

Article

Influence of the Azulene Ring on the Enantioseparation of 1,5-Diols

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Abstract: The enantioseparation of a series of six azulene-centered 1,5-diol enantiomers was studied employing two cellulose-based chiral stationary phases under normal phase conditions (isopropanol/hexanes). The separations were generally quite good on Chiralcel-OD-H, with α values ranging from 1.2 to 8.4 (average 4.0) and resolution values of 0.4–8.3 (average 4.7). Only one of the six enantiomer pairs was not well resolved, but was well separated on Lux cellulose 2 (α 1.4, R_s 8.7). It was observed that the enantioseparations of the RS/SR diastereomers (ave α = 7.8, R_s = 8.2) were dramatically better than that of the corresponding RR/SS diastereomers (ave α = 2.1, R_s = 3.0) on Chiralcel-OD-H. The better-resolved diastereomer pairs correspond to the more strongly retained diastereomers on silica gel. The enantiomers of two benzene 1,5-diols were much more poorly separated on both stationary phases, suggesting that the unusual polarity of the azulene ring enhances critical interactions with these phases.

Keywords: chiral azulene; chiral high-pressure liquid chromatography; enantioseparation

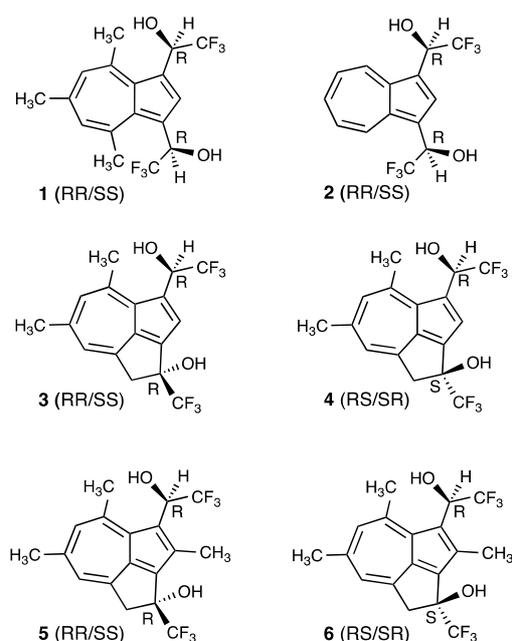
1. Introduction

Over the past two decades, the ability to accomplish enantiomer separations chromatographically has become an extremely valuable analytical tool and, to a lesser extent, preparatively useful [1,2]. There are many important applications, particularly with respect to pharmaceuticals, ranging from quality control in production to monitoring the environmental fate of these materials [3,4].

Azulene is isomeric with the relatively common naphthalene ring, yet is dramatically different in some of its properties. Azulenes are almost unique among hydrocarbons in having large (~ 1 D) dipole moments [5], roughly equivalent to that of HCl and over half that of water. They also represent one of the smallest conjugated systems having a visible chromophore; azulenes are typically blue or purple, though derivatives can be of nearly any color [6]. The azulene ring has been incorporated into a variety of structures, including pharmaceuticals (sodium azulene sulfonate) [7]. Remarkably, however, very few chiral azulene derivatives have been reported in the literature [8–13], and only one where the enantiomers were separated chromatographically [13].

During studies of chromatographic separations of a series of racemic azulene 1,5-diols (**1–6**, Figure 1), we found [14] that the diastereomers were remarkably well separated ($\Delta R_f \sim 0.22$ – 0.46) on silica gel TLC. Subsequently, the chromatographic enantiomer separation using chiral High Performance Liquid Chromatography (HPLC) was undertaken partly to determine if the azulene ring was advantageous in cellulose-cased enantiomer separations. The results of this study are reported here.

Figure 1. Azulene-1,5-diols employed in this study (all racemic).



2. Experimental Section

2.1. Instrumentation

High Performance Liquid Chromatography (HPLC) was run at ambient temperature using a Beckman System Gold 126 Solvent Module instrument with a 5 μ m Chiralcel OD-H column, 2.1 mm ID \times 150 mm, and a 5 μ m Phenomenex Lux Cellulose 2 column, 4.6 mm ID \times 150 mm (with SecurityGuardTM precolumn filter, 3 mm ID \times 4 mm), with isopropanol (IPA) and hexanes (Hex) as the mobile phase, with a Beckman System Gold 168 UV Detector.

Ultraviolet (UV) spectroscopic data was collected utilizing a Shimadzu UV-2550 spectrometer over a wavelength range of 400 to 200 nm. Circular Dichroism (CD) spectra were collected using a JASCO 810 spectrometer over a wavelength range of 400 to 200 nm.

2.2. Example Procedure for Separation and Analysis of Enantiomers, $I_{RS/SR}$

Sample for HPLC was prepared by dissolving 3.8 mg of the racemic azulene diol into 1.5 mL of 10% IPA:Hex. Then 20 μ L of the solution was injected into the HPLC utilizing the 5 μ m Phenomenex Lux cellulose 2 column with the column pressure maximum at 2 atm and a flow rate of 1.0 mL/min at ambient temperature. The eluent solution gradient began at a concentration of 5% IPA:Hex and was increased to 20% IPA:Hex evenly over 20 min. The enantiomers eluted at 5.9 min for the faster, and 7.92 min for the slower. UV spectra were gathered by taking the collected fractions and diluting them with methanol (MeOH). To obtain CD spectra the IPA:Hex was removed under vacuum and the compounds were dissolved in MeOH. First a blank of pure MeOH was run in a 1 mm quartz cuvette from 195 to 400 nm (CD parameters: sensitivity high (5 mdeg), data pitch 0.1, scanning mode: continuous, scanning speed: 100 nm/min, response 0.25 s, band width: 1 nm, accumulation 5, room temperature). The Cotton Effect observed for the enantiomers was greatest at 349.8 nm with 11.64 for the faster and -6.47 for the slower eluting enantiomers, respectively.

3. Results and Discussion

The synthesis of diols **1–6** has been recently reported [14]. These diols were formed as mixtures of diastereomers (RR/SS vs. RS/SR) that were easily separated by silica gel chromatography. All of these are chiral compounds except for the achiral *meso* (RS = SR) diastereomers of diols **1** and **2** (not shown). Note that diols **3/4**, and **5/6** are diastereomers of one another. We now find that under normal-phase conditions (IPA in hexanes) Chiralcel-OD-H (cellulose-tri-(3,5-dimethyl phenyl) carbamate) coated on 5 μ m silica gel gave baseline resolutions ($R_s \sim 3–8$) for five (**2–6**) of the six pairs of enantiomers (Table 1, examples shown in Figures 2 and 3).

Table 1. Summary of chiral High Performance Liquid Chromatography (HPLC) data for each of the enantiomers. Samples were eluted with isopropanol (IPA):hexanes (Hex) on a Chiralcel OD-H column.

Compound	R_f Value *	Rt 1 (min)	Rt 2 (min)	$w_{1/2}$ 1 (min)	$w_{1/2}$ 2 (min)	Dead Volume	R_s	α
1	0.59	1.43 ^f	1.61 ^f	0.30	0.30	0.41 mL	0.35	1.17
2	0.39	4.70 ^e	9.80 ^e	0.41	0.90	0.38	4.61	2.18
3	0.63	1.75 ^f	2.97 ^f	0.16	0.31	0.41	3.10	1.91
4	0.40	1.30 ^g	8.13 ^g	0.21	0.76	0.38	8.28	8.43
5	0.58	1.88 ^b	4.73 ^b	0.27	0.63	0.40	3.74	2.93
6	0.38	1.87 ^c	11.15 ^c	0.39	0.97	0.38	8.05	7.25
7	0.31	6.92 ^b	7.68 ^b	0.70	1.14	0.46	0.49	1.12
8	0.10	2.78 ^a	3.82 ^a	0.16	0.30	0.80	2.65	1.53

* R_f values published in Horgen *et al.* 2014 [14]. Solvent gradients: ^a isocratic 5% IPA in hexanes; ^b 5% to 10% IPA in hexanes over 22 min; ^c 10% to 20% IPA in hexanes over 22 min; ^d isocratic 20% IPA in hexanes; ^e 7.5% to 10% IPA in hexanes over 22 min; ^f 2.75% to 5% IPA in hexanes over 22 min; ^g 10% to 15% IPA in hexanes over 22 min.

Figure 2. Separation of racemic compound **2** on Chiralcel-OD-H (solvent: 7.5% IPA in hexanes to 10% IPA over 22 min), representing an average separation on this column.

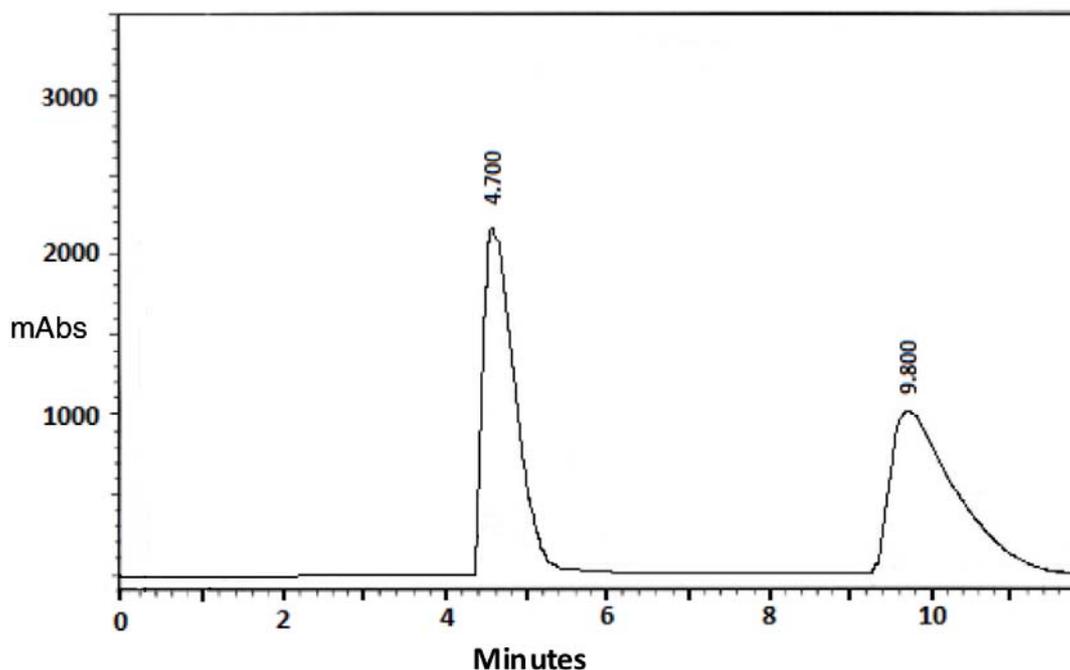
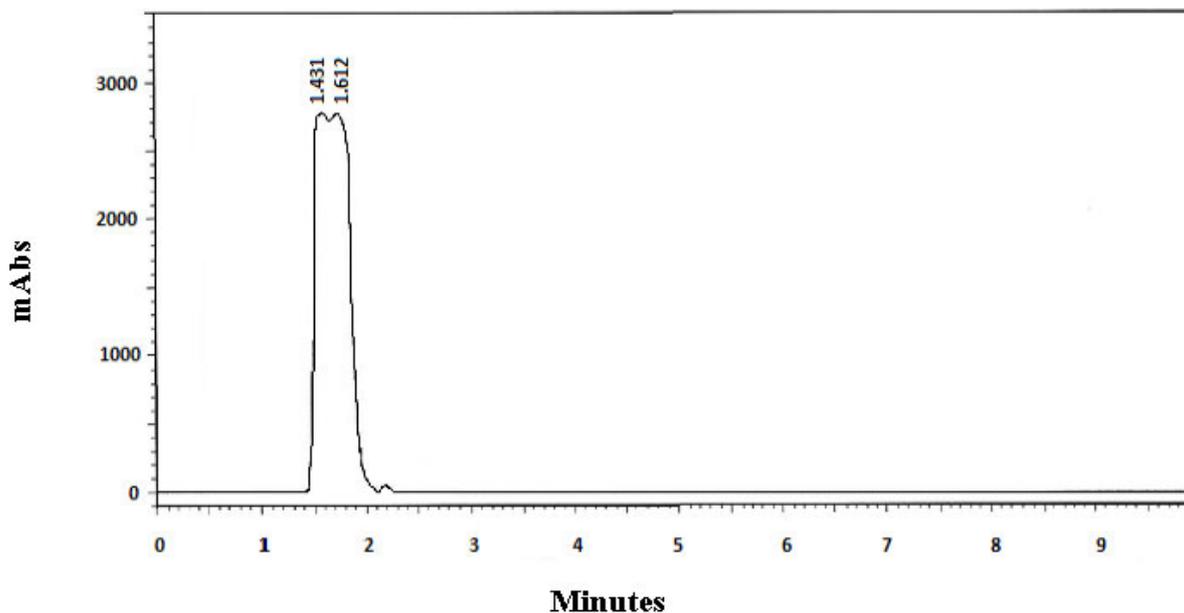


Figure 3. HPLC for compound **1** on the Chiralcel OD-H column using isocratic 5% IPA in hexanes.

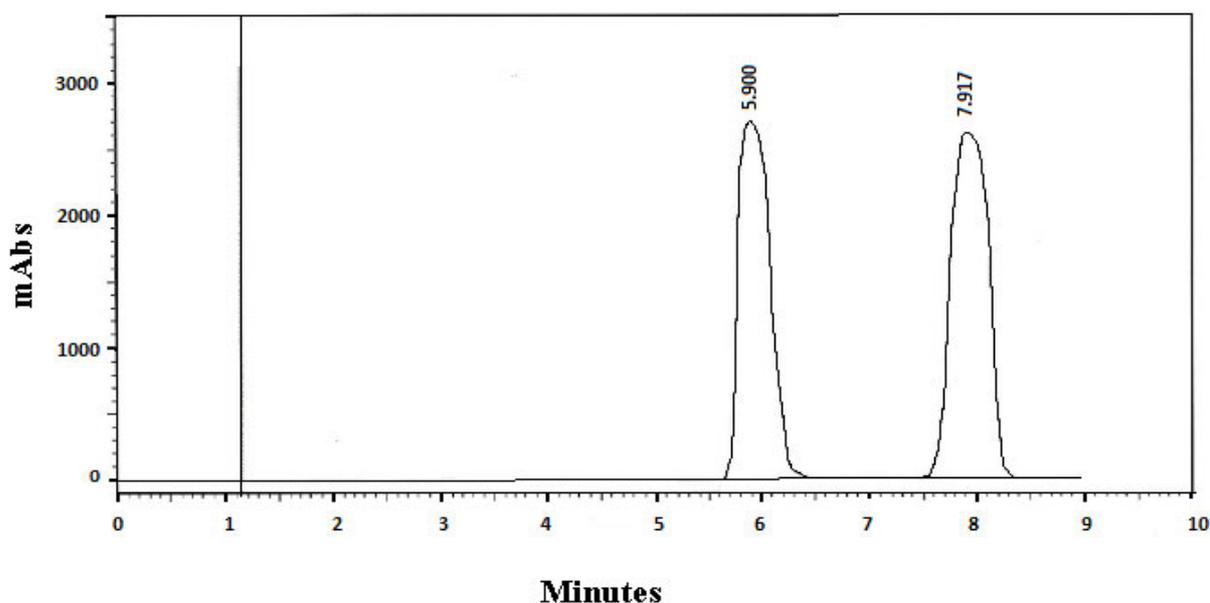


A chromatogram with typical resolution is shown in Figure 2. The remaining diol (**1**) enantiomers were resolved (R_s 8.7) by the Phenomenex Lux cellulose 2 column (Table 2, example shown in Figure 4), again under normal-phase conditions. Three of these compounds (**1**, **2** and **4**) were screened on other chiral columns and under various conditions; these data are given in Table 3.

Table 2. Results from compounds run on a Phenomenex Lux Cellulose 2 column eluted with IPA in hexanes.

Compound	R _f value *	Rt 1 (min)	Rt 2 (min)	w _{1/2} 1 (min)	w _{1/2} 2 (min)	Dead Volume	R _s	α
1	0.59	5.90 ^d	7.92 ^d	0.16	0.19	1.14 mL	8.71	1.42
2	0.39	3.23 ^c	3.53 ^c	0.46	0.46	1.13	0.38	1.14
3	0.63	5.32 ^b	6.10 ^b	0.21	0.24	1.25	2.06	1.19
4	0.40	2.28 ^c	2.85 ^c	0.55	0.55	1.15	0.61	1.50
5	0.58	3.82 ^b	4.93 ^b	0.19	0.25	1.45	2.98	1.47
6	0.38	6.65 ^b	7.77 ^b	0.36	0.38	1.40	1.78	1.21
7	0.31	3.75 ^b	3.98 ^b	0.12	0.24	1.27	0.76	1.09
8	0.10	9.70 ^b	10.4 ^b	0.51	0.70	1.20	0.63	1.08

* R_f values published in the Horgen *et al.* 2014 [14]. Solvent gradients: ^a isocratic 5% IPA in hexanes; ^b 5% to 10% IPA in hexanes over 22 min; ^c 10% to 20% IPA in hexanes over 22 min; ^d isocratic 20% IPA in hexanes; ^e 7.5% to 10% IPA in hexanes over 22 min. ^f 2.75% to 5% IPA in hexanes over 22 min. ^g 10% to 15% IPA in hexanes over 22 min.

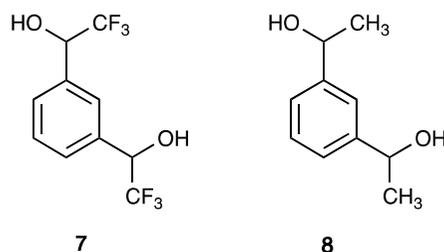
Figure 4. HPLC for compound 1 on the Lux column using isocratic 20% IPA in hexanes.

For comparison, the closely related benzene compounds **7** and **8** (Figure 5) were prepared by known [15,16] methods to study the enantioseparation of the azulene ring relative to a phenyl ring. These benzene diols consistently exhibited inferior resolution relative to the azulene diols, particularly on Chiralcel-OD-H. The average α value for the azulene diols was 3.98 (Chiralcel-OD-H) and 1.32 (Lux cellulose 2), while the benzene diols averaged 1.33 and 1.09 on the same phases, respectively.

Table 3. Results from compounds screened by Phenomenex, Regis Technologies and Chiral Technologies on various columns with both reverse and normal mobile phases.

Compound	Column Name	Stationary Phase	Solvent System	R _s	α
1	Phenomenex Lux 5u Cellulose 4	Cellulose tris-(4-chloro-3-methylphenylcarbamate)	60:40:0.1 AmmBi:ACN:DEA ^c	4.40	1.23
1	Phenomenex Lux 5u Cellulose 3	Cellulose tris-(4-methylbenzoate)	60:40:0.1 AmmBi:ACN:DEA ^c	2.90	1.21
1	Phenomenex Lux 5u Cellulose 2	Cellulose tris-(3-chloro-4-methylphenylcarbamate)	60:40:0.1 AmmBi:ACN:DEA ^c	1.03	1.14
1	Phenomenex Lux 5u Amylose 2	Amylose tris-(5-chloro-2-methylphenylcarbamate)	60:40:0.1 AmmBi:ACN:DEA ^c	0.82	1.07
1	Phenomenex Lux 5u Cellulose 2	Cellulose tris-(3-chloro-4-methylphenylcarbamate)	90:10:0.1 Hex:IPA:DEA ^c	4.45	1.52
1	Phenomenex Lux 5u Amylose 2	Amylose tris-(5-chloro-2-methylphenylcarbamate)	90:10:0.1 Hex:IPA:DEA ^c	3.20	1.80
2	(S,S)-Whelk-O 1 (Regis Tech.)	4-(3,5-dinitrobenzamido) tetrahydrophenathrene on silica	90:10 Hex:IPA	4.22	1.23
2	RegisPack ^a	Tris-(3,5-dimethylphenyl) carbamoyl amylose	90:10 Hex:MeOH	4.64	1.22
2	RegisCell ^b	Tris-(3,5-dimethylphenyl) carbamoyl cellulose	85:15 Hex:EtOH ^c	9.51	1.78
4	CHIRALPAK IB-3 (Chiral Tech.)	Cellulose tris-(3,5-dimethylphenylcarbamate)	80:20 Hex:EtOH ^c	10.64	1.31

^a Same stationary phase as the ChiralPack AD.; ^b Same stationary phase as the Chiralcel OD; ^c The acronyms used above represent the following: ammonium bicarbonate (AmBi), acetonitrile (ACN), diethanolamine (DEA), ethanol (EtOH).

Figure 5. Benzene diols and benzene and naphthalene mono-alcohols for comparison.

The corresponding naphthalene diols might have made a better comparison (naphthalene being isomeric with azulene), but they would lack the C_2 symmetry of the azulene and benzene compounds, and neither the CF_3 nor CH_3 naphthalene diols have been reported. Thus, azulene enantiomers appear to exhibit better resolution than comparable phenyl compounds. It is likely that the azulene dipole moment enhances attraction of these molecules to the stationary phase, enforcing diastereomeric interactions more effectively and thus enhancing the enantioseparations.

Since four of these racemic azulene diols (**3–6**) represent two sets of diastereomers (RR/SS vs. SR/RS), we can compare the degree of resolution for diastereomeric pairs of enantiomers. On Chiralcel-OD-H, the enantiomers of the RR/SS diastereomers **3** and **5** averaged an R_s of 3.4 and α of 2.4. In contrast, the RS/SR diastereomers **4** and **6** gave an average R_s of 8.1 and α of 7.8. In both cases, the more polar (based on normal phase TLC mobility) RS/SR diastereomers were much better resolved than the less polar RR/SS diastereomers. This may be related to the polarities, as a rough correlation ($r^2 = 0.65$) exists between silica TLC R_f and R_s on Chiralcel OD-H. The earlier study of these compounds [13] revealed that the RS/SR diastereomers favor a conformation in which both OH groups are oriented toward the same face of the planar azulene system, which is the origin of the higher affinity for silica. Specifically how this might influence enantioselectivity is unclear. While some enantioseparations have been reported [17–19] for diastereomeric molecules, these studies have not attempted to correlate diastereomeric differences (e.g., polarity) with observed resolution. Thus, whether more polar diastereomers are generally better resolved than less polar ones is an open question. With the Lux cellulose 2 column, no large differences in resolution between diastereomers was evident.

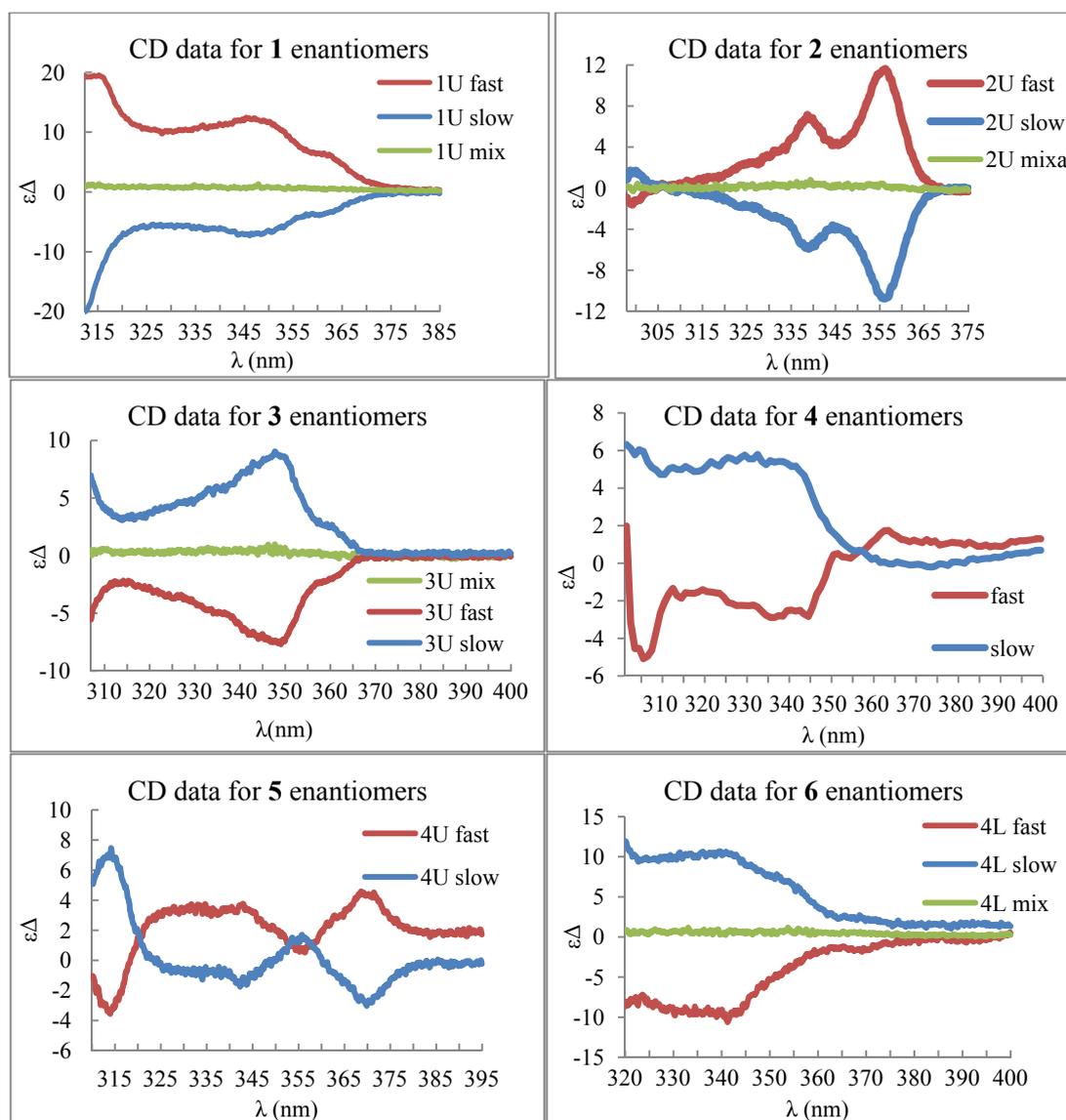
The enantiomers of these compounds had not been separated prior to this work, and so there exists no comparison data. Characterization was complicated by the fact that traditional polarimetry at 589 nm was not possible for these purple compounds because of their strong absorption of yellow light. To avoid this problem, circular dichroism (CD) spectra were obtained on the separated enantiomers (Table 4).

Table 4. Summary of Circular Dichroism (CD) spectrum for each of the separated enantiomers. Each enantiomer is labeled by its order of elution from the HPLC. Retention times are given from the HPLC chromatograms obtained with the Chiralcel-OD-H column.

	Compound	λ (nm)	$\Delta\epsilon$
1	Faster—1.43 min	349.8	11.64
	Slower—1.61 min	349.8	−6.47
2	Faster—4.70 min	356.3	11.23
	Slower—9.80 min	356.3	−10.66
3	Faster—1.75 min	349.7	−7.45
	Slower—2.97 min	349.7	8.60
4	Faster—1.30 min	345.0	−2.83
	Slower—8.13 min	345.0	4.17
5	Faster—1.88 min	314	−3.56
	Slower—4.73 min	314	7.12
6	Faster—1.87 min	339.7	−9.67
	Slower—11.15 min	339.7	10.70

Each enantiomer was collected over several analytical runs and concentration was determined by UV spectroscopy. CD was run on each of the separated enantiomers, giving roughly equal but opposite curves of molar extinction *versus* wavelength, particularly in the region of 300–400 nm (Figure 6). However, lack of CD spectra of comparison compounds prevents identification of the configuration of the enantiomers.

Figure 6. CD spectrum for each of the separated enantiomers, 1–6.



4. Conclusions

The enantioseparation of a series of azulene-centered 1,5-diols has been accomplished on two chiral HPLC columns, with Chiralcel OD-H generally being superior to the Lux cellulose 2 column. The azulene compounds were much better resolved than comparable phenyl compounds, suggesting that the polarity of the azulene ring is important in enforcing diastereomeric interactions with the stationary phase. In addition, an interesting diastereomer dependence on the enantioseparation was observed, with the more polar RS/SR diastereomers being much better resolved than the RR/SS enantiomers on Chiralcel OD-H.

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Authors Contributions

Dana Horgen: Performed separations/measurements and analyzed data, Charles Garner: designed the study, analyzed data and wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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