2.40; Rs = 2.9), while the carbonate derivative **2b** gave the best results on AD-H (α = 1.65; Rs = 2.7). For the purpose of comparison, we also investigated archival compounds **3a-c** from our in-house collection. The naphthyl **3b** and benzyl **3c** afforded significantly larger separation factors on the AS-H CSP (α = 3.60 and 4.44, respectively) compared to phenyl substituted hydroxyl phosphonate **3a**. Enantiomers of compound **3b** were also well separated on the Whelk-O 1 and AD-H CSP.

To gain insight into the structural requirements for enantioselectivity of substituted hydroxyallylphosphonates on CSPs, we prepared a set of aryl substituted compounds **4–8** (Fig. 3). Treatment of dimethylphosphonate with commercially available aryl acrylaldehydes in the presence of triethylamine afforded the target hydroxyphosphonates **4–8**. Enantiomerically enriched samples of the analytes were

**TABLE 1. Chromatographic data of α-hydroxyallylphosphonates 1a-f, 2a-c, and 3a-c on four CSPs.**[a]

| Compound | Whelk-O 1 | | | | AS-H | | | | AD-H | | | | IC-3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | %I/H | k' | α | Rs | %I/H | k' | α | Rs | %I/H | k' | α | Rs | %I/H | k' | α | Rs |
| 1a | 10 | 3.36 | NS | | 5 | 3.64(−) | 1.68 | 1.3 | 10 | 3.87(+) | 1.18 | 0.9 | 10 | 4.01 | NS | |
| | | | | | | | | | | | | | SFC[b] | 5.40 | NS | |
| 1b | 10 | 5.53 | NS | | 5 | 0.68 | NS | | 10 | 1.91(+) | 1.34 | 1.9 | 10 | 13.0 | NS | |
| | | | | | | | | | | | | | SFC[b] | | 1.14 | 1.5 |
| 1c | 10 | 6.26 | NS | | 5 | 1.36 | NS | | 5 | 3.08(+) | 1.13 | 0.5 | 10 | 16.0 | NS | |
| | | | | | | | | | | | | | SFC[b] | | 1.38 | 3.6 |
| 1d | 20 | 11.4 | NS | | 20 | 4.58(−) | 1.16 | 0.6 | 10 | 5.95(−) | 1.50 | 4.9 | 25 | 53.7(−) | 1.06 | 0.8 |
| 1e | 20 | 2.5 | NS | | 20 | 1.13(−) | 1.20 | 0.4 | 5 | 5.10 | NS | | 10 | 12.4(+) | 1.15 | 0.9 |
| 1f | 20 | 4.80(−) | 1.07 | 0.7 | 5 | 5.92(−) | 1.33 | 1.2 | 5 | 5.83(+) | 1.06 | 0.7 | 10 | 18.6(−) | 1.07 | 0.8 |
| 2a | 20 | 1.44(−) | 1.43 | 1.9 | 10 | 6.51(+) | 2.40 | 2.9 | 5 | 10.48 | NS | | 10 | 6.60(+) | 1.04 | 0.4 |
| 2b | 20 | 6.16(+) | 1.08 | 1.0 | 10 | 6.66 | 1.07 | ND | 10 | 4.55(−) | 1.65 | 2.7 | 10 | 17.29(+) | 1.32 | 1.9 |
| 2c | 20 | 6.75(+) | 1.13 | 1.7 | 10 | 4.04(+) | 1.31 | 0.6 | 5 | 7.58(−) | 1.29 | 2.3 | 10 | 13.47(+) | 1.63 | 5.9 |
| 3a | 20 | 2.16(−) | 1.20 | 1.5 | 10 | 4.87(+) | 1.32 | 1.0 | 5 | 15.9(−) | 1.11 | 1.2 | 10 | 11.75 | NS | |
| 3b | 20 | 3.63(−) | 1.21 | 1.9 | 25 | 2.27(+) | 3.60 | 3.5 | 5 | 26.7(−) | 1.56 | 7.5 | 10 | 15.7(−) | 1.07 | 0.8 |
| 3c | 5 | 16.7 | NS | | 25 | 1.34(−) | 4.44 | 3.5 | 5 | 10.7(+) | 1.11 | 1.0 | 10 | 5.51(−) | 1.27 | 2.0 |

[a]See Experimental section for chromatographic conditions. The %I/H indicates the percent isopropyl alcohol in hexanes. Detection for 1a and 1c: UV at 210 nm; 1b by refractive index and remaining analytes 1d-f, 2 and 3 by UV at 254 nm. Polarimetric sign of rotation of the first eluted enantiomer is listed in parenthesis following k'. Compounds 1a-f: (−) indicates R enantiomer; compounds 2–3: (+)indicates R enantiomer. 'NS' indicates no observed separation of the enantiomers.

[b]LCMS data using supercritical fluid chromatography (SFC) was obtained from a previous report (Ref. 1).

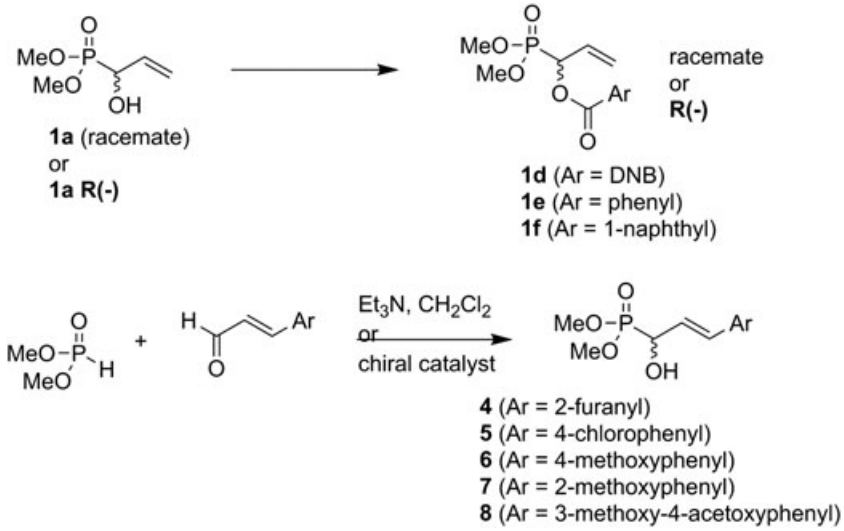


**Fig. 3.** Preparation of substituted α-hydroxyallylphosphonates **1d–f** and **4–8**.

prepared by asymmetric synthesis using either quinine[24,25] or titanium isopropoxide/dimethyl tartrate[19] as a catalyst. Chromatographic resolution of the racemic compounds was screened on several CSPs with the best results obtained on Whelk-O 1, AS-H, and OD phases (Table 2). Separation factors for all six analytes on the Whelk-O 1 were similar, ranging from 1.32 for **4** (Ar = 2-furyl) to 1.51 for **6** (Ar = 4-methylphenyl). In all cases, the (S)-(−) enantiomer eluted first. Larger separation factors were observed on the AS-H CSP with the furyl substituted **4** providing an α value of 6.54. Compound **4** has the most electron-deficient aryl group of the set and analytes **5**, **6,** and **7,** with electron-donating groups exhibiting progressively lower separation factors. In all cases, baseline resolution was observed on AS-H CSP, with the (R)-(+) enantiomer eluted first. The six aromatic analytes **3–8** showed less affinity for the OD CSP, requiring only a 5% isopropanol in hexanes mobile phase to achieve reasonable chromatographic retention. Resolution (Rs) was lower for most analytes on OD CSP; however, reasonable α-values were obtained for *o*-methyl substituted **7** (α = 1.72) and diphenyl substituted **8** (α = 1.92).

The unusual selectivity observed for furanyl substituted phosphonate **4** and the desire to optimize the separation of the parent compounds **1a–b**, encouraged us to carry out a temperature study to determine the thermodynamic parameters associated with the enantioseparation (Table 3).[26,27] Separation factors were obtained for seven individual chromatographic runs collected in 10 °C increments from 2 °C to 55 °C using a jacketed Peltier temperature controller. The measured α values of furan substituted **4** showed a significant impact, with temperature with measurements at 2 °C affording a separation factor of 9.19 and decreasing to 2.90 at 55 °C. Differences in the enthalpy ($\Delta(\Delta H^o)$) and entropy ($\Delta(\Delta S^o)$) of adsorption of the two enantiomers by the AS-H CSP can be determined by the following equation:

$$\ln\alpha = -\Delta(\Delta H^o)/RT + \Delta(\Delta S^o)/R$$

The van't Hoff plot of ln α versus the reciprocal of temperature in Kelvin gave a linear correlation coefficient greater than 0.95 in all three cases. Enthalpic and entropic contributions to the enantioselective adsorption of the enantiomers of **4** were calculated from the van't Hoff plot as $\Delta\Delta H^o = -15.7$ kJ/mol and $\Delta\Delta S^o = -39.0$ J/Kmol, respectively. Since the contribution from the entropic term ($-T\Delta(\Delta S^o)$) is

**TABLE 2. Chromatographic data for aryl substituted α-hydroxyallylphosphonates 2a and 4–8 on three CSPs.**[a]

| Compound[b] | WhelkO-1 | | | AS-H | | | OD | | |
|---|---|---|---|---|---|---|---|---|---|
| | k' | α | Rs | k' | α | Rs | k' | α | Rs |
| **2a** | 1.44(−) | 1.43 | 1.9 | 2.36(+) | 2.49 | 2.5 | 6.91(−) | 1.30 | 1.2 |
| **4** | 2.31(−) | 1.32 | 2.0 | 4.79(+) | 6.54 | 5.2 | 5.90 | NS | |
| **5** | 2.03(−) | 1.34 | 1.6 | 2.49(+) | 4.40 | 3.0 | 6.10(−) | 1.08 | 0.5 |
| **6** | 4.08(−) | 1.51 | 3.4 | 5.10(+) | 1.93 | 1.6 | 9.83(−) | 1.24 | 1.1 |
| **7** | 3.62(−) | 1.36 | 1.8 | 3.11(+) | 1.53 | 1.2 | 2.77(+)[c] | 1.72 | 0.6 |
| **8** | 11.59(−) | 1.35 | 2.8 | 5.67(+) | 1.46 | 1.0 | 8.39(−)[d] | 1.92 | 3.0 |

[a]For key to chromatographic data, see Table 1.
[b]Compounds 2–8 exhibit (+) sign of rotation for R enantiomer. Mobile phase isopropanol/hexanes (I/H) composition for CSPs: Whelk-O 1, 20% I/H; AS-H, 25% I/H and OD at 5% I/H, unless otherwise stated.
[c]10% I/H.
[d]20% I/H.

**TABLE 3. Thermodynamic values for chromatographic separations of hydroxyphosphonate 1a, carbonate 1b, and furan 4**[a]

| Compound | $\Delta(\Delta H^o)$ (kJ/mol) | $\Delta(\Delta S^o)$ (J/mol K) | $-T\Delta(\Delta S^o)$ (kJ/mol) | $\Delta(\Delta G^o)$ (kJ/mol) |
|---|---|---|---|---|
| **1a** | −3.5 | −8.8 | 2.6 | −0.9 |
| **1b** | −0.68 | −0.07 | 0.02 | −0.66 |
| **4** | −15.7 | −39.0 | 11.4 | −4.3 |

[a]See Figure 4 for experimental details.

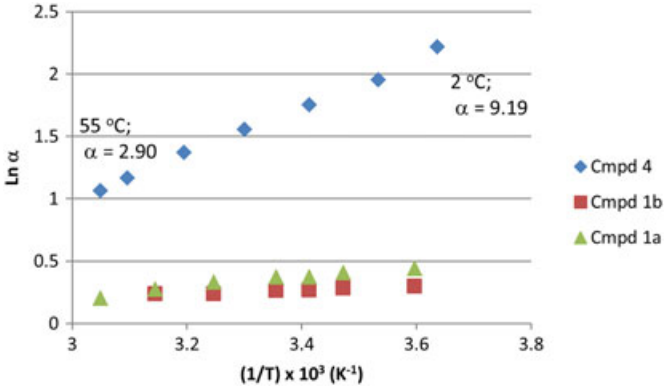


**Fig. 4.** Temperature study van't Hoff plot of ln α vs. 1/T for resolution of **1a**, **1b,** and **4** on CSPs. The CSP and mobile phase isopropyl alcohol in hexane (I/H) composition: **1a**) CSP: AS-H, 20% I/H; **1b**) AD-H, 10% I/H; **4**) AS-H, 10% I/H.

smaller in value than the enthalpic term ($\Delta(\Delta H^o)$), the enantioselective adsorption in enthalpy controlled and the α value will decrease with increasing temperature. The retention times of both enantiomers also decreased with higher temperatures, indicating enthalpic control for analyte retention. From a practical standpoint, optimal resolution as measured by Rs was observed at 20 °C. Lower temperatures improved the separation factor, but also gave rise to band broadening, which negatively impacted the peak shape and resolution. Parent hydroxyphosphonate **1a** exhibited a similar ratio of the contributions of enthalpy and entropy to the free energy value ($\Delta(\Delta G^o)$); however, the overall values were smaller corresponding to smaller separation factor α. We investigated the resolution of carbonate **1b** on the AD-H CSP and found the relative contributions of $\Delta(\Delta H^o)$ and $\Delta(\Delta S^o)$ were significantly different compared to resolutions of **4** or **1a** on the AS-H CSP. While the separation of **1b** is enthalpically controlled, the entropic contribution is much smaller. As a result, the separation factors for **1b** are not greatly influenced by small changes in temperature.

## CONCLUSION

We have demonstrated a useful and practical HPLC method for analysis of enantiomeric purity of dimethyl α-hydroxylallyl phosphonate **1a** and dimethoxyphosphoryl allyl methyl carbonate **1b** using sequential refractive index, polarimetric, and UV detectors. These methods can be used for the direct analysis of reaction mixtures for the preparation of substituted hydroxyphosphonates as valuable synthetic building blocks. Comparison of seven chiral stationary phases demonstrated that the AS-H CSP provided superior resolution of α-hydroxyphosphonates, while AD-H was preferred for the resolution of carbonate derivatives such as **1b** or **2b**. Although separation factors were lower, the Whelk-O 1 CSP consistently provided baseline resolution of the aryl substituted α-hydroxylphosphonates **2–8**. A temperature study of the resolution of furanyl-α-hydroxy allylphosphonate **4** and parent compound **1a** on the AS-H CSP allowed determination of enthalpic control for the resolution and retention of the enantiomers.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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