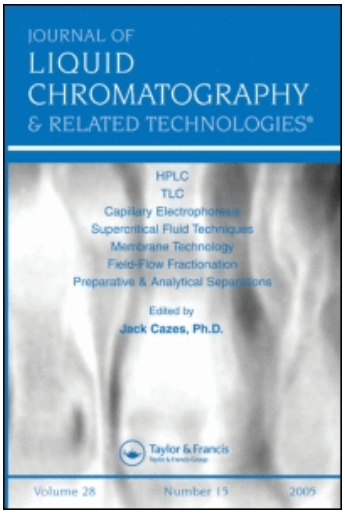


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

MOLECULAR MODELING OF ENANTIOMERIC RESOLUTION OF METHYLPHENIDATE ON CELLULOSE *TRIS* BENZOATE CHIRAL STATIONARY PHASE

Hassan Y. Aboul-Enein[a]; Imran Ali[a]; Michel Laguerre[b]; Guy Felix[c]
[a] Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia [b] Institut Europeen de Chimie et de Biologie (IECB), Ecole Polytechnique, ENSCPB, Pessac Cedex, France [c] ENSCPB, Talence, France

Online publication date: 10 January 2002

To cite this Article Aboul-Enein, Hassan Y. , Ali, Imran , Laguerre, Michel and Felix, Guy(2002) 'MOLECULAR MODELING OF ENANTIOMERIC RESOLUTION OF METHYLPHENIDATE ON CELLULOSE *TRIS* BENZOATE CHIRAL STATIONARY PHASE', Journal of Liquid Chromatography & Related Technologies, 25: 18, 2739 — 2748
To link to this Article: DOI: 10.1081/JLC-120014946
URL: http://dx.doi.org/10.1081/JLC-120014946

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
Vol. 25, No. 18, pp. 2739–2748, 2002

MOLECULAR MODELING OF ENANTIOMERIC RESOLUTION OF METHYLPHENIDATE ON CELLULOSE *TRIS* BENZOATE CHIRAL STATIONARY PHASE

Hassan Y. Aboul-Enein,[1,*] Imran Ali,[1] Michel Laguerre,[2] and Guy Felix[3]

[1]Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Center, P.O. Box 3354, Riyadh-11211, Saudi Arabia
[2]Institut Europeen de Chimie et de Biologie (IECB), Ecole Polytechnique, ENSCPB, 16 Avenue Pey-Berland, 33607 Pessac Cedex, France
[3]ENSCPB, 16 Avenue Pey-Berland (B.P. 108), F-33402 Talence, France

ABSTRACT

The enantiomeric resolution of (±)-threo methylphenidate (MPH) (Ritalin®) has been achieved on Chiralcel OB column using the mobile phase hexane–ethanol–methanol–trifluoroacetic acid (480 : 9.75 : 9.75 : 0.5, v/v/v/v), containing benzoic acid and phenol as mobile phase additives. Ultraviolet detection was

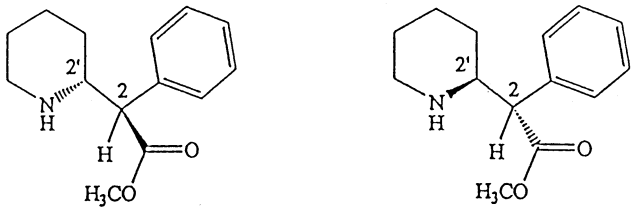
*Corresponding author. E-mail: enein@kfshrc.edu.sa

DOI: 10.1081/JLC-120014946
1082-6076 (Print); 1520-572X (Online)

carried out at 230 nm. Molecular modeling was carried out to explain the chiral resolution mechanism of MPH enantiomers.

INTRODUCTION

The chiral resolution of the drugs, pharmaceuticals, and agrochemicals is an important area nowadays.[1] HPLC has been used widely for the enantiomeric resolution of various racemates.[1,2] Although, various chiral stationary phases (CSPs) have been developed for the enantiomeric resolution, the chiral resolution is still a challenging job.[1,2] Therefore, we have tried to resolve the enantiomers of methylphenidate [methyl-α-(2-piperidyl) hydrochloride, MPH, Fig. 1] on Chiralcel OB CSP by using some mobile phase additives. Methylphenidate is a sympathomimetic agent and used in the treatment of attention deficient disorder, narcolepsy, analgesia induced sedation in cancer patients, and as an adjunct in some eating disorders,[3–8] cocaine abuse, and cocaine dependence.[9–11] Methylphenidate (MPH) has two chiral centres and, therefore, has four stereoisomers, i.e., (+)- and (−)-threo MPH and (+)- and (−)-erythro MPH. Methylphenidate (Ritalin®) is marketed as a racemic mixture of (±)-threo MPH.[4] It has also been reported, that the (+)-threo MPH is 5 to 38 times more active than the (−)-threo antipode[12] and, hence, the plasma concentration of (+)-threo MPH was found to be much higher than the (−)-threo enantiomer.[13] Recently, Abou-Enein and Ali have developed a method for the chiral resolution of this drug on different polysaccharides phases.[14] In the present work, attempts have been made to explain the mechanism of the chiral resolution of MPH on cellulose *tris* benzoate CSP (Chiralcel OB column) by using the results of the molecular modeling. The results of this paper are presented herein.



(+)-threo (2R,2'R)-MPH (−)-threo (2S,S'S)-MPH

Figure 1. The structures of (+)- and (−)-threo methylphenidate (MPH).

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

EXPERIMENTAL

Chemicals and Reagents

The solutions (1 mg/mL) of racemic mixture and pure enantiomers of MPH were prepared in ethanol. Benzoic acid, methanol, and hexane of HPLC grade, were purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Ethanol was obtained from E. Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA), benzoyl formic acid, phenylalanine, and phenol were supplied by Aldrich Chemical Co., USA.

Chromatographic Conditions

An aliquot of 20 μL of each solution was injected on to an HPLC system consisting of a Waters solvent delivery pump (Model 510), Waters injector (Model WISP 710B), Waters tunable absorbance detector (Model 484), and Waters integrator (Model 740). The columns used was Chiralcel OB (25 cm × 0.46 cm, i.d., particle size 10 μm.) [Cellulose *tris* benzoate] and was obtained from Daicel Chemical Industries, Tokyo, Japan. The mobile phase used in this study was hexane–ethanol–methanol–trifluoroacetic acid (480 : 9.75 : 9.75 : 0.50, v/v/v/v). Benzoic acid (0.2 mM), phenol (0.2 mM), benzoyl formic acid (0.2 mM), and phenylalanine (0.2 mM) were separately used as mobile phase additives on this column. The mobile phase was filtered and degassed before the use. The mobile phase flow rate was 1.0 mL/min and the experiments were carried out at 23 ± 1°C. The chart speed was kept constant at 0.1 cm per minute. UV detection was carried out at 230 nm. The chromatographic parameters such as retention factor (k), separation factor (α), and resolution factor (R_s) were calculated.

Molecular Modeling

The calculations were performed on a SGI Octane R10K workstation running Macromodel[15] version 6.5 (Schrodinger Inc.). Conformational minima were found using the modified MM3* (1991 parameters) force field as implemented and completed in the MacroModel program. Built structures were minimized to a final RMS gradient $\leq 0.005\,\mathrm{kJ\,\AA^{-1}\,Mol^{-1}}$, via the Truncated Newton Conjugate Gradient (TNCG) method (1000 cycle).

The calculations were performed with the GB/SA continuum solvation model.[16] The solvent chosen was chloroform ($\varepsilon = 4$), which mimics efficiency and a polar medium like the solvent used, which contains 96% hexane. In all

cases, the extended cutoff option was used (VdW = 8 Å, electrostatic = 20 Å and H-bond = 4 Å).

Stochastic Dynamic Simulation

This variant of molecular dynamics (forces from the force field) are augmented by frictional and random forces, which simulate some of the properties of a solvent medium[17] implemented by MacroModel. The chosen temperature was 300K, the time step was 1.5 fs, the total simulation time was 1 ns, and 500 snapshots were saved for each run.

Systems Building

Starting from templates available in MacroModel, a pentamer of O-benzoylated glucose was built and quickly minimized. Then six phenols, or six benzoic acids, were added randomly around this parameter, and the whole assembly was thoroughly minimized and submitted to a 1 ns stochastic dynamics simulation run.

RESULTS AND DISCUSSION

The chromatographic parameters for the resolved enantiomers of (±)-threo MPH on Chiralcel OB are given in Table 1. Typical chromatograms showing

Table 1. The Chromatographic Parameters, Retention Factor (k), Separation Factor (α) and Resolution Factor (R_s) for Enantiomeric Resolution of (±)-Threo Methylphenidate (Ritalin) on Chiralcel OB Column Using Hexane–Ethanol–Methanol–Trifluoroacetic Acid (480 : 9.75 : 9.75 : 0.5, v/v/v/v) Containing Benzoic Acid and Phenol as the Mobile Phase

k_1 (−)	k_2 (+)	α	R_s
*1.55	1.92	1.30	1.19
$^{+}$1.50	1.86	1.24	1.10

* and $^{+}$: Mobile phases containing benzoic acid (0.2 mM) and phenol (0.2 mM) respectively.
For details see experimental section.

MPH enantiomers separation on Chiralcel OB, are shown in Fig. 2. The resolved enantiomers were identified by analyzing individual (+)-threo and (−)-threo MPH enantiomers under the same chromatographic conditions. The (−)-threo enantiomer eluted first, followed by the (+)-threo enantiomer. The enantiomers of MPH have been resolved successfully on different polysaccharides CSPs.[14]

The chiral recognition mechanism at a molecular level on the cellulose based CSPs is still unclear, although, it has been reported that the chiral resolution by these CSPs is achieved through the different hydrogen, π–π and dipole–dipole induced interactions between the chiral stationary phase and the

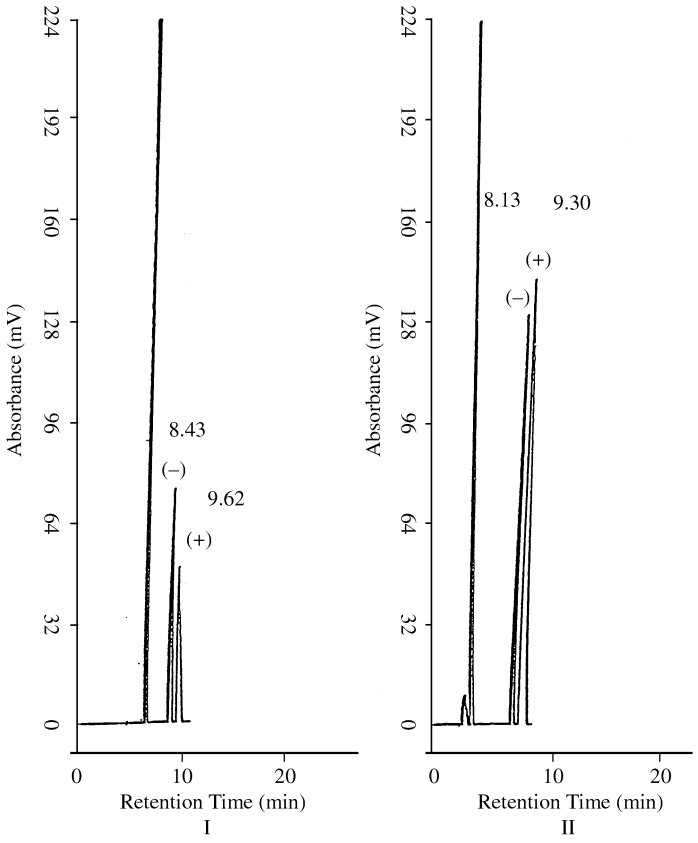


Figure 2. The chromatograms of the resolved enantiomers of (±)-threo methylphenidate (Ritalin) on Chiralcel OB columns using hexane–ethanol–methanol–trifluoroacetic acid (480 : 9.75 : 9.75 : 0.50, v/v/v/v) as the mobile phase containing 0.2 mM benzoic acid (I) and 0.2 mM phenol (II).

enantiomers.[18–20] Cellulose based chiral stationary phases are semi-synthetic polymers, which contain polymeric chains of derivatized *D*-(+) glucose residues with β-1,4 linkage. These chains lie side by side in a linear fashion in cellulose. The structure of the MPH (Fig. 1) contains electronegative atoms namely nitrogen and oxygen, along with one aromatic ring. Therefore, the resolution of MPH enantiomers most likely occurred due to hydrogen bonding and dipole–dipole induced interactions of different magnitudes between the electronegative atoms of MPH and the −CO−O− groups of benzoate moieties of cellulose CSP derivative (Chiralcel OB). Furthermore, it has also been reported,[18,19] that π–π interactions of different magnitude occur between the substituted phenyl moieties of benzoate of the chiral selector and the aromatic ring of the selected analytes. Therefore, one aromatic ring of each MPH enantiomers may fit stereogenically, in a different fashion, into the chiral grooves of the stationary phase. Each in turn, is stabilized by the π–π interactions of different magnitude for both (+) and (−) enantiomers and, hence, the resolution of enantiomers occurred.

A comparison of the enantiomeric resolution of MPH was carried out on a variety of polysaccharide based chiral stationary phases.[14] Complete resolution occurred on Chiralpak AD and Chiralcel OD, while the partial resolution was obtained using Chiralcel OJ and Chiralcel OC columns. No enantiomeric resolution was achieved on the Chiralcel OB column. These observations may, in part, be explained on the basis of the π–π interactions between the chiral stationary phases and the enantiomers of MPH. The chemical structures of these chiral stationary phases have been described by Aboul-Enein,[21] which can be used to explain the resolution of MPH enantiomers on these phases. Accordingly, partial resolution of the MPH enantiomers on Chiralcel OJ and Chiralcel OC, and no separation on the other chiral stationary phases including Chiralcel OB, could be due to poor π–π interactions between these CSPs and the enantiomers of MPH.

In order to show the importance of π–π interactions discussed above, benzoic acid, phenol, nitrophenol, and benzoyl formic acid were used as mobile phase additives. Different concentrations of these mobile phase additives were used. The complete resolution of the MPH enantiomers was observed on Chiralcel OB using benzoic acid (0.2 mM) and phenol (0.2 mM) as mobile phase additives, while a partial resolution occurred when using benzoyl formic acid (0.2 mM). A partial resolution of MPH enantiomers occurred when using 0.05, 0.10, and 0.15 mM concentrations of benzoic acid and phenol. No improvement in the enantiomeric resolution of MPH enantiomers was observed by using higher concentrations (more than 0.20 mM) of benzoic acid and phenol. Moreover, with benzoic acid and phenol the peaks were sharp, while they were broad using benzoyl formic acid. It is also interesting to note that, by using these mobile phase additives, partial resolution of MPH enantiomers was also observed on other chiral stationary phases (Chiralcel OK, Chiralcel OF, Chiralpak AS and Chiralpak AR).

The resolution of the enantiomers of MPH using these mobile phase additives may be due to the hydrogen bonding between MPH eanantiomers, Chiralcel OB, and mobile phase additives. It is interesting to note here, that the interaction of mobile phase additives with MPH enantiomers and Chiralcel OB CSP provides one additional phenyl group, which makes the π–π interaction strong and, hence, results in the complete resolution of the enantiomers of MPH. The value of α when using benzoic acid (1.30), was greater than the value of α when using phenol (1.24), which clearly indicates that benzoic acid improves the enantiomeric separation compared to phenol. It may be due to the extra hydrogen bonding in case of benzoic acid, as it contains $-CO-$ group. The broad peaks, when using benzoyl formic acid, may be due to the stronger hydrogen bonding between the chiral stationary phases and the enantiomers of MPH. The stronger hydrogen bonding is due to the presence of two $-CO-$ groups in benzoyl formic acid, in comparison to benzoic acid. The partial enantiomeric resolution of MPH on the other chiral stationary phases (Chiralcel OK, Chiralcel OF, Chiralpak AS and Chiralpak AR), which occurred when using benzoic acid and phenol, may be due to the steric effects. The steric interactions between MPH enantiomers–benzoic acid or MPH enantiomers–phenol and the other chiral stationary (other than Chiralcel OB) phases, may be due to the presence of different groups attached to the phenyl moieties of these chiral stationary phases. There was no enantiomeric separation of MPH enantiomers when using phenylalanine and nitrophenol as mobile phase additives, which could be due to the steric effect again imposed by phenylalanine, and strong electron withdrawing of the nitro group in nitrophenol.

To explain the experimental results of this work, molecular modeling was carried out. In order to gain insight on the influence of phenol or benzoic acid in the mobile phase, we performed several molecular dynamics runs. The substrate chosen was a fully benzoylated pentamer of glucose. As it extremely difficult to know what is the exact ratio of additives vs. benzoyl moieties, we decided to use six molecules randomly positioned around the pentamer. It is important to note that it is not possible to simulate the solvent flux, as molecular dynamics always proceeds with a constant number of particles. Thus, the simulations performed are essentially static, even if velocities are applied on each run. To mimic the presence of solvent, we used a continuum solvation model[16] (BG/SA model). The solvent chosen was chloroform with an $\varepsilon \approx 4$: the mixture hexane–ethanol–methanol (480 : 9.75 : 9.75, v/v/v) has an ε, which can be estimated around 2–3, but it is not easy to estimate the influence of the 0.1% of trifluoroacetic acid in the mobile phase.

In the two cases studied (phenol or benzoic acid), and even if the additive molecules are randomly positioned, at the beginning of the simulations the situation is almost the same; it appears that additive molecules exchanges around two H-bonds with the substrate. In all cases, these H-bonds are between an OH

(phenol or carboxylic acid) of the additive and the carbonyl of the benzoyl moities. Moreover, all six additive molecules are in a close vicinity of the substrate. At the end of the simulations, as can be seen in the figure, the final situation is strikingly different between the two different additives. With phenol, all additives are found at a very large distance of the substrate (minimum distance: 10 Å); it seems that the phenol has a very low affinity for benzoylcellulose (at least for the simulation conditions) and, thus, can exert a very low influence on the efficiency of the stationary phase. In the opposite, with benzoic acid, at the end of the simulation (Figs. 3 and 4), all six benzoic molecules are in a very close vicinity of the substrate (all distance are less than 3 Å); the average number of H-bonds is always 2 and there are several interactions between aromatic rings of the additives and the substrate via π–π or σ–π stacking. It seems that benzoic acid has a very high affinity with the substrate (always within the simulation conditions) and, thus, can exert a noticeable influence on the efficiency of the stationary phase.

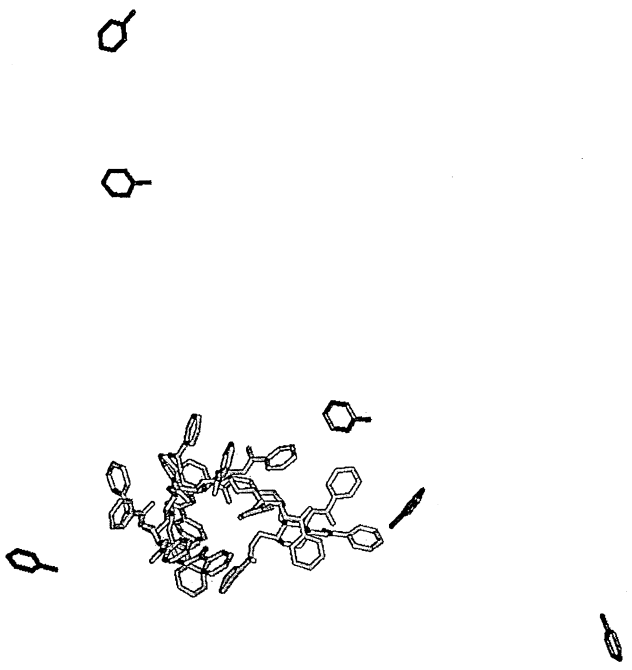


Figure 3. The final position after 1 ns molecular dynamics run; the pentamer substrate is in light gray and phenol molecules are in black. The hydrogens have been omitted for clarity.

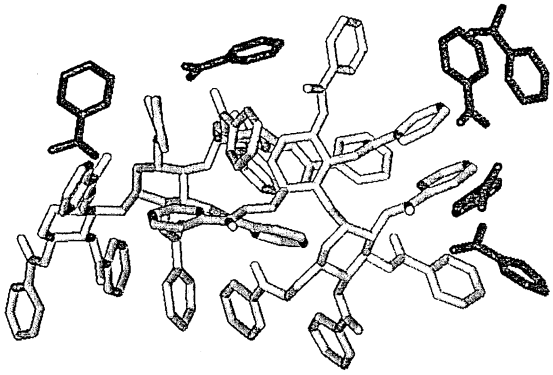


Figure 4. The final position after 1 ns molecular dynamics run; the pentamer substrate is in light gray and benzoic acid molecules are in black. The hydrogens have been omitted for clarity.

CONCLUSION

The enantiomers of MPH were resolved on Chiralcel OB using benzoic acid and phenol as the mobile phase additives. Benzoic acid and phenol provides the extra π–π and hydrogen bonding, which resulted in the complete resolution of enantiomers of MPH on Chiralcel OB CSP. The molecular modeling studies indicates the interaction of mobile phase additives, which resulted in an improved resolution of MPH. Taking into the consideration the results obtained, one can conclude that the enantiomeric resolution of MPH on these chiral stationary phases may be governed by the π–π, σ–π stacking. However, hydrogen bonding and dipole induced dipole interactions are also essential for the enantiomeric resolution.

ACKNOWLEDGMENT

The authors (I.A. and H.Y.A-E.) would like to thank the King Faisal Specialist Hospital and Research Center, Riyadh administration for their support for the Pharmaceutical Analysis Laboratory Research Program.

REFERENCES

1. Yashima, E.; Okamoto, Y. Chiral Recognition Mechanism of Polysaccharides Chiral Stationary Phases. In *The Impact of Stereochemistry on Drugs Development and Use*; Aboul-Enein, H.Y., Wainer, I.W., Eds.; John Wiley & Sons: New York, USA, 1997; Vol. 142, 345.

2. Beesley, T.E.; Scott, R.P.W. *Chiral Chromatography*; John Wiley & Sons: New York, USA, 1998; 221.
3. Barkley, R.J. Child Psychol. Psychiatry Allied Discip. **1977**, *18*, 137.
4. Giarman, N.J. In *Drill's Pharmacology in Medicine*, 3rd Ed.; McGraw-Hill: New York, USA, 1965; 368.
5. Spencer, T.S.; Wilens, T.; Biederman, J.; Faraone, S.V.; Ablon, S.; Lapey, K.A. Arch. Gen. Psychiatry **1995**, *52*, 434.
6. Klein, R.G. Arch. Gen. Psychiatry **1995**, *52*, 429.
7. Bruera, D.; Renneis, C.; Paterson, A.H.G.; MacDonald, R.N. Am. J. Nurs. **1988**, *11*, 1555.
8. Greenhill, L.L.; Osman, B.B. *Ritalin: Theory and Patient Management*; Liebert: New York, USA, 1991.
9. Levin, F.R.; Evans, M.S.; McDowell, D.M.; Kleber, H.D. J. Clin. Psychiatry **1998**, *59*, 300.
10. Grabowski, J.; Roache, J.D.; Schmitz, J.M.; Rhoades, H.; Creson, D.; Korszun, A. J. Clin. Psychopharmacol. **1997**, *17*, 485.
11. Khantzian, E.J.; Gawin, F.H.; Riordan, C.; Kleber, H.D. J. Subs. Abuse Treat. **1984**, *1*, 107.
12. Maxwell, R.A.; Chaplin, E.; Eckardt, S.B.; Soares, J.R.; Hite, G.J. J. Pharmacol. Exp. Ther. **1970**, *173*, 158.
13. Srinivas, N.R.; Quinn, D.; Hubbard, J.W.; Midha, K.K. J. Pharmacol. Exp. Ther. **1987**, *241*, 300.
14. Aboul-Enein, H.Y.; Ali, I. Chirality **2002**, *14*, 47.
15. Mohamadi, F.; Richards, N.G.J.; Guida, W.C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrikson, T.; Still, W.C. J. Comp. Chem. **1990**, *11*, 441.
16. Still, W.C.; Tempczyk, A.; Hawley, R.C.; Hendrikson, T. J. Am. Chem. Soc. **1990**, *112*, 6127.
17. Van Gunsteren, W.F.; Berendsen, H.J.C. Molec. Simul. **1988**, *1*, 173.
18. Wainer, I.W.; Alembic, M.C. J. Chromatogr. **1986**, *358*, 85.
19. Wainer, I.W.; Stiffin, R.M.; Shibata, T. J. Chromatogr. **1987**, *411*, 139.
20. Yamamoto, C.; Yashima, E.; Okamoto, Y. Bull. Chem. Soc. Jpn. **1999**, *72*, 1815.
21. Aboul-Enein, H.Y. Biomed. Chromatogr. **1998**, *12*, 116.

Received April 16, 2002
Accepted June 8, 2002
Manuscript 5857