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Journal of Chromatography A, 1097 (2005) 188–191
JOURNAL OF CHROMATOGRAPHY A
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Short communication

Unusual resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids on a chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid

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Received 17 August 2005; received in revised form 5 October 2005; accepted 17 October 2005
Available online 2 November 2005

Abstract

While HPLC chiral stationary phases (CSPs) based on chiral crown ethers have been known useful for the resolution of only racemic primary amino compounds or some secondary amino compounds, in this study, we first demonstrated that the CSP based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid is also useful for the resolution of *N*-benzoyl-α-amino acids, which do not contain a primary or secondary amino group. Especially, *N*-(3,5-dinitrobenzoyl)-α-amino acids were resolved better than corresponding *N*-(3-nitrobenzoyl)- or *N*-benzoyl-α-amino acids, the separation (α) and the resolution factors (R_S) for the resolution of eight *N*-(3,5-dinitrobenzoyl)-α-amino acids being in the range of 1.06–1.81 and 0.54–2.81, respectively. The optimum mobile phase condition was the mixture of acetic acid–triethylamine–acetonitrile with the ratio of 0.05/0.25/100 (v/v/v).
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Keywords: Chiral stationary phase; (+)-(18-Crown-6)-2,3,11,12-tetracarboxilic acid; Enantiomer separation; *N*-(3,5-Dinitrobenzoyl)-α-amino acids

1. Introduction

Crown ether-based CSPs have been applied mostly to the resolution of racemic primary amino compounds [1,2]. Even though the chiral recognition mechanism is still controversial, the protonation of the primary amino group of analytes in a mobile phase containing a certain amount of acidic modifier and the complexation of the resulting primary ammonium ion ($R\text{-}NH_3^+$) of analytes inside the cavity of chiral crown ether ring of the CSP have been known essential for the chiral recognition [3]. In this instance, the presence of primary amino group of analytes is essential for the chiral recognition on crown ether-based CSPs. For example, CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** (Fig. 1) [4,5] or chiral pseudo-18-crown-6 ether [6] have been utilized for the liquid chromatographic resolution of racemic primary amino compounds. Especially, CSP **2** (Fig. 1) developed in our laboratory was successfully applied to the resolution of various racemic primary amino compounds [5,7–11].

While application of crown ether-based CSPs has been limited only to the resolution of racemic primary amino compounds, the resolution of racemic secondary amino compounds including β-blockers on CSP **2** was recently reported [12,13]. As an effort to extend the use of CSP **2** further, in this study, we wish to first report the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids on CSP **2**. *N*-(3,5-Dinitrobenzoyl)-α-amino acids do not contain any primary or secondary amino group, but contain a free carboxylic acid group. In this instance, the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids on CSP **2** is quite unusual and surprising.

2. Experimental

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7725i injector with a 20 μl sample loop, a YoungLin M720 Absorbance detector (variable wavelength) and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The temperature of the chiral column was set to 20 °C by using a Julabo F30 Ultratemp 2000 cooling circulator. Chiral column (150 mm × 4.6 mm I.D.) packed with CSP **2** was available from previous study [7].

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doi:10.1016/j.chroma.2005.10.036

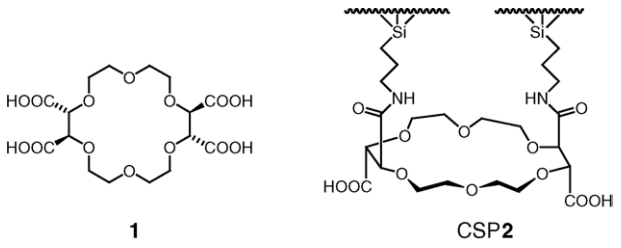

Fig. 1. Structures of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** and CSP **2**.

N-(3,5-Dinitrobenzoyl)- (**3**-**10**), *N*-(3-nitrobenzoyl)- (**11**-**14**) and *N*-benzoyl-α-amino acids (**15**,**16**) (Fig. 2) were prepared through the treatment of 3,5-dinitrobenzoyl, 3-nitrobenzoyl or benzoyl chloride (1.0 mmol) with α-amino acids (1.2 mmol) in the presence of propylene oxide (3.0 mmol) in tetrahydrofuran at room temperature for 30 min, filtration of the reaction mixture, evaporation of the solvent and the final recrystallization of the solid residue from chloroform. Injection samples were prepared by dissolving each of racemic or optically active *N*-(3,5-dinitrobenzoyl)-, *N*-(3-nitrobenzoyl)- and *N*-benzoyl-α-amino acids in tetrahydrofuran at a concentration of 1 mg/ml and an injection size of 2 μl was typically used.

3. Results and discussion

The chromatographic results for the resolution of eight *N*-(3,5-dinitrobenzoyl)-α-amino acids (**3**-**10**) on CSP **2** are summarized and compared with those for the resolution of *N*-(3-nitrobenzoyl)- (**11**-**14**) and *N*-benzoyl-α-amino acids (**15**,**16**) on CSP **2** in Table 1. The resolution results summarized in Table 1 are the best that could be obtained at the present state of the art. The elution orders determined by injecting configurationally known samples were always consistent, the (D)-enantiomers being retained longer than (L)-enantiomers. Mobile phase was the mixture of acetic acid–triethylamine–acetonitrile with the ratio of 0.05/0.25/100 (v/v/v). As shown in Table 1, each of *N*-(3,5-dinitrobenzoyl)- (**3**-**10**), *N*-(3-nitrobenzoyl)- (**11**-**14**) and *N*-benzoyl-α-amino acids (**15**,**16**) was resolved reasonably well. These resolution results have never been expected because analytes (**3**-**16**) do not contain any primary or secondary amino group. In this instance, the resolution results in Table 1 are quite surprising and unusual.

Table 1
Resolution of *N*-(3,5-dinitrobenzoyl)- (**3**-**10**), *N*-(3-nitrobenzoyl)- (**11**-**14**) and *N*-benzoyl-α-amino acids (**15**,**16**) on CSP **2** with a mobile phase of the mixture of acetic acid-triethylamine-acetonitrile, 0.05/0.25/100, v/v/v)[a]

Analytes[b]	k_1	k_2	α	R_S
3 (Alanine)	6.81 (D)	8.17 (L)	1.20	0.76
4 (Valine)	3.22 (D)	5.83 (L)	1.81	2.81
5 (Leucine)	4.56 (D)	7.16 (L)	1.57	2.01
6 (Phenylglycine)	3.56 (D)	5.23 (L)	1.47	1.49
7 (Phenylalanine)	5.11 (D)	8.23 (L)	1.61	1.78
8 (Serine)	21.25 (D)	22.52 (L)	1.06	0.54
9 (Threonine)	8.85 (D)	10.89 (L)	1.23	0.74
10 (Tyrosine)	36.20 (D)	45.97 (L)	1.27	0.73
11 (Alanine)	5.71 (D)	6.51 (L)	1.14	0.60
12 (Valine)	3.06 (D)	4.47 (L)	1.46	1.40
13 (leucine)	4.26 (D)	6.18 (L)	1.45	1.68
14 (Phenylglycine)	2.86 (D)	3.58 (L)	1.25	0.97
15 (Valine)	3.35 (D)	3.72 (L)	1.11	0.41
16 (Leucine)	3.68 (D)	4.20 (L)	1.14	0.55

[a] Flow rate: 0.5 ml/min; detection: 254 nm UV; temperature, 20 °C; k_1, retention factor of the first eluted enantiomer (in the parentheses the absolute configuration of the first eluted enantiomer is given); k_2, retention factor of the second eluted enantiomer (in the parenthesis the absolute configuration of the second eluted enantiomer is given); α, separation factor; R_S, resolution factor.
[b] In the parentheses, the names of the original α-amino acids are given.

The retention (k_1 and k_2), the separation (α) and the resolution factors (R_S) were significantly dependent on the presence and the number of the nitro group on the *N*-benzoyl derivatizing group of α-amino acids. As shown in Table 1, *N*-(3,5-dinitrobenzoyl)-α-amino acids were retained longer on the chiral column than corresponding *N*-(3-nitrobenzoyl)- or *N*-benzoyl-α-amino acids. In addition, *N*-(3,5-dinitrobenzoyl)-α-amino acids were resolved better in terms of the separation (α) and the resolution factors (R_S) than corresponding *N*-(3-nitrobenzoyl)- or *N*-benzoyl-α-amino acids. As an example, the chromatograms shown in Fig. 3 clearly demonstrate that *N*-(3,5-dinitrobenzoyl)leucine **5** is retained longer and resolved better on CSP **2** than *N*-(3-nitrobenzoyl)leucine **13** and *N*-benzoylleucine **16**. From these results, we can conclude that the nitro groups on the *N*-benzoyl ring of analytes do play an important role for the retention and for the chiral recognition. When the *N*-derivatizing group of leucine was changed to *N*-t-Boc group, retention factor (k) was diminished quite much and no resolution was observed on CSP **2** (k = 1.39, α = 1.00).

Under the mobile phase condition (pH 9.14), the two free carboxylic acid groups of the CSP are expected to be deprotonated

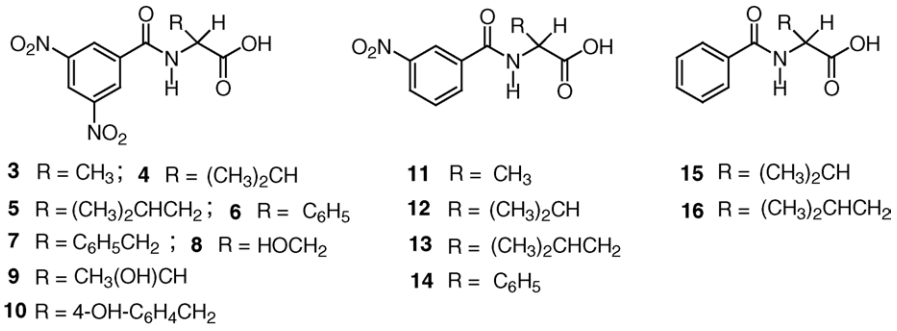

Fig. 2. Structures of *N*-(3,5-dinitrobenzoyl)- (**3**-**10**), *N*-(3-nitrobenzoyl)- (**11**-**14**) and *N*-benzoyl-α-amino acids (**15**,**16**) used in this study.

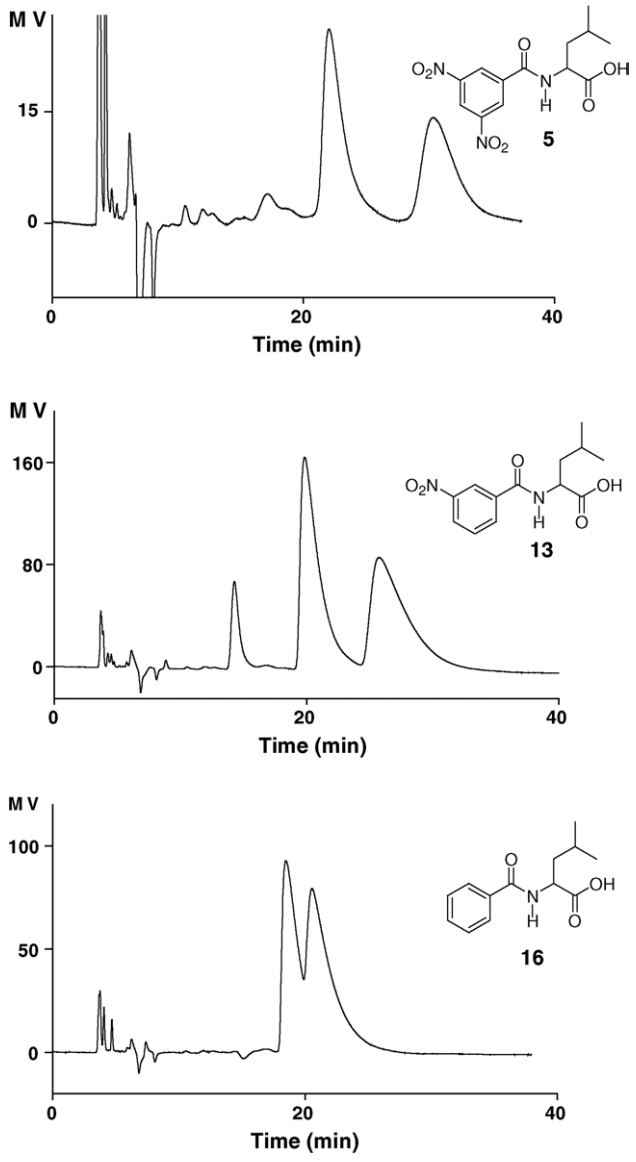

Fig. 3. Representative chromatograms for the resolution of (top) *N*-(3,5-dinitrobenzoyl)leucine **5**, (middle) *N*-(3-nitrobenzoyl)leucine **13** and (bottom) *N*-benzoylleucine **16** on CSP **2**. For the chromatographic condition, see the footnote to Table 1.

to carboxylate groups. In this instance, the ionic or dipole interaction between the carboxylate anions of the CSP and the nitro group of analytes might be responsible for the longer retention of analytes and for the better chiral recognition. However, at the present time, the exact chiral recognition mechanism is not clear.

The effect of the variation of the mobile phase composition on the chromatographic resolution behaviors on CSP **2** was investigated with selected two analytes, *N*-(3,5-dinitrobenzoyl)leucine **5** and *N*-(3,5-dinitrobenzoyl)phenylglycine **6** and the chromatographic results were summarized in Table 2. When polar protic solvent was added (up to 20 %) to the mobile phase, the retention factor (k_1) was reduced quite much (see entries a–e in Table 2). From these results, we assume that the retention factors (k_1) decreases as the polarity of the mobile phase increases. The separation factors (α) were not influenced significantly by adding polar protic solvent to the mobile phase. However, the resolution factors (R_S) were reduced quite significantly by adding polar protic solvent to the mobile phase.

The use of formic acid or trifluoroacetic acid instead of acetic acid reduced the separation (α) and the resolution factors (R_S) quite significantly while it affected the retention factors (k_1) only slightly (see entry a, f, and g in Table 2).

Increasing the content of triethylamine (entry a and h in Table 2) or increasing the content of both acetic acid and triethylamine in the mobile phase (entry a and i in Table 2) was found to decrease all of the retention (k_1), the separation (α) and the resolution factors (R_S). In contrast, decreasing the content of triethylamine (see entry a and j in Table 2) or decreasing the content of both acetic acid and triethylamine in the mobile phase (see entry a and k in Table 2) was found to increase the retention factors (k_1), but to decrease the separation (α) and the resolution factors (R_S). The pH value of the mobile phase was also dependent on the mobile phase composition, but the chromatographic parameters were not dependent significantly on the pH value of the mobile phase as shown in Table 2.

By adding protic polar solvent to the mobile phase or increasing the content of acetic acid or triethylamine in the mobile phase, the polarity or the ionic strength of the mobile phase is increased. In this instance, the solvation of polar analytes by

Table 2
Resolution of *N*-(3,5-dinitrobenzoyl)leucine **5** and *N*-(3,5-dinitrobenzoyl)phenylglycine **6** on CSP **2** with the variation of the mobile phase composition[a]

Entry	Mobile phase	**5**			**6**		
		k_1	α	R_S	k_1	α	R_S
a	Acetic acid–triethylamine–acetonitrile: 0.05/0.25/100 (pH 9.14)	4.56	1.57	2.01	3.56	1.47	1.49
b	Acetic acid–triethylamine–methanol–acetonitrile: 0.05/0.25/20/80 (pH 8.39)	0.06	1.64	0.64	0.05	1.00	
c	Acetic acid–triethylamine–ethanol–acetonitrile: 0.05/0.25/10/80 (pH 8.64)	0.80	1.57	1.21	0.61	1.32	0.61
d	Acetic acid–triethylamine–ethanol–acetonitrile: 0.05/0.25/20/80 (pH 8.62)	0.33	1.64	0.89	0.26	1.34	0.34
e	Acetic acid–triethylamine–2-propanol–acetonitrile: 0.05/0.25/20/80 (pH 8.72)	0.74	1.63	1.18	0.67	1.38	0.49
f	Formic acid–triethylamine–acetonitrile: 0.05/0.25/100 (pH 8.08)	2.76	1.16	0.79	3.27	1.28	1.07
g	Trifluoroacetic acid–triethylamine–acetonitrile: 0.05/0.25/100 (pH 8.81)	4.73	1.18	0.52	2.50	1.17	0.46
h	Acetic acid–triethylamine–acetonitrile: 0.05/0.50/100 (pH 9.16)	3.63	1.52	1.56	2.55	1.45	0.98
I	Acetic acid–triethylamine–acetonitrile: 0.10/0.50/100 (pH 9.00)	2.68	1.47	1.41	1.90	1.38	0.85
j	Acetic acid–triethylamine–acetonitrile: 0.05/0.05/100 (pH 8.56)	6.50	1.25	1.24	3.86	1.31	0.90
k	Acetic acid–triethylamine–acetonitrile: 0.01/0.05/100 (pH 8.86)	28.49	1.52	1.98	28.17	1.43	1.20

[a] Flow rate: 0.5 ml/min; detection: 254 nm UV; temperature, 20 °C; k_1: retention factor of the first eluted enantiomer; α: separation factor; R_S: resolution factor.

the mobile phase is increased and the polar interaction between the analytes and the stationary phase is shielded and consequently the polar analytes should be eluted faster. In contrast, by decreasing the content of acetic acid or triethylamine in the mobile phase, the polarity of the mobile phase is decreased and consequently the polar analytes are eluted slower. However, adding polar protic solvent to the mobile phase or increasing or decreasing the content of acetic acid or triethylamine in the mobile phase utilized for the data in Table 1 (entry a in Table 2) usually diminishes the chiral resolution efficiency of CSP **2** as shown in Table 2. In this instance, the optimum mobile phase for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids on CSP **2** can be concluded as the mixture of acetic acid/triethylamine/acetonitrile with the ratio of 0.05/0.25/100 (v/v/v).

Acknowledgement

This work was supported by KOSEF, Korea (NRL Program: M1-0318-00-0005).

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