Original

Enthalpy−entropy compensation for enantio−separation on cellulose and amylose *tris* (3, 5−dimethylphenylcarbamate) derivatives as stationary phases

Masato Kazusaki*, Toshiya Shoda and Hirofumi Kawabata

Chemical Analysis Research Group, Pharmaceutical Research & Technology Center, Dainippon Pharmaceutical Co., Ltd.

1−5−51 Ebie, Fukushima−ku, Osaka, 553−0001, Japan

Received July 17, 2003. Revised manuscript received October 1, 2003. Accepted October 3, 2003

Abstract

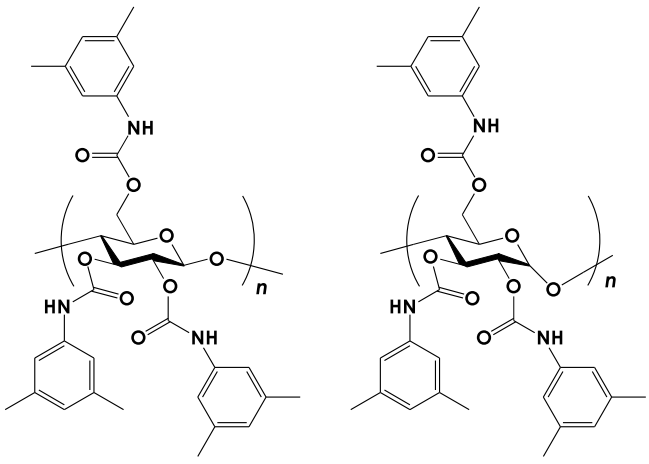
The thermodynamics of enantio−separation on cellulose and amylose *tris* (3, 5−dimethylphenylcarbamate) derivatives (CHIRALCEL OD−RH and CHIRALPAK AD−RH) was studied in the reversed−phase mode. The differences of enthalpic and entropic changes of enantio−selective adsorption are discussed with the aim to rationalize their possible contributions to the overall chiral recognition. On the cellulose−derived OD−RH column chiral recognition was completely enthalpy−driven with a negative entropic contribution. However, positive entropic contribution for enantio−separation was observed on an amylose−derived AD−RH column. Enthalpy−entropy compensation concerning enantio−separation indicates that enthalpic gain/loss was substantially cancelled out by the entropic loss/gain on both columns. This relationship leaded to constant $\Delta\Delta G^{\circ}$ for enantio−separation. It was also revealed that the entropic contribution caused the greater capability for enantio−separation on an amylose−derived AD−RH column than on a cellulose−derived OD−RH column.

Keywords: CHIRALCEL OD−RH, CHIRALPAK AD−RH, enthalpy−entropy compensation, van't Hoff plot

Introduction

The development of analytical methods that separate and quantify the enantiomers of pharmaceutical compounds continues to play an important role in the drug development process [1]. Many chiral stationary phases have been developed and commercialized for the detection of the enantiomer as the potential impurity in the optically active drug substance. In the past, we reported the separation of pharmaceutical compound and the corresponding enantiomer on cellulose *tris* (3, 5−dimethylphenylcarbamate) (CHIRALCEL OD−RH) and amylose *tris* (3, 5−dimethylphenylcarbamate) (CHIRALPAK AD−RH) [2]. While the amylose−based CHIRALPAK AD−RH had the same derivatization group (3, 5−dimethylphenylcarbamate) as its cellulose−based counter part (CHIRALCEL OD−RH) did, the elution order of enantiomers on the CHIRALPAK AD−RH was reversed compared to that on CHIRALCEL OD−RH.

The structures of the derivatized subunits of the chiral stationary phases (CSPs) were shown in Figure 1. Okamoto's research



Cellulose derivative Amylose derivative

Figure 1. Chemical structures of chiral selectors
Cellulose derivative: CHIRALCEL OD−RH
Amylose derivative: CHIRALPAK AD−RH

Tel: 06−6454−8177, Fax: 06−6458−3723
E−mail: masato−kazusaki@dainippon−pharm.co.jp

group has reported numerous examples in which cellulose−based and amylose−based columns showed different chiral recognition abilities [3−5]. The difference in chiral recognition ability between the two CSPs is attributed to the conformational difference between cellulose−derived and amylose−derived stationary phases.

Temperature has a major impact on retention, selectivity (enantio−selectivity), resolution, and column efficiency for chromatographic separation. Consequently, the variation of the column operation temperature has been frequently exploited as an optimization parameter in gas and liquid chromatographic separations of enantiomers [6−9]. Analysis based on van't Hoff plots of retention and separation derived from variable−temperature operation is routinely used to access thermodynamic functions of enantio−selective adsorption, which may be interpreted in terms of mechanistic aspects of chiral recognition. Numerous studies devoted to the elucidation of effects of temperature on the separation characteristics of chiral selector systems, such as protein [10, 11], crown ethers [12, 13], cyclodextrins [14] and cellulose− and amylose−derivatives [15−19].

Under linear chromatographic conditions the temperature dependence on the retention of a given analyte can be expressed with the following functional thermodynamic equation:

$$\ln k = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln\Phi \quad (1)$$

where k is the retention factor, R the universal gas constant, T the absolute temperature in Kelvin, ΔH° and ΔS° the molar enthalpy and molar entropy of absorption, respectively, and Φ the phase volume ratio. Combing Eq. (1) with the expression for the enantio−separation factor ($\alpha = k_s/k_f$, with s and f arbitrarily referring the second and first eluted enantiomers, respectively) and the Gibbs−Helmholtz relationship (Eq (2)):

$$-\Delta\Delta G^\circ = RT \ln \alpha \quad (2)$$

gives Eq. (3):

$$\ln \alpha = -\Delta\Delta H^\circ/RT + \Delta\Delta S^\circ/R \quad (3)$$

This expression relates the temperature and the experimentally easily accessible α value with the molar differential enthalpy ($\Delta\Delta H^\circ$) and entropy ($\Delta\Delta S^\circ$) of enantio−selective adsorption.

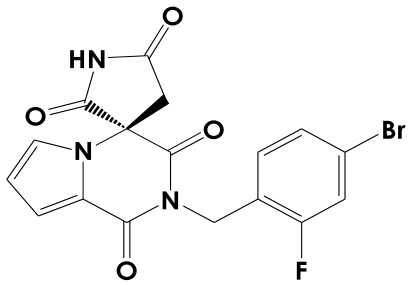


Figure 2. Chemical structure of *R*−2−(4−bromo−2−fluorobenzyl)−(1, 2, 3, 4−tetrahydropyrrolo [1, 2−a] pyrazine−4−spiro−3'−pyrrolidine)−1, 2', 3, 5'−tetrone (*R*−enantiomer).

Provided that these equations are temperature−independent, graphical analysis of ln α and inverse of absolute temperature gives linear plots (van't Hoff plots), from which the molar differential enthalpy ($\Delta\Delta H^\circ$) and entropy ($\Delta\Delta S^\circ$) can be extracted from the slope ($-\Delta\Delta H^\circ/R$) and the intercept ($\Delta\Delta S^\circ/R$), respectively.

In this paper we explore the mechanism of enantio−separation of potential drug substance (*R*−2− (4−bromo−2−fluorobenzyl)−(1, 2, 3, 4−tetrahydropyrrolo [1, 2−a] pyrazine−4−spiro−3'−pyrrolidine)−1, 2', 3, 5'−tetrone) (Figure 2) [20] and corresponding (*S*)−enantiomer as the probe compounds on the chiral columns, CHIRALCEL OD−RH and CHIRALPAK AD−RH, respectively.

Experimental

Materials

R−2− (4−Bromo−2−fluorobenzyl)−(1, 2, 3, 4−tetrahydropyrrolo [1, 2−a] pyrazine−4−spiro−3'−pyrrolidine)−1, 2', 3, 5'−tetrone and corresponding *S*−enantiomer were synthesized in the laboratory of Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). Ammonium acetate was of reagent grade from Wako Pure Chemical Industries Ltd. (Osaka, Japan). HPLC grade acetonitrile were also purchased from Wako Pure Chemical Industries Ltd. Acetonitrile was chosen for organic modifier due to the poor durability of cellulose− and amylose−columns for pressure. The water was deionized prior to usage.

Instrumentation

The HPLC system consisted of an L−7100 intelligent pump, an L−7400 UV−visible spectrophotometric detector, an L−7200 auto sampler and D−7000 interface and the chromatographic data were processed using HSM 7000 chromatography data station software (Hitachi, Tokyo, Japan). The columns used in this study were CHIRALCEL OD−RH and CHIRALPAK AD−RH packed with chemically modified cellulose and amylose as the stationary phase, respectively. These columns were purchased from Daicel Chemical Ltd. (Tokyo, Japan).

Chromatographic conditions

The mobile phase consisted of 0.01 M acetate buffer (pH 4.7) and HPLC−grade acetonitrile that were pre−mixed before use. The mobile phase flow−rate was 0.5 mL/min. The column temperature was 11−45℃. UV detection was performed at 297 nm. The sample solutions were prepared in acetonitrile at a concentration of 0.1−0.2 mg/mL. A 10−μL volume was injected. The retention factor was determined as $k = (t_R - t_0)/t_0$. The t_0 was determined by injecting methanol, noting the time of first perturbation due to methanol.

Results and discussion

The chromatograms of the enantio−separation of probe enanti-

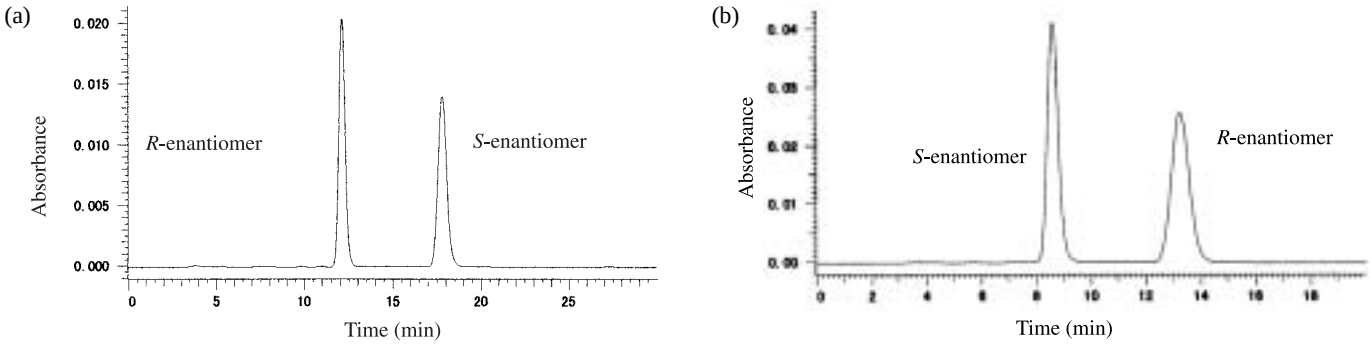


Figure 3. Separation of *R*− and *S*−enantiomers on (a) CHIRALCEL OD−RH and (b) CHIRALPAK AD−RH. HPLC conditions: column, CHIRALCEL OD−RH and CHIRALPAK AD−RH; column temperature, 25℃ for CHIRALCEL OD−RH and 40℃ for CHIRALPAK AD−RH; mobile phase, acetonitrile−0.01 M acetate buffer (pH 4.7) (1:1); flow rate, 0.5 mL/min; detection, 297 nm.

omeric pairs on the CHIRALCEL OD−RH and CHIRALPAK AD−RH columns in the reversed−phase mode are shown in Figure 3, respectively. Acetonitrile concentration in the mobile phase was altered to change the magnitude of interaction between the chiral stationary phase and the enantiomers. It was noted that the separation factors observed on the CHIRALPAK AD−RH column are always greater than those on the CHIRALCEL OD−RH column. Figure 4 shows plots of the separation factor versus inverse temperature on the CHIRALCEL OD−RH and CHIRALPAK AD−RH column using a mixture of acetonitrile and 0.01 M acetate buffer (1:1) as the mobile phase. Linear and non−linear van't Hoff plot were observed on the CHIRALCEL OD−RH column and CHIRALPAK AD−RH, respectively. Non−linear plot could be divided into approximately two linear regions. Slopes of all plots observed in this study are positive, suggesting that the differences of enthalpy change ($\Delta\Delta H^\circ$) are negative. This fact indicates that enantio−separation is

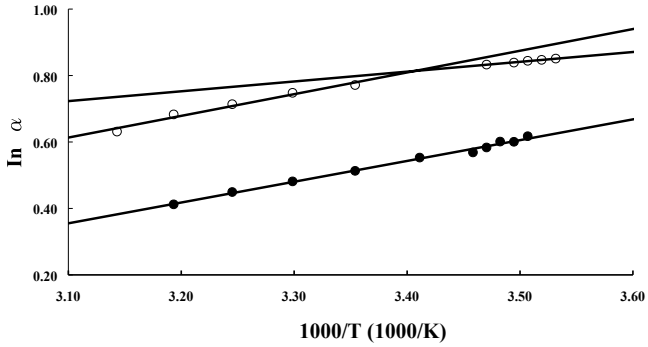


Figure 4. van' Hoff plots of enantio−separation on CHIRALCEL OD−RH (filled circle) and CHIRALPAK AD−RH (hollow circle). HPLC conditions: column, CHIRALCEL OD−RH and CHIRALPAK AD−RH; column temperature, variable; mobile phase, acetonitrile−0.01 M acetate buffer (pH 4.7) (1:1); flow rate, 0.5 mL/min; detection, 297 nm.

Table 1. Thermodynamic quantities of the enantio−separation on CHIRALCEL OD−RH and CHIRALPAK AD−RH

Column	Volume fraction of acetonitrile (%)	Q [$\Delta\Delta H^\circ / T\Delta\Delta S^\circ$]	$-\Delta\Delta G^\circ$ (kcal/mol)	$-\Delta\Delta H^\circ$ (kcal/mol)	$T\Delta\Delta S^\circ$ (kcal/mol)
CHIRALCEL OD−RH	50	1.34	0.312	1.236	−0.924
	55	1.35	0.307	1.175	−0.868
	57	1.37	0.312	1.152	−0.840
	60	1.40	0.308	1.076	−0.768
	65	1.43	0.321	1.070	−0.749
	70	1.47	0.297	0.932	−0.635
HIRALPAK AD−RH	45[a]	1.69	0.443	1.087	−0.644
	50[a]	5.12	0.473	0.588	−0.115
	55[a]	−0.16	0.493	0.068	0.425
	45[b]	1.53	0.452	1.301	−0.849
	50[b]	1.55	0.462	1.298	−0.836
	55[b]	1.66	0.458	1.155	−0.697

Mobile phase consisted of acetonitrile and 0.01 M acetate buffer, pH 4.7. $\Delta\Delta G^\circ$, $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ represent the difference of free energy, enthalpy and entropy between enantiomers concerning transfer from the mobile phase to the stationary phase. $\Delta\Delta G^\circ$ and $T\Delta\Delta S^\circ$ are evaluated at 25℃. Column temperature: a) below 20℃, b) above 20℃.

enthalpically favorable process on these columns. Using these data obtained, thermodynamic properties were calculated to understand the mechanism for enantio−separation on both chiral columns.

The thermodynamic parameters for enantio−separation and enthalpy−entropy ratios Q [$Q = \Delta\Delta H^\circ / T\Delta\Delta S^\circ$] were calculated and listed in Table 1. This arbitrary factor (Q) represents the relative contributions of enthalpic vs. entropic effects to $\Delta\Delta G^\circ$ at 25℃ [21]. As described above, the $\Delta\Delta H^\circ$ values obtained in this study are negative. Therefore, positive Q values indicate that the favorable enthalpic contribution to enantio−separation was counterbalanced by unfavorable entropic contribution. The Q values obtained from CHIRALCEL OD−RH column, and CHIRALPAK AD−RH column at column temperature above 20℃ reveal that the enantio−separation under this condition is governed by enthalpy (Q ranges between 1.34 and 1.47 on CHIRALCEL OD−RH, and 1.53 and 1.66 on CHIRALPAK AD−RH, respectively). However, on the CHIRALPAK AD−RH column at column temperature below 20℃, entropy−dominated enantio−separation was observed. As is evident from the data listed in Table 1, the $\Delta\Delta G^\circ$ value was kept relatively constant on each column, especially irrespective of two kind of mechanism for enantio−separation on the CHIRALPAK AD−RH column.

The differences of the entropy changes ($T\Delta\Delta S^\circ$) are plotted against the differences of enthalpy changes ($\Delta\Delta H^\circ$) give straight lines with correlation coefficients of 1.000 on both chiral columns, as shown in Figure 5. As proposed previously [22], the empirical linear relationship between $\Delta\Delta H^\circ$ and $T\Delta\Delta S^\circ$ means that the resulting change in $T\Delta\Delta S^\circ$ is proportional to the accompanying change in $\Delta\Delta H^\circ$, which leads to following Eq. (4). Integration of Eq. (4) gives Eq. (5), where β and $T(\Delta\Delta S_0^\circ)$ correspond to the slope and intercept of the plots shown in Figure 5. Eq. (5) indicates that the difference of entropy change consists of two terms one of which is proportional to the difference of enthalpy change and the other independent of it. Inserting Eq. (4) in the differential form of the Gibbs−Helmholtz Eq (6), Eq (7) is obtained.

$$T\Delta(\Delta\Delta S^\circ) = \beta\,\Delta(\Delta\Delta H^\circ) \quad (4)$$

$$T(\Delta\Delta S^\circ) = \beta(\Delta\Delta H^\circ) + T(\Delta\Delta S_0^\circ) \quad (5)$$

$$\Delta(\Delta\Delta G^\circ) = \Delta(\Delta\Delta H^\circ) - T\Delta(\Delta\Delta S^\circ) \quad (6)$$

$$\Delta(\Delta\Delta G^\circ) = (1-\beta)\,\Delta(\Delta\Delta H^\circ) \quad (7)$$

As can be seen from Eq. (7), only a proportion $(1-\beta)$ of the increment in $\Delta\Delta H^\circ$ contributes toward the enantio−separation ($\Delta\Delta G^\circ$). The intercept of the plot can be related to the difference of the degree of conformational change and the extent of desolvation induced upon enantio−separation. Eq. (5) indicates that as far as the intercept ($T\Delta\Delta S_0^\circ$) is positive, as is indeed the case in this study, enantio−separation can take place even in the absence of enthalpic gain ($-\Delta\Delta H^\circ$).

In our study, very large values of the slope close to unit (1.03 for amylose−deriven CHIRALPAK AD−RH column and 0.96 for cellulose−derived CHIRALCEL OD−RH column, respectively) were obtained. This fact indicates that by the modification of the mobile phase composition, the enhanced enthalpic force for enantio−separation is effectively cancelled out by the entropic force, resulting in the constant enantio−separation on these columns.

As for the entropic contribution, larger $T\Delta\Delta S_0^\circ$ value of 0.49 kcal/mol was calculated on an amylose−derived column, however, that value for a cellulose−derived column was 0.27 kcal/mol. Inserting Eq (5) in the Gibbs−Helmholtz Eq (8), Eq (9) is obtained.

$$\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S_0^\circ \quad (8)$$

$$\Delta\Delta G^\circ = (1-\beta)\,\Delta\Delta H^\circ - T\Delta\Delta S_0^\circ \quad (9)$$

In our study β is nearly equal to unit, indicating the difference of free energy is exclusively defined by the entropic term in this system. Owing to the difference of the entropic contribution CHIRALCEL OD−RH column, which consists of the 3, 5−dimethyl−phenylcarbamate moiety bound to the cellulose backbone instead of amylose, exhibited less chiral recognition ability than CHIRALPAK AD−RH column (Table 1).

Chiral discrimination between the enantiomers is due to the differences in their steric fit in the chiral cavities [3, 4]. Many authors discussed the importance of the higher order structure of the polysaccharide−based CSPs in chiral recognition [23−26]. Based on the experimental data, the importance of steric fitness in the chiral cavity is demonstrated by the comparison of entropic contribution ($T\Delta\Delta S_0^\circ$) on cellulose−derived CHIRALCEL OD−RH column and amylose−derived CHIRALPAK AD−RH columns. Dalgliesh established 3−point theory for chiral discrimination attributed by the differences of interactions between the chiral absorption sites and enantiomers [27]. This theory is valid well in the normal−phase mode. We suggest that the participation of the entropic contribution for chiral discrimination (Table 1) and the pos-

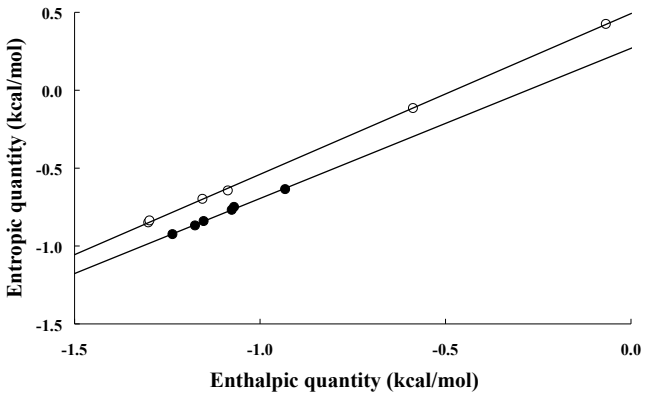


Figure 5. Enthalpy−entropy compensation plot of the enantio−separation on cellulose−derived CHIRALCEL OD−RH (filled circle) and amylose−derived CHIRALPAK AD−RH (hollow circle).

sibility of enantio−separation without the differences of the molar enthalpic change ($\Delta\Delta H^{\circ} \neq 0$) (Figure 5) in the reversed−phase mode.

References

[1] ICH, International Conference on Harmonization Guideline. Specifications: Test procedures and Acceptance Criteria for Drug Substances and Drug Products: Chemical Substances. http://www.nhi.go.jp/dig/ich/quality/q 6 a/q 6 a−e.pdf

[2] Kazusaki, M.; Kawabata, H.; Matsukura, H. *J. Liq. Chrom. & Rel. Tech.* **2000**, *23,* 2819 − 2828.

[3] Okamoto, Y; Yashima, E, *Angew Chem. Int. Ed.* 37 (1998) 1020.

[4] Okamoto, Y.; Kaida, Y. *J. Chromatogr. A* **1994**, *666,* 403−419.

[5] I. W. Wainer, R. M. Stiffin, T. Shibata, *J. Chromatogr.* 411 (1987) 65.

[6] Schurig, V. *J. Chromatogr. A* **2001**, *906,* 275−299.

[7] Cabrena, K.; Lubda, D. *J. Chromatogr. A* **1994**, *666,* 433−438.

[8] Kusters, E.; Loux, V.; Schmidt, E.; Floersheim, P. J. *Chromatogr. A* **1994**, *666,* 421−432.

[9] Kirkland, K. M.; McCombs, D. A. *J. Chromatogr. A* **1994**, *666,* 211−219.

[10] Fornstedt, T.; Sajonz, P.; Guiochon, G. *J. Am. Chem. Soc.* **1997**, *119,* 1254−1264.

[11] Karlsson, A.; Charron, C. *J. Chromatogr. A* **1996**, *732,* 245 − 253.

[12] Naemura, K; Fuji, J.; Ogashara K.; Hirose, K.; Tobe, Y. *Chem. Commun.* **1996**, 2749−2750.

[13] Machida, Y.; Nishi, H.; Nakamura, K.; Nakai, H.; Sato, T. *J. Chromatogr. A* **1998**, *805,* 85−92.

[14] N. Morin, N.; Guiaume, Y. C.; Peirin, E.; Rouland J. −C. *J. Chromatogr. A* **1998**, *808,* 51−60..

[15] Yashima, E.; Sahavattanapong, P.; Okamoto, Y. *Chirality* **1996**, *8,* 446−451.

[16] Smith, R. J.; Taylor, D. R.; Wilkins, S. M. *J. Chromatogr. A* **1995**, *697,* 591−596.

[17] O'Brien, T.; Crocker, L.; Thompson, R.; Torma P. H.; Conlon D. A.; Feibush, B.; Moeder, C.; Bicker G.; Grinberg, N. *Anal. Chem.* **1997**, *69,* 1999−2007..

[18] Kazusaki, M.; Shoda, T.; Kawabata, H.; Matsukura, H. J. *Liq. Chorom. & Rel. Technol.* **2001**, *24,* 141−151.

[19] Kazusaki, M.; Kawabata, H.; Matsukura, H. *J. Liq. Chrom. & Rel. Technol.* **2000**, *23,* 2937−2946.

[20] Negoro, T; Murata, M.; Ueda, S.; Fujitani, B.; Ono, Y.; Kuriyama, A.; Komiya, M.; Suzuki, K.; Matsumoto, J. *J. Med. Chem.* **1998**, *41,* 4118−4129.

[21] Oberleitner, W. R.; Maier, N. M.; Lindner, W. J. *Chromatogr. A* **2002**, *960,* 97−108.

[22] Kazusaki, M.; Shoda, T.; Kawabata, H.; Yamaguchi, T. *Chromatography* **2003**, *24,* 13−19.

[23] Hesse, G.; Hagel, R. *Chromatographia,* **1973**, *6,* 277−280.

[24] Ichida, A.; Shibata, T.; Okamoto, I.; Yuki, Y.; Namikoshi, H.; Toga, Y. *Chromatographia* **1984**, *19,* 280−284.

[25] Shibata, T.; Sei, T.; Nishimura, H.; Degichi, K. *Chromatographia* **1987**, *24,* 552−554.

[26] Francotte, E.;Zhang, T. J. *Chromatogr. A* **1995**, *718,* 257−266.

[27] Dalgliesh, C. E. J. *Chem. Soc.* **1952**, 3940−3942.