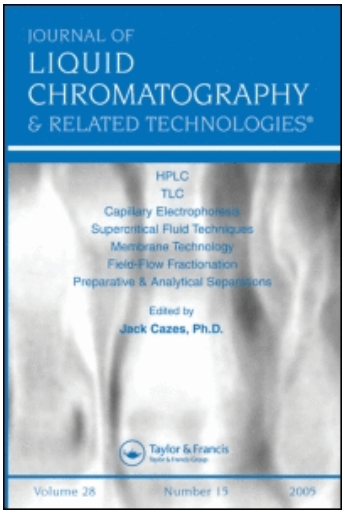

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Chiral HPLC Separations of 1-Azabicyclo[2.2.1]Heptan-3-One and 1-Alkoxycarbonylalkyl-Pyrrolidine-3-Carboxylic Acid Alkyl Ester Enantiomers on Polysaccharide-Based Stationary Phases

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CHIRAL HPLC SEPARATIONS OF 1-AZABICYCLO[2.2.1]HEPTAN-3-ONE AND 1-ALKOXYCARBONYLALKYL-PYRROLIDINE-3-CARBOXYLIC ACID ALKYL ESTER ENANTIOMERS ON POLYSACCHARIDE-BASED STATIONARY PHASES

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ABSTRACT

Chiralcel OD-H, OJ, Chiralpak AD and AS columns were screened for the enantiomeric separation of 1-azabicyclo-[2.2.1]heptan-3-one (**5**) and 1-alkoxycarbonylalkyl-pyrrolidine-3-carboxylic acid alkyl ester intermediates (**1**, **2**, **3** & **4**, see Figure 1 for structures) during the large-scale synthesis of PD 151832. PD 151832 is a highly potent m1 subtype selective muscarinic agonist expected to be useful for patients with Alzheimer's disease. Cellulose-based columns such as Chiralcel OD-H and OJ are in general less efficient than amylose-based columns such as Chiralpak AD and AS for separation of these types of compounds. The optimal column for separation of compounds **3** and **5** is a Chiralpak AD column using hexane/2-propanol/diethylamine as mobile phase, while a Chiralpak AS column works the best for compounds **1**, **2** and **4** using the same solvent mixture.

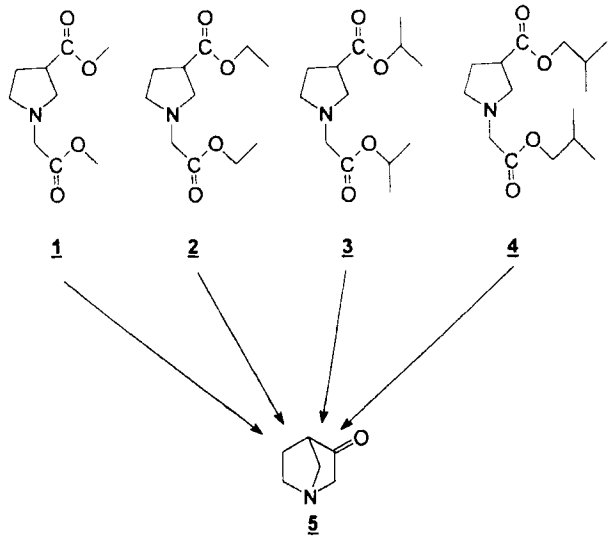

Figure 1. Scheme for Synthesis of 1-Azabicyclo[2.2.1]Heptan-3-One (**5**) from 1-Alkoxycarbonylalkyl-Pyrrolidine-3-Carboxylic Acid Alkyl Esters (**1**, **2**, **3** and **4**).

INTRODUCTION

Enantiomerically pure (R)-1-azabicyclo[2.2.1]heptan-3-one is a synthetic intermediate for making potentially useful biologically active agents.[1] In particular, (R)-1-azabicyclo[2.2.1]heptan-3-one is an important intermediate towards the synthesis of PD 151832, a potential cognition activator under active development for the treatment of neurodegenerative disorders.[2] One synthetic approach to this intermediate involved the cyclization of 1-alkoxycarbonylalkyl-pyrrolidine-3-carboxylic acid alkyl esters (**1**, **2**, **3** & **4**) to give 1-azabicyclo[2.2.1]heptan-3-one (**5**). It was our desire to resolve these early intermediates. Chiral analytical methods are vital in exploring resolution conditions for these intermediates and to ensure the enantiomeric purity of compound **5**.

Chiralcel OD-H, OJ, Chiralpak AD and AS columns were evaluated for the enantiomeric separation of compounds 1, 2, 3, 4 and 5. The effect of the structure of mobile phase alcohol modifier on enantiomeric resolution was also studied.

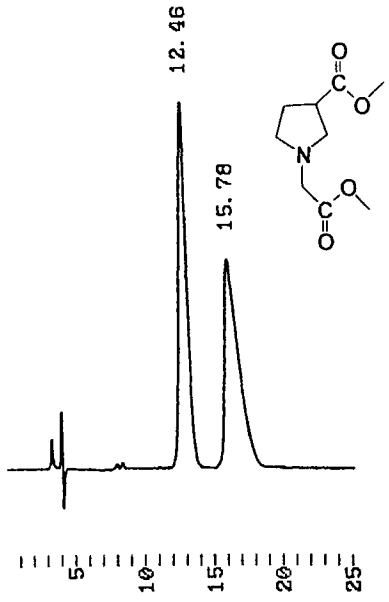

Figure 2. Separation of a Racemic Mixture of Compound **1**; Column: Chiralpak AS, Mobile Phase: Hexane/IPA/DEA (950/50/1), Flow Rate: 1.0 mL/min, Detection: UV @ 230 nm, Sample Amount Injected: 90 μg.

EXPERIMENTAL

Equipment

The liquid chromatographic system consisted of a Hitachi L-6200 intelligent pump, a Micromeritics 728 autosampler, a Valco injector with a 20 μL loop, a Hitachi L-4000H variable wavelength UV detector, a Waters 410 Differential Refractometer equipped with a column oven, and a Hitachi D-2500 Chromato-integrator.

The analytical columns were Chiralcel OD-H, OJ, and Chiralpak AS and AD. All of the columns were 250 x 4.6 mm I.D., and 10 microns in particle size except OD-H which was 5 microns. They were purchased from Chiral Technologies, Inc, Exton, PA.

Chemicals

Hexane and 2-propanol (HPLC grades) were obtained from EM Science, Gibbstown, NJ. Ethanol (absolute) was purchased from Aaper Alcohol and Chemical Company, Shelbyville, KY. Diethylamine (redistilled, 99.5%) was obtained from Aldrich Chemical Company, Milwaukee, WI. Racemic 1-azabicyclo[2.2.1]heptan-3-one, (R)-1-azabicyclo [2.2.1]heptan-3-one, (S)-1-azabicyclo[2.2.1]heptan-3-one, and racemic 1-alkoxycarbonylalkyl-pyrrolidine-3-carboxylic acid alkyl esters were synthesized in the Chemical Development Department, Parke-Davis Pharmaceutical Research Division, Holland, MI.

HPLC Conditions

The mobile phase was either hexane/2-propanol (IPA)/diethylamine (DEA) or hexane/ethanol (EtOH)/diethylamine (DEA) in an appropriate volume ratio. The flow rate was either 1.0 or 0.6 mL/min. The detection was UV @ 230 nm for compounds **1**, **2**, **3** and **4**, and 220 nm for compound 5 when the modifier was 2-propanol (IPA). Refractive index detection (RI) was used for compound **5** when the modifier was ethanol (EtOH). The column temperature was maintained at 30°C. The sample was dissolved in mobile phase. The amount of sample injected was 10 to 100 μg unless otherwise stated.

The capacity factor of the first eluted peak, k_1', the separation factor, α, and the resolution factor, R_s were calculated as follows: $k_1' = (t_1 - t_0)/t_0$; $\alpha = (t_2 - t_0)/(t_1 - t_0)$; $R_s = 2(t_2 - t_1)/(w_1 + w_2)$; where t_0 is the time at void volume, t_1 is the retention time of the first eluted peak, t_2 is the retention time of the second eluted peak, w_1 and w_2 are the widths at baseline for the first and second eluted peaks, respectively, and they were obtained by extrapolating the relatively straight sides of the peaks to the baseline.

RESULTS AND DISCUSSION

The ability of polysaccharide derivative based stationary phases to achieve separation of enantiomers appears to depend on the conformation of the polysaccharide chain and the structure of the substituents.[3] Okamoto et al.[4] and more recently Wainer et al.[5] have provided further insights into the chiral recognition mechanism. However, the actual chiral recognition mechanism remains far from clear.

Table 1

Enantiomeric Separation of Compounds 1, 2, 3, and 4, and Effect of 2-propanol Concentration in Mobile Phase With a Flow Rate of 1.0 mL/min

	Compound 1			Compound 2			Compound 3			Compound 4		
Column	k_1'	α	R_S	k_1'	α	R_S	k_1'	α	R_S	k_1'	α	R_S
Hexane/IPA/DEA: (950/50/1)												
OD-H	1.51	1.07	0.85	----	----	<0.5	No Separation			No Separation		
OJ	----	----	<0.5	No Separation			No Separation			No Separation		
AD	----	----	<0.5	0.93	1.10	1.19	0.55	1.11	1.09	0.62	1.13	1.07
AS	2.80	1.34	4.01	1.28	1.22	2.23	No Separation			0.56	1.25	1.65
Hexane/IPA/DEA (980/20/1)												
OD-H	2.70	1.08	1.20	1.89	1.05	0.67	No Separation			No Separation		
OJ	No Separation			No Separation			----	----	<0.5	No Separation		
AD	----	----	<0.5	1.67	1.09	1.39	1.00	1.11	1.26	1.13	1.11	1.38
AS	4.95	1.38	4.72	2.35	1.24	2.95	No Separation			1.03	1.28	2.59

Table 2

Effect of the Flow Rate on the Enantiomeric Separation of Compounds 1, 2, 3 and 4 Using a Mobile Phase of Hexane/IPA/DEA (950/50/1)

	Compound 1			Compound 2			Compound 3			Compound 4		
Column	k_1'	α	R_S	k_1'	α	R_S	k_1'	α	R_S	k_1'	α	R_S
1.0 mL/min												
OD-H	1.51	1.07	0.85	----	----	<0.5	No Separation			No Separation		
OJ	----	----	<0.5	No Separation			No Separation			No Separation		
AD	----	----	<0.5	0.93	1.10	1.19	0.55	1.11	1.09	0.62	1.13	1.07
AS	2.80	1.34	4.01	1.28	1.22	2.23	No Separation			0.56	1.25	1.65
0.6 mL/min												
OD-H	1.56	1.07	1.20	----	----	<0.5	No Separation			No Separation		
OJ	----	----	<0.5	No Separation			No Separation			No Separation		
AD	1.35	1.05	0.65	0.96	1.10	1.42	0.58	1.11	1.07	0.65	1.12	1.32
AS	2.86	1.35	4.42	1.32	1.22	2.53	No Separation			0.59	1.24	1.97

Table 3

Effectof Mobile Phase Alcohol Modifier on the Enantiomeric Separation of Compounds 1, 2, 3 and 4 with a Flow Rate of 1.0 mL/min

	Compound 1			Compound 2			Compound 3			Compound 4		
Column	k_1'	α	R_S	k_1'	α	R_S	k_1'	α	R_S	k_1'	α	R_S
Hexane/IPA/DEA (980/20/1)												
OD-H	2.70	1.08	1.20	1.89	1.05	0.67	No Separation			No Separation		
OJ	No Separation			No Separation			----	----	<0.5	No Separation		
AD	----	----	<0.5	1.67	1.09	1.39	1.00	1.11	1.26	1.13	1.11	1.38
AS	4.95	1.38	4.72	2.35	1.24	2.95	No Separation			1.03	1.28	2.59
Hexane/EtOH/DEA (980/20/1)												
OD-H	----	----	<0.5	No Separation			No Separation			No Separation		
OJ	No Separation			No Separation			No Separation			----	----	<0.5
AD	4.46	1.04	0.90	----	----	<0.5	1.17	1.07	1.04	1.39	1.11	1.31
AS	1.93	1.23	3.09	0.99	1.13	1.33	No Separation			0.46	1.12	0.77

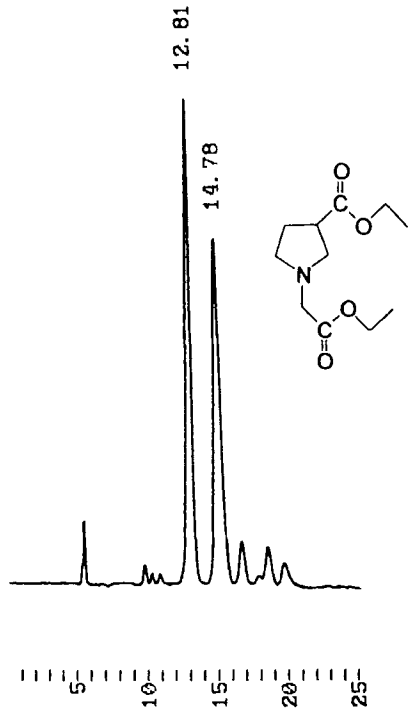

Figure 3. Separation of a Racemic Mixture of Compound **2**; Column: Chiralpak AS, Mobile Phase: Hexane/IPA/DEA (950/50/1), Flow Rate: 0.6 mL/min, Detection: UV @ 230 nm, Sample Amount Injected: 25 μg.

The enantiomeric separation for compounds **1**, **2**, **3** and **4** was carried out using hexane /IPA/DEA. A small amount of diethylamine added in the mobile phase reduced the peak tailing. As shown in Table 1 with hexane /IPA/ DEA (950/50/1). the best results for compounds **1**, **2** and **4** were obtained using a Chiralpak AS column. On the other hand, the same column would not resolve compound **3**.

The drastic difference observed in chiral recognition for compound **3** is probably caused by the added bulk of the methyl substituent on the carbon directly linked to the oxygen on the carboxylic acid ester. This appears to prevent the necessary interactions for chiral recognition to occur. Nevertheless, compound **3** can be resolved using a Chiralpak AD column.

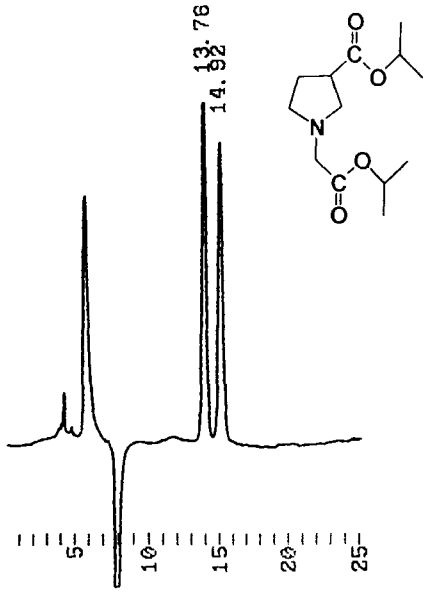

Figure 4. Separation of a Racemic Mixture of Compound **3**; Column: Chiralpak AD, Mobile Phase: Hexane/IPA/DEA (990/10/1), Flow Rate: 0.6 mL/min, Detection: UV @ 230 nm, Sample Amount Injected: 30 μg.

Table 4

Enantiomeric Separation of Compound 5 and Effect of Mobile Phase Alcohol Modifier on a Chiralpak AD Column with a Flow Rate of 1.0 mL/min

Column	k_1'	α	R_S
Hexane/IPA/DEA			
700/300/1	1.47	2.15	11.91
800/200/1	2.17	2.13	12.46
900/100/1	4.28	2.09	13.25
Hexane/EtOH/DEA			
700/300/1	3.45	1.48	5.46
800/200/1	4.67	1.47	6.60

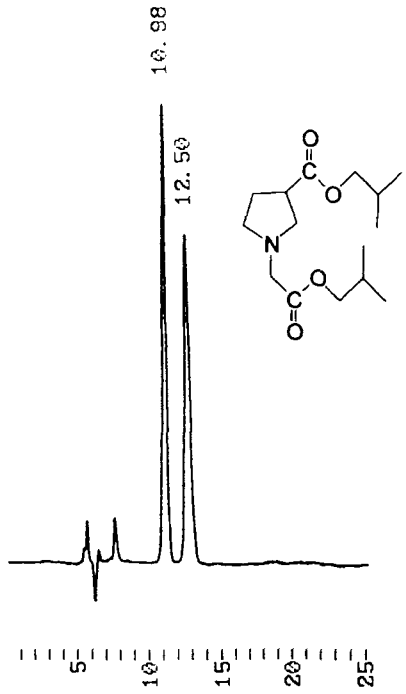

Figure 5. Separation of a Racemic Mixture of Compound **4**; Column: Chiralpak AS, Mobile Phase: Hexane/IPA/DEA (980/20/1), Flow Rate: 0.6 mL/min, Detection: UV @ 230 nm, Sample Amount Injected: 30 μg.

With hexane/IPA/DEA (980/20/1), a decrease in IPA concentration in mobile phase resulted in a corresponding increase in retention and the resolution was generally improved as seen in Table 1. The effect of flow rate was also studied (Table 2).The resolution is increased at 0.6 mL/min for those compounds which give resolution at 1.0 mL/min. The only exception is compound **3** on a Chiralpak AD column where the resolution remains unchanged.

The structure of the mobile phase alcohol modifier is observed to change enantiomeric resolution depending on compound and column type.[6-16] When 2-propanol was replaced with ethanol, resolutions of all four compounds on all four different columns were significantly decreased except for compound **1** where resolution was slightly increased (Table 3). In addition, retention for all four compounds on a Chiralpak AD column increased with the more polar ethanol while it decreased on Chiralcel OD-H, OJ and Chiralpak AD columns.

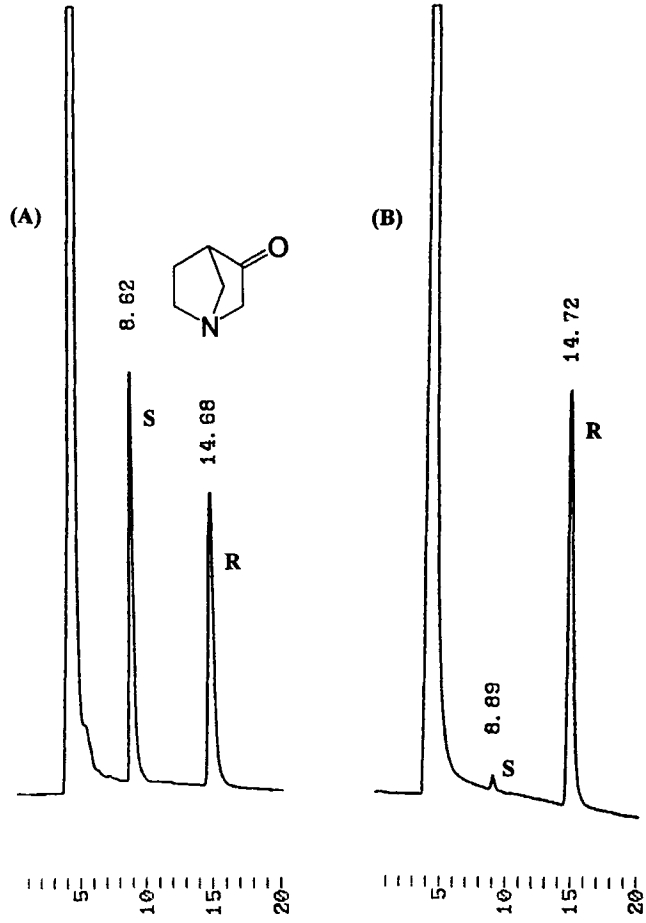

Figure 6. Separations of (A) a Racemic Mixture of Compound **5** and (B) an Enriched Sample of Compound **5**/Chiral Acid Salt; Column: Chiralpak AD, Mobile Phase: Hexane/IPA/DEA (700/300/1), Flow Rate: 1.0 mL/min, Detection: UV @ 220 nm, Sample Amount Injected: 90 μg (Sample was dissolved in IPA).

Separation of the enantiomers of target compound **5** was unsuccessful on Chiralcel OD-H and OJ columns. A Chiralpak AS column gave only partial resolution. Excellent resolution was achieved using a Chiralpak AD column. A decrease in IPA concentration in mobile phase significantly increases retention of compound **5**, and to a lesser extent, resolution. The use of ethanol in mobile phase increases retention of compound **5** while drastically decreasing the resolution (Table 4).

As a result of this study, we were able to select conditions to perform efficient separations of all five compounds of interest. Representative chromatograms for all five compounds are shown in Figures 2-6, respectively.

CONCLUSIONS

Four different polysaccharide derivative-based stationary phases were rapidly screened for enantiomeric separation of five different intermediates in the synthesis of PD 151832. Amylose-based Chiralpak AD and AS columns appear to be superior to the cellulose-based Chiralcel OD-H and OJ columns for separation. A Chiralpak AS column works the best for compounds **1**, **2** and **4**, and a Chiralpak AD column works best for compounds **3** and **5**. 2-Propanol is superior to ethanol as a mobile phase modifier.

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