Enantiomer Separation of *N*-Protected α-Amino Acids on Covalently Immobilized Cellulose Tris(3,5-chlorophenylcarbamate) Chiral Stationary Phase in HPLC

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Chiral stationary phases (CSPs) based on polysaccharide derivatives have been widely and successfully used for enantiomer separation by HPLC.[1-3] These CSPs are produced by physical coating of the chiral selectors on silica support. Therefore, they are not compatible to all solvents in normal phase and the use of various solvents such as ethyl acetate, tetrahydrofuran, toluene and halogenated solvents as mobile phases or analyte solvents is prohibited for column safety.[1,2] Consequently, a reaction performed in any of the prohibited solvents cannot be directly monitored by HPLC unless these solvents are removed. To improve these problems of the coated CSPs, the covalently immobilized type CSPs derived from polysaccharide derivatives have been developed and applied.[4-11] Very recently, a new covalently immobilized type CSP, Chiralpak IC based on cellulose tris(3,5-chlorophenylcarbamate) has been introduced.[12] In this study, we present the liquid chromatographic enantiomer separation of *N*-protected phthaloyl (PHT) and fluorenylmethoxycarbonyl (FMOC) α-amino acids[13-15] as well as their applications to the on-line reaction monitoring on Chiralpak IC.

Tables 1 and 2 show liquid chromatographic results for enantiomer separation of *N*-protected PHT and FMOC α-amino acids on Chiralpak IC, respectively. In general, Chiralpak IC affords fairly good separation factors for the enantiomer resolution of *N*-PHT and FMOC α-amino acids. Eleven *N*-PHT α-amino acids (α = 1.09-2.13, Rs = 1.04-11.03) except for *N*-PHT 2-aminocaprylic acid and sixteen *N*-FMOC α-amino acids (α = 1.20-1.73, Rs = 1.75-7.19) except for *N*-FMOC glutamic acid and glutamine were baseline resolved. The elution orders of these analytes are shown in Table 1 and Table 2. The L-enantiomers of all *N*-PHT α-amino acids were preferentially retained except for *N*-PHT phenylglycine (Table 1, entry 11), while the D-enantiomers of the examined *N*-FMOC α-amino acids were preferentially retained.

The chromatographic method in this study was applied for determination of the enantiomeric purity of three commercially available analytes (*N*-PHT L-glutamic acid, *N*-PHT L-phenylalanine and *N*-FMOC L-phenylglycine) as well as other synthesized *N*-protected α-amino acids on Chiralpak IC. The enantiomeric impurities of 0.42, 0.38 and 0.50% for these three commercially available analytes were determined, respectively. Also the degree of racemization for other synthesized *N*-protected PHT and FMOC α-amino acids was determined, as shown in Tables 1 and 2. The degree of racemization of *N*-protected FMOC α-amino acids prepared in this study was pretty lower (< 0.77%) than that of *N*-protected PHT α-amino acids.[16] Since the enantiomeric purities of nine *N*-FMOC α-amino acids analytes were not detected in Table 2, it was concluded that the racemization of nine analytes among the examined seventeen *N*-FMOC α-amino acids analytes did not occur during FMOC protecting procedure of amino group. In the case of *N*-protected

Table 1. Enantiomer Separation of *N*-PHT Protected α-Amino Acids

Entry	Analyte	α^a	$k'_1{}^b$	k'_2	Rs[c]	Conf.[d]	Enantiomeric impurity[e]
1	Alanine	1.11	7.00	7.79	1.87	L	0.21%
2	2-Aminobutyric acid	1.17	5.44	6.35	2.44	L	0.13%
3	2-Aminocaprylic acid	1.03	3.46	3.58	0.18	-	-
4	Glutamic acid	2.13	2.57[f]	5.49	7.29	L	0.42%
5	Isoleucine	1.14	3.02	3.45	1.74	L	0.43%
6	Leucine	1.09	3.33	3.62	1.04	L	4.20%
7	Methionine	2.12	3.96[g]	8.40	11.03	L	1.40%
8	Norleucine	1.08	4.08	4.40	1.04	L	2.15%
9	Norvaline	1.15	4.42	5.10	1.56	L	0.57%
10	Phenylalanine	1.12	7.44	8.34	1.82	L	0.38%
11	Phenylglycine	1.58	3.96[g]	6.28	6.51	D	45.48%
12	Valine	1.32	3.41	4.49	3.16	L	0.37%

Mobile phase: 5% 2-propanol/hexane(V/V) with 0.1% TFA; Flow rate = 1 mL/min; Detection UV 254 nm. [a]Separation factor. [b]Capacity factor for the first eluted enantiomer. [c]Resolution factor. [d]Absolute configuration of the second eluted enantiomer. [e]Average value of three times determined. [f]20% 2-propanol/hexane(V/V) with 0.1% TFA. [g]10% 2-propanol/hexane(V/V) with 0.1% TFA.

Table 2. Enantiomer Separation of *N*-FMOC Protected α-Amino Acids

Entry	Analyte	α^a	$k'_1{}^b$	k'_2	Rs[c]	Conf.[d]	Enantiomeric impurity[e]
1	Alanine	1.37	2.55	3.49	4.32	D	n. d.[g]
2	2-Aminobutyric acid	1.42	2.92	4.15	4.33	D	0.48%
3	2-Aminocaprylic acid	1.33	2.35	3.14	3.18	–	–
4	Asparagine	1.23	9.08[f]	11.20	1.95	D	n. d.[g]
5	Aspartic acid	1.58	1.34[f]	2.12	3.59	D	n. d.[g]
6	Glutamic acid	1.06	6.28	6.65	0.43	D	n. d.[g]
7	Glutamine	1.08	8.36[f]	9.03	0.62	D	n. d.[g]
8	Isoleucine	1.55	2.16	3.35	5.44	D	0.37%
9	Leucine	1.73	2.28	3.95	5.95	D	n. d.[g]
10	Methionine	1.27	4.46	5.65	3.05	D	0.37%
11	Norleucine	1.35	2.61	3.52	3.42	D	n. d.[g]
12	Norvaline	1.44	2.81	4.04	4.94	D	0.40%
13	Phenylalanine	1.28	3.68	4.72	3.35	D	0.10%
14	Phenylglycine	1.46	3.38	4.93	4.99	D	0.50%
15	Serine	1.71	5.33	9.14	7.19	D	0.77%
16	Threonine	1.50	3.96	5.95	4.89	D	0.31%
17	Tyrosine	1.20	10.86	12.99	1.75	D	n. d.[g]
18	Valine	1.39	2.15	2.99	3.86	D	n. d.[g]

Mobile phase: 10% 2-propanol/hexane(V/V) with 0.1% TFA; Flow rate = 1 mL/min; Detection UV 254 nm. [a]Separation factor. [b]Capacity factor for the first eluted enantiomer. [c]Resolution factor. [d]Absolute configuration of the second eluted enantiomer. [e]Average value of three times determined. [f]20% 2-propanol/hexane(V/V) with 0.1% TFA. [g]not detected (< 0.01%).

PHT α-amino acids, however. *N*-PHT phenylglycine enantiomer having a benzyl carbon on the chiral center was nearly racemized and other *N*-PHT α-amino acids showed relatively high degree of racemization (0.13-4.20%), compared to *N*-FMOC α-amino acids. Based on our experimental results, although commercially available *N*-protected α-amino acid enantiomers for asymmetric synthesis precursors are used, their potential enantiomeric impurities should be carefully considered.

The solvent versatility of Chiralpak IC was investigated on a typical example of direct HPLC monitoring for the preparation of *N*-protected PHT α-amino acids.[10,11] Figure 1 shows before and after work-up HPLC chromatograms of determination of the enantiomeric purity during *N*-PHT L-valine preparation with phthalic anhydride and L-valine in toluene. For the use of coated polysaccharides derived CSPs, a work-up together with the removal of toluene used as reaction solvent in preparation of PHT α-amino acids should be strictly required before injection in HPLC for the coated column safety. As shown in Figure 1, however, the solvent versatility of Chiralpak IC, the bonded polysaccharides derived CSP in monitoring reactions performed in any kind of organic solvents or aqueous solution allows to afford new applications of enantiomer separation using direct analysis techniques without further purification or work-up. Also, it is notable that the enantiomeric purity of the reaction

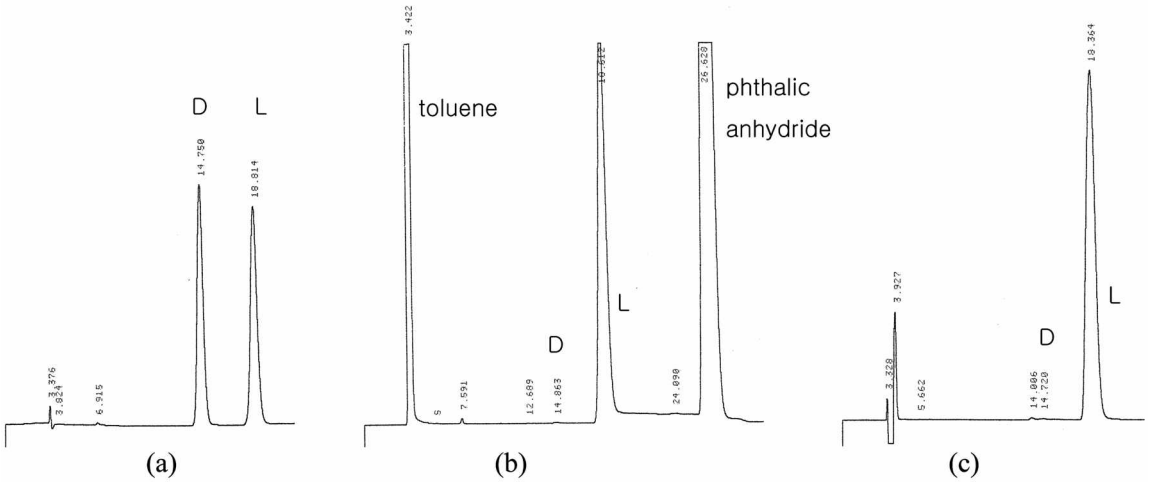

Figure 1. (a) Chromatogram of enantiomer separation of *N*-PHT racemic valine (injected amount: 20 μg) (b) before work-up HPLC chromatogram of determination of the enantiomeric purity (D:L = 0.37:99.63) during *N*-PHT L-valine preparation with phthalic anhydride and L-valine in toluene (c) after work-up HPLC chromatogram of determination of the enantiomeric purity (D:L = 0.37:99.63) of *N*-PHT L-valine on Chiralpak IC. See Table 1 for chromatographic conditions.

mixture of *N*-PHT L-valine before work-up is identical to that of the isolated *N*-PHT L-valine analyte after work-up. which indicates that this analyte was not racemized during isolation procedure.

Experimental Section

Chromatographic analysis was performed at room temperature using an HPLC consisting of a Waters model 510 pump. a Rheodyne model 7125 injector with a 20 μL loop, a variable wavelength UV detector Waters 490 set at 254 nm and an HP 3396 series II recorder. Chiralpak IC column (250 mm L × 4.6 mm I.D.) were purchased from Daicel Chemical Company (Tokyo. Japan). HPLC-grade hexane and 2-propanol were obtained from J. T. Baker (Phillipsburg. NJ). Trifluoroacetic acid (TFA) was obtained from Aldrich (Milwaukee, WI). The racemic (or enantiomerically pure) *N*-PHT and FMOC protected α-amino acids were prepared according to the conventional methods.[13,16] *N*-PHT L-glutamic acid. *N*-PHT L-phenylalanine and *N*-FMOC L-phenylglycine were obtained from Fluka company.

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