Direct Resolution of *N-tert*-Butoxycarbonyl and Benzyloxycarbonyl α-Amino Acids on a Chiral Stationary Phase

Byoung-Hyoun Kim and Wonjae Lee*

LG Chemical Ltd., Research Park, P.O. Box 61 Yu Sung, Science Town, Taejon 305-380, Korea
Received January 12, 1998

N-Protected α-amino acids have been widely used as important chiral building blocks in the fields of pharmaceutical chemistry and biochemistry. *t*-BOC (*tert*-butoxycarbonyl) and CBZ (benzyloxycarbonyl) moieties are the most commonly used protecting groups among a great many developed amino protecting groups for α-amino acids.[1] Owing to the importance of optical purity of *N-t*-BOC and CBZ α-amino acids, convenient and accurate methods to determine the enantiopurity of these compounds have been required and developed. Several methods for the direct chromatographic separation of the enantiomers of *N*-CBZ α-amino acids have been reported using various techniques.[2-10] However, very few results for the direct resolution of *N-t*-BOC α-amino acids have been reported.[10,11] A chiral stationary phase (CSP) derived from β-cyclodextrin derivative showed good enantioselectivity (α=1.13-1.69) for the direct resolution of *N-t*-BOC α-amino acids using reversed mobile phases.[11] Recently, CSPs derived from amino acid urea derivatives were reported to afford poor enantioselectivity (α= 1.04-1.10) for these compounds.[10] Here, we present the direct liquid chromatographic resolution of *N-t*-BOC as well as CBZ amino acids on polysaccharide derived Chiralpak AS column under normal phase conditions.[12]

Table 1 shows chromatographic results for the direct separation of the enantiomers of several *N-t*-BOC α-amino acids. Chiralpak AS affords high enantioselectivity for the resolution of *N-t*-BOC α-amino acids. The separation factors shown in Table 1 are substantially greater than those afforded by CSPs derived from β-cyclodextrin and amino acid urea derivatives.[10,11] It is observed that the base-line enantioseparation of the examined *N-t*-BOC α-amino acids is generally provided. Table 2 shows chromatographic results for the direct separation of the enantiomers of several *N*-CBZ α-amino acids. Chiralpak AS exhibits excellent resolving ability for all *N*-CBZ α-amino acids used in this study, where separation factors range from 2.0 to 6.5. Other

Table 1. Direct separation of the enantiomers of *N-t*-BOC α-amino acids

Analyte	α	k'_1	k'_2	Retained*
N-t-BOC alanine	1.82	2.44	4.45	D(+)
N-t-BOC valine	2.37	1.56	3.68	D(−)
N-t-BOC leucine	4.50	2.05	9.20	D(+)
N-t-BOC isoleucine	2.27	1.92	4.35	D(−)
N-t-BOC phenylglycine	2.05	4.09	8.38	D(−)
N-t-BOC phenylalanine	1.17	4.51	5.29	D(−)
N-t-BOC methionine	1.84	6.08	11.19	D(−)

Mobile phase: 2-propanol/hexane=4/96 (V/V) with 0.1% trifluoroacetic acid; Flow rate=1.0 mL/min; UV 220 nm; Temperature ambient (about 25 °C); Injection volume 10 μL of 10 mg/mL; *indicates absolute configuration and the sign of optical rotation of the second eluted enantiomer determined by an in-line polarimetric detector (Shodex OR-1M) set at 589 nm.

Table 2. Direct separation of the enantiomers of *N*-CBZ α-amino acids

Analyte	α	k'_1	k'_2	Retained*
N-CBZ alanine	2.00	3.27	6.53	D(−)
N-CBZ valine	5.18	1.81	9.35	D(−)
N-CBZ leucine	6.15	2.30	14.16	D(+)
N-CBZ isoleucine	6.53	2.05	13.40	D(−)
N-CBZ phenylglycine	4.86	5.76	28.00	D(−)
N-CBZ phenylalanine	2.64	4.79	12.64	D(−)
N-CBZ methionine	2.92	7.85	22.91	D(−)

Mobile phase: 2-propanol/hexane=10/90 (V/V) with 0.1% trifluoroacetic acid; Flow rate=1.0 mL/min; UV 220 nm; Temperature ambient (about 25 °C); Injection volume 10 μL of 10 mg/mL; *indicates absolute configuration and the sign of optical rotation of the second eluted enantiomer determined by an in-line polarimetric detector (Shodex OR-1M) set at 589 nm.

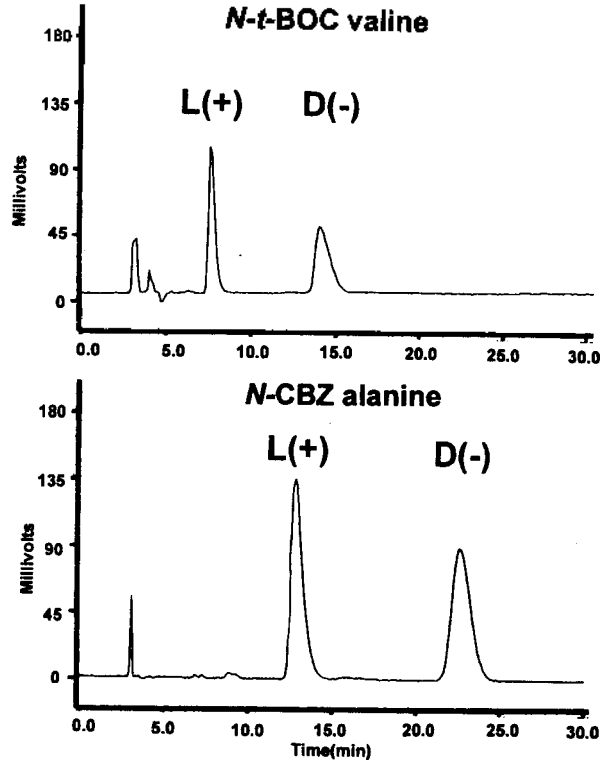

Figure 1. Chromatograms of the direct enantiomer separation of *N-t*-BOC valine and *N*-CBZ alanine; See Tables 1 and 2 for chromatographic conditions.

reported methods using ion-pair chromatography, ligand exchange chromatography and CSPs derived from acetylquinone, macrocyclic antibiotics and amino acid derivatives do not provide a high level of enantioselectivity.[2-10] In addition, Chiralpak AS shows superior performance to a previously employed CSP (Chiralcel OD), which failed to resolve *N*-CBZ phenylalanine.[6] Chiralpak AS column is amylose based, whereas Chiralcel OD column is derived from cellulose derivative. Basically, the structural differences between a rigid linear structure of cellulose and a helical structure of amylose might be responsible for the observed differences in resolution.[13] It is notable that Chiralpak AS shows a consistent elution order for both *N*-*t*-BOC and CBZ α-amino acids studied, where the L-isomers elute first in all cases. Typical chromatograms of *N*-*t*-BOC and CBZ α-amino acids are presented in Figure 1.

In summary, we demonstrated the direct liquid chromatographic separation of enantiomers of several *N*-protected *t*-BOC and CBZ α-amino acids. Excellent resolution of *N*-*t*-BOC and CBZ α-amino acids used in this study was obtained using polysaccharide derived Chiralpak AS. It is expected that Chiralpak AS will be useful for direct resolution of other *N*-*t*-BOC as well as CBZ α-amino acids.

References

1. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd Ed.; John Wiley & Sons. Inc.: New York, 1991.
2. Petterson, C.; No, K. *J. Chromatogr.* **1983**, *282*, 671.
3. Petterson, C. *J. Chromatogr.* **1984**, *316*, 553.
4. Yuki, Y.; Saigo, K.; Kimoto, H.; Tachibana, K.; Hasegawa, M. *J. Chromatogr.* **1987**, *400*, 65.
5. Petterson, C.; Gioeli, C. *J. Chromatogr.* **1987**, *398*, 247.
6. Okamoto, Y.; Aburatani, R.; Kaida, Y.; Hatada, K. *Chem. Lett.* **1988**, 1125.
7. Ishikawa, A.; Shibata, T. *J. Liq. Chromatogr.* **1993**, *16*, 859.
8. Armstrong, D. W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill, C.; Chen, J.-R. *Anal. Chem.* **1994**, *66*, 1473.
9. Armstrong, D. W.; Liu, Y.; Ekborgott, K. H. *Chirality* **1995**, *7*, 474.
10. Oi, H.; Kitahara, H.; Aoki, F.; Kisu, N. *J. Chromatogr. A* **1995**, *689*, 195.
11. Chang, S. C.; Wang, L. R.; Armstrong, D. W. *J. Liq. Chromatogr.* **1992**, *15*, 1411.
12. Chiralpak AS column purchased from Daicel Chemical Company (250 mm L×4.6 mm I.D., 10 μm, Tokyo, Japan) is based upon amylose tris[(S)-α-methylbenzyl carbamate] coated on a silica support. Be careful that only ethanol or 2-propanol in hexane as a mobile phase should be used.
13. Okamoto, Y.; Kaida, Y. *J. Chromatogr. A* **1994**, *666*, 403.