Preparation of novel beta-cyclodextrin chiral stationary phase based on click chemistry

ARTICLE *in* JOURNAL OF CHROMATOGRAPHY A · JUNE 2008

Impact Factor: 4.17 · DOI: 10.1016/j.chroma.2007.11.018 · Source: PubMed

Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1191 (2008) 188–192
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Preparation of novel β-cyclodextrin chiral stationary phase based on click chemistry

Yongping Zhang [a], Zhimou Guo [b], Jinxing Ye [a], Qing Xu [b], Xinmiao Liang [a,b,*], Aiwen Lei [c,**]

[a] *School of Pharmacy, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China*
[b] *Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China*
[c] *College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China*

Available online 17 November 2007

Abstract

A facile strategy based on click chemistry for preparation of the structurally well-defined native β-cyclodextrin (β-CD) based chiral stationary phase (CSP) was proposed. The β-CD CSP was evaluated by enatioseparation of benzoin, *trans*-stilbene oxide, Troger's base, bendroflumethiazide, ketoprofen, chlorthalidone, three flavanone compounds and two β-adrenergic blocking agents under reversed phase high performance liquid chromatography. The chromatographic results demonstrate the chiral separation ability of click β-CD CSP and illustrate the usefulness of click chemistry in the preparation of β-CD based CSP.

Keywords: β-Cyclodextrin; Chiral stationary phases; Click chemistry; Chiral separation

1. Introduction

During the past two decades there has been great interest in the development and application of chiral chromatographic methods, particularly in the pharmaceutical industries [1–3]. The versatility of chiral stationary phases and its effective application in both analytical and preparative-scale enantioseparation have been well developed [4–6]. The stability and separation ability of the chiral stationary phase, to some extent, may be affected by the synthetic methods [7,8]. Therefore, it is necessary and challenging to develop an efficient synthetic strategy for chiral stationary phase (CSP).

Click chemistry was coined by Sharpless and co-workers and has been applied in many fields [9]. According to this concept, a series of silica based [10,11] and polymer support [12]. HPLC separation materials prepared by this method have been proved to be suitable and efficient. Internal stucture and ordering array of selectors on the silica support, which is the key factor affecting the performance of CSPs, is made possible due to the high selectivity and few side reactions of this reaction. Recently, immobilization of Cinchona alkaloid derivatives on silica gel for the preparation of chiral stationary phase via click chemistry was reported by Kacprzak et al. [13].

Native and derivatized β-cyclodextrin (β-CD) chiral stationary phases have been applied in the separation of a large amount of enantiomers [14–16]. Its preparation methods were various, such as immobilization of β-CD on silica gel through amine and urethane linkage [17,18] or amide linkage [19]. However, the problem was their poor stability in an aqueous mobile phase. Armstrong used the stable ether linkages to immobilize CDs [20]. In these immobilization methods, the hydroxyl groups at 2-, 3- and 6-position could react with the functional group on the solid supports, and multi-linked CDs chiral stationary phases would be formed [7]. In addition, the batch-to-batch reproducibility of these non-selective immobilization reactions is not easy to be achieved. To overcome this problem, some selective reactions were developed for preparation of the structurally well-defined β-CD based CSPs. A good example is the Staudinger reaction, in which only one active group modified on the CDs can react with the functional group on the silica gel [21,22]. However, the reaction conditions of the most selective methods are complicated, especially for preparation of the native β-CD based CSP due to side reactions generated by competitions

* Corresponding author at: Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China. Tel.: +86 411 84379519; fax: +86 411 84379539.
** Corresponding author. Tel.: +86 27 6875 4672; fax: +86 27 6875 4672.
E-mail addresses: liangxm@dicp.ac.cn (X. Liang), aiwenlei@whu.edu.cn (A. Lei).

0021-9673/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2007.11.018

of hydroxyl groups at the other positions of β-CD. Therefore, further improvement of approaches for the preparation of β-CD based CSPs is necessary.

Very recently, we reported a facile strategy for immobilization of the alkyne-modified β-CD on the azide-modified silica as hydrophilic interaction liquid chromatography separation material [11]. In this study, a novel approach based on click chemistry for immobilization of the azide-modified β-CD on the alkyne-modified silica as chiral separation material was presented. This column (denoted as click β-CD CSP) was evaluated by separation of racemates under reversed phase and expected to show chiral separation ability.

2. Experimental

2.1. Chemicals

Silica gel (5 μm, 10 nm, 270 m^2/g) was purchased from Fuji Silysia Chemical Ltd. (Aichi, Japan). 3-Isocyanatopropyltriethoxysilane was from ABCR. The racemic compounds of *trans*-stilbene oxide, benzoin, bendroflumethiazide, alprenolol, propranolol and Troger's base were obtained from Sigma; flavanone, 6-hydroxyflavanone and 6-methoxyflavanone were from Acros, ketoprofen and chlorthalidone were donated by Department of Pharmaceutical chemistry of Friedrich-Schiller-University Jena.

The molecular structures of these enantiomers are presented in Fig. 1.

2.2. Synthesis of click β-CD CSP

The alkyne-modified silica gel (3) was synthesized as shown in Fig. 2. Propargylamine (42.0 mmol, 2.31 g) was added to the solution of 3-isocyanatopropyltriethoxysilane (35.0 mmol, 8.65 g) in 100 mL anhydrous DMF. The mixture was stirred at 80 °C for 12 h. After the mixture was cooled to room temperature, 10 g silica gel was added. The suspension was stirred for another 24 h at 80 °C and then cooled to room temperature. The mixture was filtered and washed by 500 mL methanol and 150 mL acetone, and dried in high vaccum. The product was analyzed by FT-IR spectra, elemental analysis and ^{13}C CP-MAS. The data of elemental analyses showed (%): C 3.19; N 0.99; H 1.39. According to the macroanalysis data, the surface concentration of the alkyne on the silica supports is calculated to be 1.45 μmol/m^2. ^{13}C CP-MAS: δ_c (9.210, 23.039, 30.150, 36.032, 42.224, 60.114, 67.246, 81.064, 164.493).

The key intermediate mono-(6-azido-6-deoxy)-β-CD (4) is prepared according to literature [23,24].

The click immobilization process to prepare the click β-CD CSP was depicted as shown in Fig. 2. To a solution of mono-(6-azido-6-deoxy)-β-CD (3.0 mmol, 4.5 g) in 60 mL methanol–56 mL water, sodium ascorbate (0.45 mmol, 0.09 g)

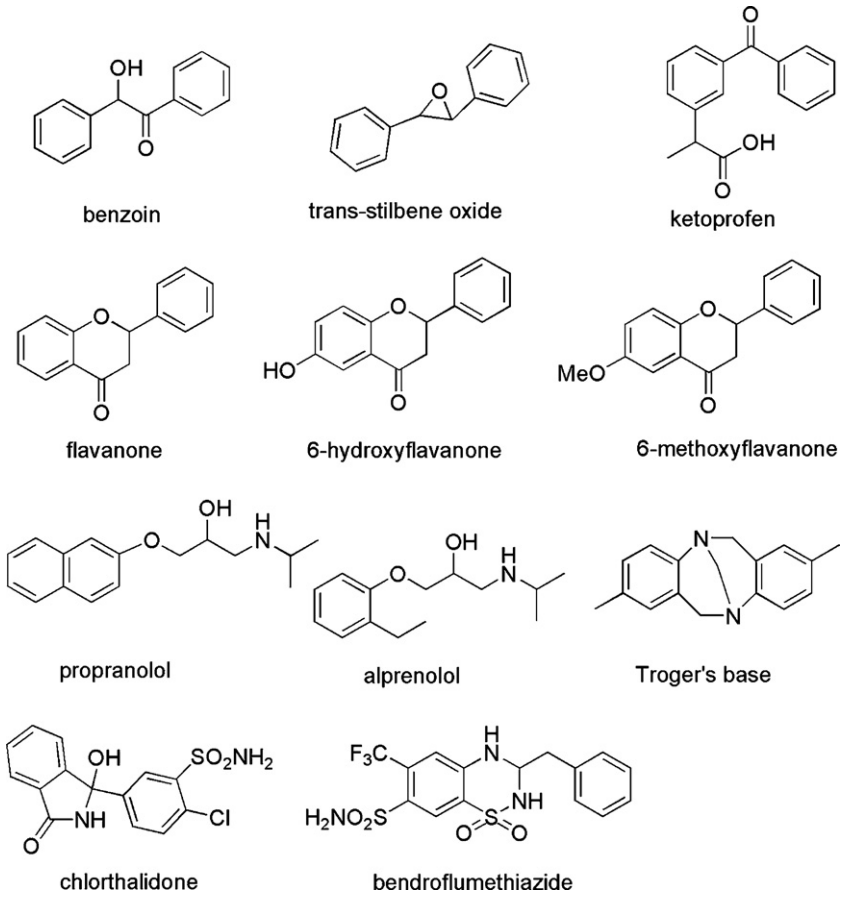

Fig. 1. Molecular structures of the test enantiomers.

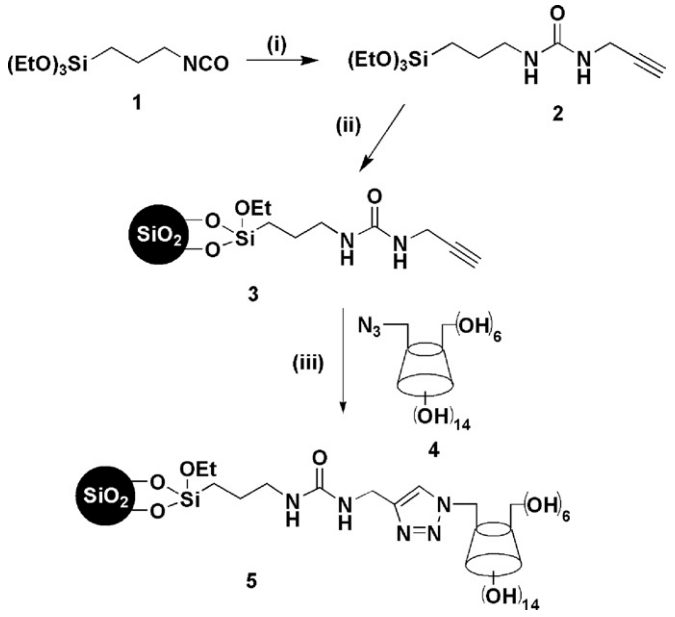

Fig. 2. Reagents and conditions in the preparation process. (i) Propargylamine, DMF, 80–90 °C; (ii) silica beads, DMF, 80–90 °C; (iii) $CuSO_4$ (5 mol%), sodium ascorbate (15 mol%), methanol–H_2O, 1:1, RT.

(dissolved in 2 mL water), $CuSO_4$ (0.15 mmol, 0.024 g) (dissolved in 2 mL water) and alkyne-modified silica gel (5.0 g) were added. After stirring for 72 h at RT, the obtained materials were filtrated and washed by 500 mL water, 300 mL 10% EDTA (the excess of 10% EDTA was used to oxidize Cu^+ ion into Cu^{2+} ion until the color of washing solution changed from blue to colorless), 500 mL water and 150 mL acetone successively. The product was characterized by FT-IR spectra, elements analysis and ^{13}C CP-MAS. The data of elemental analyses showed (%): C 4.90, N 1.11, H 0.97. ^{13}C CP-MAS: δ_c (9.359, 23.182, 42.832, 60.730, 73.185, 81.665, 88.770, 102.850, 161.603).

2.3. Apparatus and chromatography

Evaluation of the click β-CD CSP column was performed using Agilent HPLC system, which is comprised of an Agilent 1100 series, G1379A degasser, G1311A pump, G1313A Autosampler and G1315B DAD. All chromatographic experiments were carried out at 30 °C. The UV absorbance detection was performed at 210–310 nm and the flow rate of the mobile phase was 1.00 mL/min.

The CSPs were dispersed into acetone and packed into stainless-steel column (150 mm × 4.6 mm I.D.) by a slurry packing technique.

All the test probes were prepared to about 1 mg/mL concentration using methanol–water (v/v, 1:1). The injected volume was 2 μL.

3. Results and discussion

The click β-CD CSP was analyzed by FT-IR spectra and elements analysis. The FT-IR spectrum of β-CD CSP is similar to that of the alkyne-modified silica gel (not shown), probably due to the low surface concentration and the weak infrared absorption of alkyne group on the silica support. The carbon content increases from 3.19% of alkyne-silica gel to 4.90% of β-CD CSP (5) based on elemental analysis. It shows that the cyclodextrin moiety has been successfully immobilized onto the silica support surface. In terms of the macroanalysis data, the surface concentration of the native β-CD on the silica supports is calculated to be 0.13 μmol/m^2. Compared with ^{13}C CP-MAS of (3), new peaks appearing at δ_c = (73.185, 102.850) of (5) also provide the proof of successful bonding.

Table 1
Chromatographic data for separation of enantiomers on click β-CD CSP

Racemates	Mobile phase	k'_1	α	Rs
1. Benzoin	A	24.82	1.11	1.87
2. *trans*-Stilbene oxide	B	2.27	1.13	0.82
3. Ketoprofen	C	10.3	1.09	0.89
4. Flavanone	D	2.80	1.47	3.67
5. 6-Hydroxyflavanone	E	10.7	1.13	1.69
6. 6-Methoxyflavanone	F	16.7	1.05	0.86
7. Propranolol	G	8.82	1.05	0.57
8. Alprenolol	G	7.46	1.05	0.54
9. Troger's base	F	18.6	1.09	1.05
10. Chlorthalidone	H	3.03	1.33	1.71
11. Bendroflumethiazide	I	7.04	1.11	1.19

Mobile phase: (A) methanol/1% TEAA (pH 4.94) (10/90, v/v); (B) methanol/1% TEAA (pH 4.94) (35/65, v/v); (C) acetonitrile/water (0.5% acetic acid) (15/85, v/v); (D) acetonitrile/1% TEAA (pH 4.94) (25/75, v/v); (E) acetonitrile/1% TEAA (pH 4.94) (15/85, v/v); (F) methanol/1% TEAA (pH 4.94) (25/75, v/v); (G) methanol/1% TEAA (pH 4.94) (5/95, v/v); (H) acetonitrile/water (0.5% acetic acid) (8/92, v/v); (I) methanol/1% TEAA (pH 4.94) (15/85, v/v).

In the click immobilization process of the β-CD, only the azide group at the C-6 position of β-CD could react with the alkyne group on the silica supports. Therefore, the side reaction generated by competitions of hydroxyl groups at other position of the β-CD can be avoided, and the resulting β-CD bonded on the silica surface was envisioned to keep its internal structure and performances. Moreover, the immobilization of azide-modified β-CD on alkyne-modified silica gel takes place under mild conditions in aqueous solution.

The chromatographic evaluation of the click β-CD CSP was performed in methanol/water and acetonitrile/water eluents containing buffer of triethylammonium acetate (TEAA), respectively. The results are listed in Table 1, and some representative chromatograms are shown in Fig. 3. It can be seen that click β-CD CSP demonstrated enantioselectivity in the separation of most of the tested compounds.

In order to obtain β-CD CSP with the desired performances, many derivatized β-CD CSPs have been prepared [7,22,25]. The chiral recognition behaviors of flavanone compounds on the derivatized β-CD (example for permethyl β-CD [25], peracetyl β-CD and perphenylcarbamoyl β-CD [22]) based CSPs were investigated previously. In this study, the enantioseparation of three flavanone compounds was selected to investigate the chiral recognition behaviors of this kind of compounds on the native β-CD based CSP. The results show that click β-CD CSP exhibited a good ability in the enantioresolution of flavanone compounds, especially for flavanone, with a high

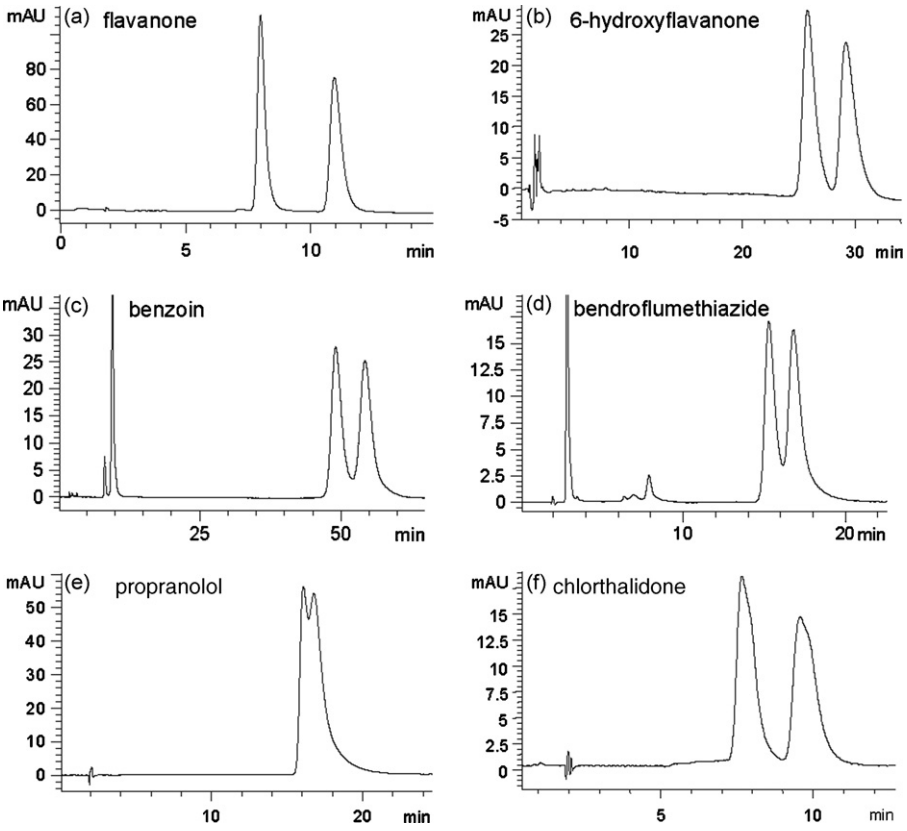

Fig. 3. Chromatograms for some racemic compounds on the column (150 mm × 4.6 mm I.D.) packed with click β-CD CSP. Conditions: (a) acetonitrile/1% TEAA (pH 4.94) (25/75, v/v); (b) acetonitrile/1% TEAA (pH 4.94) (15/85, v/v); (c) methanol/1% TEAA (pH 4.94) (10/90, v/v); (d) methanol/1% TEAA (pH 4.94) (15/85, v/v); (e) methanol/1% TEAA (pH 4.94) (5/95, v/v); (f) acetonitrile/water (0.5% acetic acid) (8/92, v/v); flow rate 1.00 mL/min, UV detector λ = 254 nm.

resolution (Rs) as 3.67. Some racemates, e.g. bendroflumethiazide and propranolol, could be well resolved using urea-bonded perphenylcarbamoyl-β-CD CSPs [22,26]. That is probably not only due to hydrogen bonded formation occurring between the −NH and $-NHSO_2$ groups in racemates and the urea bond on the spacer arm, but also because of the formation of π–π interactions between phenylcarbamate groups and the analyte [7]. However, this click β-CD CSP may only form hydrogen bonds by urea bond with these racemates without π–π interactions. So we got relatively low resolution factors.

Table 2
The effect of the type of immobilization on enatioselectivity of native β-CD CSP

Racemates	CSP type	Mobile phase	k'_1	α	Rs
trans-Stilbene oxide	β-CD[a]	1	2.27	1.00	–
	β-CD[b]	3	3.39	1.08	0.72
Benzoin	β-CD[a]	2	2.37	1.00	–
	β-CD[b]	3	5.16	1.09	0.97

Mobile phase: (1) Acetonitrile/NaH_2PO_4 (pH 4.6, 20 mM) (35/65, v/v), flow rate 0.30 mL/min; (2) acetonitrile/NaH_2PO_4 (pH 4.6, 20 mM) (25/75, v/v), flow rate 0.30 mL/min; (3) acetonitrile/H_2O (15/85, v/v), flow rate 1.00 mL/min.

[a] The CSP was prepared via non-selective reaction and the corresponding date about separation of *trans*-stilbene oxide and benzoin are all cited from literature [27], column size (150 mm × 4.6 mm).

[b] CSP prepared in current investigation, column size (150 mm × 4.6 mm).

Table 2 shows the separation performances of benzoin and *trans*-stilbene on two β-CD CSPs, which were with non-selectively or selectively chemically bonded, respectively. The click β-CD CSP has better resolution, while the non-selectively chemically bonded β-CD CSP has difficulty separating benzoin and *trans*-stilben oxide. It demonstrates that the selectively chemically bonded β-CD CSP has a better chiral recognition effect compared to the non-selectively chemically bonded CSP reported in the literature.

4. Conclusion

This work introduces a facile strategy for preparation of a native β-CD based CSP via click chemistry. The chromatographic results display the chiral separation ability of click β-CD CSP. Click chemistry is proved to be effective in the preparation of β-CD based CSP. The detailed evaluation of the click β-CD CSP and the preparation of derivatized β-CD based CSPs by this strategy are undergoing, and will be reported in due course.

Acknowledgements

We acknowledge the grant of key project of the knowledge innovation program supported by the Chinese Academy of Sciences (Grant: KGCX2-SW-213) and thank the start-up fund of Green Catalysis Institute from Wuhan University. Prefes-

sor Gerhard K.E. Scriba (Friedrich-Schiller-University Jena) is acknowledged for gifts of some racemic compounds.

References

[1] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.
[2] E. Yashima, J. Chromatogr. A 906 (2001) 105.
[3] Y. Makino, Y. Urano, T. Nagano, J. Chromatogr. A 947 (2002) 151.
[4] V.A. Davankav, J. Chromatogr. 66 (1994) 55.
[5] W.H. Pirkle, L.J. Briee, G.J. Terfloth, J. Chromatogr. 75 (1996) 109.
[6] Y. Okamoto, Y. Kaida, J. Chromatogr. 90 (2001) 105.
[7] I.W. Muderawan, T.T. Ong, S.C. Ng, J. Sep. Sci. 29 (2006) 1849.
[8] P. Franco, A. Senso, L. Oliveros, C. Minguillon, J. Chromatogr. A 906 (2001) 155.
[9] H.C. Kolb, M.G. Finn, K.B. Sharpless, Angew. Chem. Int. Ed. 40 (2001) 2004.
[10] Z.M. Guo, A.W. Lei, X.M. Liang, Q. Xu, Chem. Commun. 43 (2006) 4512.
[11] Z.M. Guo, A.W. Lei, Y.P. Zhang, Q. Xu, Y.X. Xue, F.F. Zhang, X.M. Liang, Chem. Commun. 24 (2007) 2491.
[12] M. Slater, M. Snauko, F. Svec, J.M.J. Frechet, Anal. Chem. 78 (2006) 4969.
[13] K.M. Kacprzak, N.M. Maier, W. Lindner, Tetrahedron Lett. 47 (2006) 8721.
[14] H. Kim, E. Radwanski, R. Lovey, C.C. Lin, A.A. Nomeir, Chirality 14 (2002) 436.
[15] Y.C. Guillaume, J.F. Robert, C. Guinchard, Talanta 55 (2001) 263.
[16] S. Rudaz, J.L. Veuthey, Chirality 11 (1999) 319.
[17] K. Fujimura, T. Ueda, T. Ando, Anal. Chem. 55 (1983) 446.
[18] K. Fujimura, S. Suzuki, K. Hayashi, S. Masuda, Anal. Chem. 62 (1990) 2198.
[19] Y. Kawaguchi, M. Tanaka, M. Nakae, K. Funaso, T. Shono, Anal. Chem. 55 (1983) 1852.
[20] D.W. Armstrong, US Patent No. 4,539, 1985, 399.
[21] L.F. Zhang, Y.C. Wong, L. Chen, C.B. Ching, S.C. Ng, Tetrahedron Lett. 40 (1999) 1815.
[22] Y.F. Poon, I. Wayan Muderawan, S.C. Ng, J. Chromatogr. A 1101 (2006) 185.
[23] B. Brady, N. Lynam, T. O'Sullivan, C. Ahern, R. Darcy, Org. Synth. 77 (2000) 220.
[24] R.C. Petter, J.S. Salek, C.T. Sikorski, G. Kumaravel, F.T. Lin, J. Am. Chem. Soc. 112 (1990) 3860.
[25] X.H. Lai, S.C. Ng, J. Chromatagr. A 1059 (2004) 53.
[26] S.C. Ng, L. Chen, L.F. Zhang, C.B. Ching, Tetrahedron Lett. 43 (2002) 677.
[27] K.Si. Ahmed, F. Tazerouti, A.Y. Badjah-Hadi-Ahmed, B.Y. Meklati, Chromatographia 62 (2005) 571.