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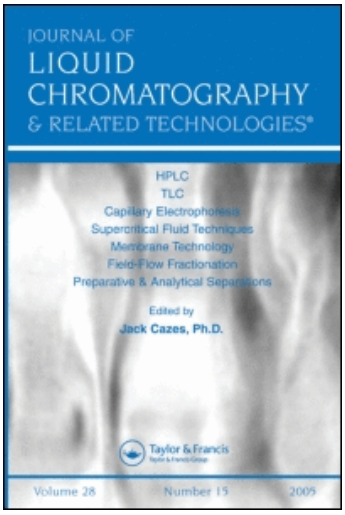

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ABSTRACT

The direct enantioseparation of several N-protected t-BOC (tert-butoxycarbonyl) and CBZ (benzyloxycarbonyl**)** α-amino acids on polysaccharide derived chiral stationary phases (CSPs) is described. Good resolution of N-protected t-BOC and CBZ α-amino acids used in this study has been achieved. Chiralpak AD shows performance superior to other CSPs for the direct separation of the enantiomers of N-t-BOC as well as N-CBZ α-amino acids. The behavior of elution order and the effects of eluent composition for the resolution of N-t-BOC and N-CBZ α-amino acids have been investigated.

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INTRODUCTION

Owing to the growing interest in peptide synthesis, N-protected α-amino acids are one of the most important building blocks in the fields of pharmaceutical chemistry and biochemistry. It is well known that t-BOC and CBZ groups are the most useful protecting moieties among a number of amino protecting groups for α-amino acids.[2] They are resistant to racemization in peptide synthesis and can be readily de-protected under mild acid hydrolysis and/or catalytic hydrogenolysis. Several studies concerning the enantiomeric resolution of these compounds have been reported using various techniques. The direct separation of the enantiomers of N-CBZ protected α-amino acids has been accomplished with a varying degree of success using ion-pair chromatography, ligand exchange method, and CSPs derived from acetylquinone, cellulose, and amino acid derivatives.[3-9]

For the direct separation of the enantiomers of N-t-BOC protected α-amino acids, however, only a few of results have been reported. The ion-pair chromatography using chiral mobile phase additives showed only one example to resolve N-t-BOC-phenylalanine.[3,4] Although a hydroxypropyl derivatized β-cyclodextrin bonded CSP exhibited good enantioselectivity (α = 1.13-1.69) for the direct resolution of the enantiomers of N-t-BOC α-amino acids, recently developed CSPs derived from amino acid urea derivatives afforded moderate enantioselectivity (α = 1.04-1.10) for these compounds.[9,10]

In this paper, we wish to report the direct liquid chromatographic enantiomer separation of N-t-BOC as well as N-CBZ protected amino acids on several polysaccharide derived CSPs.

EXPERIMENTAL

Chromatographic analysis was performed at room temperature using an HPLC consisting of a Waters model 510 pump, a Rheodyne model 7125 injector with a 20μL loop, a variable wavelength UV detector Waters 490 set at 220nm and an Axxiom pyramid chromatography manager data system (pyramid-1 v.1.933, England). Signs of optical rotation were determined using a Shodex OR-1M (Showa Denko, Japan) as an inline chromatography detector. Chiralcel OD, OG, OF, OJ, OB, and Chiralpak AD columns (250mm L x 4.6mm I.D.) were purchased from Daicel Chemical Company (Tokyo, Japan). (Caution: only proper 2-propanol/hexane as a mobile phase should be used in Chiralcel OF.) HPLC-grade hexane and 2-propanol were obtained from J. T. Baker (Phillipsburg, NJ). Trifluoroacetic acid was obtained from Aldrich (Milwaukee, WI). The racemic (or enantiomerically pure) N-BOC and CBZ protected α-amino acids were prepared according to the conventional methods.[11]

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Table 1

Direct Separation of the Enantiomers of N-t-BOC Protected α-Amino Acids

CSP		Ala	Val	Leu	Ile	PG	Phe	Met
	α	1.00	1.36	1.00	1.19	1.19	1.08	1.00
OD	k'_1	1.57	0.87	1.12	0.83	2.13	2.55	2.21
	Rs		2.31		1.10	1.47	0.38	
	retained*		D(-)		D(-)	L(+)	D(-)	
	α	1.00	1.00	1.18	1.06	1.26	1.09	1.09
OG	k'_1	1.43	0.85	0.93	0.79	2.17	1.95	2.69
	Rs			0.90	0.22	1.52	0.47	0.62
	retained*			L(-)	L(+)	L(+)	L(+)	L(+)
	α	1.18	1.37	1.00	1.24	1.53	1.06	1.04
OF	k'_1	3.48	1.77	2.40	1.83	7.32	5.82	7.36
	Rs	1.17	2.04		1.27	3.02	0.30	0.30
	retained*	D(+)	D(-)		D(-)	L(+)	D(-)	D(-)
	α	1.00	1.85	1.36	1.83	1.65	1.18	1.08
OJ	k'_1	1.36	0.59	0.52	0.45	3.41	1.26	2.03
	Rs		2.08	1.07	1.86	2.54	0.57	0.42
	retained*		D(-)	D(+)	D(-)	D(-)	D(-)	D(-)
	α	1.12	1.00	1.00	1.00	1.00	1.00	1.00
OB	k'_1	0.62	0.33	0.35	0.29	1.64	0.92	1.42
	Rs	0.40						
	retained*	D(+)						
	α	1.79	2.00	2.41	1.98	1.57	3.00	2.51
AD	k'_1	4.55	6.17	5.40	6.35	9.77	6.79	8.53
	Rs	5.10	6.50	7.67	6.02	5.98	10.36	10.46
	retained*	L(-)	L(+)	L(-)	L(+)	L(+)	L(+)	L(+)

Mobile phase: 2-propanol/hexane=4/96 (V/V) with 0.1% trifluoroacetic acid; Flow rate=1.0 mL/min; UV 220nm; Temperature ambient (about 25°C); Injection volume 10μL of 10 mg/mL.

* Indicates absolute configuration and the sign of optical rotation of more strongly retained enantiomer.

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Table 2

Direct Separation of the Enantiomers of N-CBZ Protected α-Amino Acids

CSP		Ala	Val	Leu	Ile	PG	Phe	Met
OD	α	3.04	6.23	2.63	7.16	1.94	1.04	1.81
	k'_1	2.11	1.40	1.77	1.39	6.40	4.28	3.63
	Rs	8.71	14.12	6.34	14.40	6.87	0.15	5.75
	retained*	D(-)	D(-)	D(+)	D(-)	D(-)	L(+)	D(-)
OG	α	1.12	1.15	1.00	1.00	1.00	1.00	1.00
	k'_1	2.24	1.34	1.66	1.37	3.68	2.98	4.13
	Rs	0.88	0.79					
	retained*	D(-)	D(-)					
OF	α	1.53	1.54	1.25	1.40	1.30	1.26	1.18
	k'_1	5.10	2.89	3.70	2.90	9.09	8.25	11.05
	Rs	2.05	2.60	1.42	2.08	2.07	1.58	1.35
	retained*	D(-)	D(-)	D(+)	D(-)	D(-)	D(-)	D(-)
OJ	α	1.18	2.02	1.10	2.14	1.54	1.17	1.05
	k'_1	3.92	1.70	1.80	1.20	8.03	4.34	6.97
	Rs	1.28	3.60	0.43	3.22	3.19	0.96	0.33
	retained*	D(-)	L(+)	L(-)	L(+)	L(+)	D(-)	L(+)
OB	α	1.87	1.00	1.00	1.00	1.53	1.13	1.00
	k'_1	1.96	1.03	1.34	1.13	6.62	2.79	4.74
	Rs	1.79				0.83	0.25	
	retained*	(D-)				D(-)	D(-)	
AD	α	1.99	5.17	2.19	7.08	2.11	1.20	1.64
	k'_1	3.08	3.68	3.75	3.47	6.60	5.22	5.80
	Rs	7.91	17.81	8.70	15.00	9.78	1.98	6.70
	retained*	D(-)	D(-)	D(+)	D(-)	D(-)	L(+)	D(-)

Mobile phase: 2-propanol/hexane=10/90 (V/V) with 0.1% trifluoroacetic acid; Flow rate=1.0 mL/min; UV 220nm; Temperature ambient (about 25°C); Injection volume 10μL of 10 mg/mL.

* Indicates absolute configuration and the sign of optical rotation of more strongly retained enantiomer.

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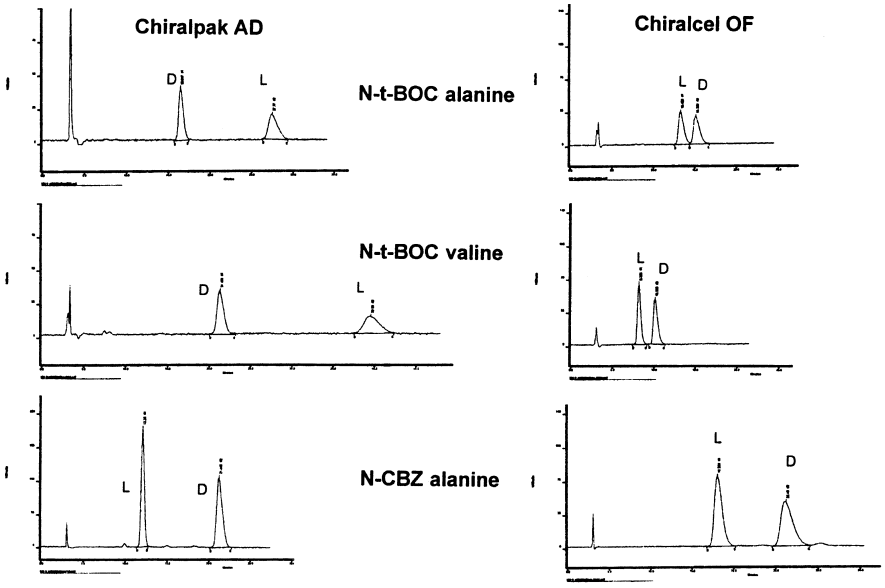

Figure 1. Chromatograms of the direct enantiomer separation of N-t-BOC alanine, N-t-BOC valine and N-CBZ alanine on Chiralpak AD and Chiralcel OF; Chromatographic conditions as in Tables 1 and 2.

RESULTS AND DISCUSSION

Table 1 shows chromatographic data for the direct separation of the enantiomers of several N-t-BOC protected α-amino acids. Chiralpak AD exhibits excellent resolving ability for all N-t-BOC protected α-amino acids used in this study, where separation factors range from 1.57 to 3.00. Chiralcel OJ and OF afford good enantioselectivity, whereas Chiralcel OB shows little enantioselectivity. Although the elution orders of the analytes on Chialpak AD and Chiralcel OG are inverted on Chiralcel OJ, consistent elution orders of the resolved analytes are observed on each of these three CSPs. Therefore, the elution of the (D)-isomers prior to the (L)-isomers on Chialpak AD and Chiralcel OG is observed, whereas the (D)-isomers are selectively retained on Chiralcel OJ.

Since the base-line enantioseparation of the examined analytes is generally provided on Chiralpak AD and Chiralcel OJ, the opposite of elution orders on Chialpak AD to those on Chiralcel OJ may provide the advantage for enantiomeric purity determination.[12] Table 2 shows chromatographic data for the direct separation of the enantiomers of several N-CBZ protected α-amino

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Table 3

Effect of Mobile Phase Composition on the Enantioseparation of Some N-t-BOC and CBZ Protected α-Amino Acids on Chiralcel OF and Chiralpak AD

CSP	Analyte	Mobile Phase*	α	k'_1	k'_2	Rs
		4%	1.18	3.48	4.11	1.17
	N-t-BOC Alanine	3%	1.21	4.96	5.98	1.25
		2%	1.23	9.27	11.41	1.64
		4%	1.37	1.77	2.42	2.04
Chiralcel OF	N-t-BOC Valine	3%	1.38	2.62	3.62	2.17
		2%	1.39	4.62	6.42	2.46
		14%	1.50	2.76	4.14	1.70
	N-CBZ Alanine	12%	1.52	3.71	5.64	1.78
		10%	1.53	5.10	7.81	2.05
		4%	1.79	4.55	8.14	5.10
	N-t-BOC Alanine	3%	1.82	5.82	10.59	5.43
		2%	1.87	8.93	16.70	5.93
		14%	1.94	1.79	3.47	7.11
Chiralpak AD	N-CBZ Alanine	12%	1.96	2.25	4.41	7.40
		10%	1.99	3.08	6.13	7.91
		14%	5.02	2.30	11.55	17.01
	N-CBZ Valine	12%	5.08	2.87	14.58	17.18
		10%	5.17	3.68	19.03	17.81

Flow rate = 1.0mL/min; UV 220nm; Temperature ambient (about 25°C).
* Indicates percent of 2-propanol in hexane (V/V) with 0.1% trifluoroacetic acid as the mobile phase.

acids. The degree of enantioselectivity of N-CBZ α-amino acids on Chiralpak AD is comparable to that on Chiralcel OD.[7] Although Chiralcel OF and OJ show lower enantioselectivity than Chiralpak AD and Chiralcel OD, they afford the base-line resolution for most of the analytes in this study. The elution orders of all analytes studied on Chiralpak AD are identical to those on Chiralcel OD, where the L-isomers elute first except for N-CBZ phenylalanine.

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It is noteworthy that Chiralpak AD shows performance superior to that of other CSPs used in our study for the direct separation of the enantiomers of N-t-BOC as well as N-CBZ α-amino acids. In addition, it is observed that Chiralcel OF and OJ afford good enantioselectivity for the direct resolution of both N-t-BOC and CBZ α-amino acids. However, Chiralcel OD affords excellent resolution of N-CBZ α-amino acids and worse resolution of N-t-BOC α-amino acids.

Typical chromatograms of N-t-BOC and CBZ protected α-amino acids on Chiralpak AD and Chiralcel OF are presented in Figure 1. The effects of mobile phase composition on the retention and the separation factor are investigated. As shown in Table 3, mobile phase compositions are varied from 2% to 4% of 2-propanol in hexane for N-t-BOC protected analytes and from 10% to 14% of 2-propanol in hexane for N-CBZ protected analytes on Chiralcel OF and Chiralpak AD. As the composition of 2-propanol in hexane decreases, the enantiomeric resolution increases with increased capacity factors.

In conclusion, we presented the direct liquid chromatographic separation of enantiomers of α-amino acids as N-protected t-BOC and CBZ derivatives, the most commonly used amino protecting groups for α-amino acids. The direct resolution of the enantiomers of N-BOC protected α-amino acids was for the first time accomplished on polysaccharide derived CSPs. Among all CSPs studied, Chiralpak AD showed excellent resolving ability for the enantiomers of N-BOC as well as N-CBZ protected amino acids.

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