Molecular Modeling of Enantio-discrimination of α-Methoxy-α-trifluoromethylphenylacetic Acid (MTPA) by Cyclomaltoheptaose (β-Cyclodextrin) and 6-Amino-6-deoxy-cyclomaltoheptaose

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Molecular modeling was performed to comprehend the chiral recognition of α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) enantiomers by cyclomaltoheptaose (β-cyclodextrin, β-CD) and 6-amino-6-deoxy-cyclomaltoheptaose (am-β-CD). Monte Carlo (MC) docking coupled to constant temperature molecular dynamics (MD) simulations was applied to the investigation for the α-methoxy-α-trifluoromethylphenylacetic acid complexation with two different CDs in terms of the relative distribution of the interaction energies. The calculated results are finely correlated with the experimental observations in chiral recognition thermodynamics. Am-β-CD as a host showed the superior enantio-discrimination ability to the native β-CD where the amino group of am-β-CD was critically involved in enhancing the ability of chiral discrimination *via* the Coulombic interaction with MTPA.

Key Words : Molecular modeling, Chiral recognition, Inclusion complex, Cyclodextrin

Introduction

Chiral discrimination has been a subject of great interest in the development, use, and action of phamaceutical agent. Most often the enantiomers of chiral drugs have different pharmacological and toxicological properties and therefore the quantitative enantiomeric composition of these drugs should be determined.[1] It is therefore important to have a method for the precise and accurate determination of the enantiomeric purity.

An important method for separating enantiomers involves chromatographic techniques with cyclodextrins (CDs) used as chiral stationary or mobile phase.[2] β-Cyclodextrin (β-CD) is a macrocyclic molecule formed by α-(1 → 4) glycosidic links between seven D-glucose monomer units, adopt a toroid shape. The resulting cavity of the CDs well characterized complexing properties with the appropriate guest molecules.[3] The inherent chirality of the CD molecules allows them to form diastereomeric complexes with enantiomeric compounds. Thus, it has been used as bonded chiral phases in liquid chromatography (LC) or as chiral mobile phase additives in LC and capillary electophoresis (CE) for the enantiomeric separation of racemic molecules.[1,4]

The most probable binding mode of native and modified β-CD with various guests involves the insertion of the less hydrophilic part of the guest molecule into the CD cavity, while the more hydrophilic, often charged, group stays just outside the primary or secondary rim of the cavity.[5,6] In many cases, the hydrophobic and van der Waals interactions are the principal intermolecular weak forces responsible for the formation of stable supramolecular complexes, although it is difficult to rigorously separate the contributions of these two forces in general[7] and particularly in the complexations of CDs.[8] The cationic β-CD enhances the binding ability

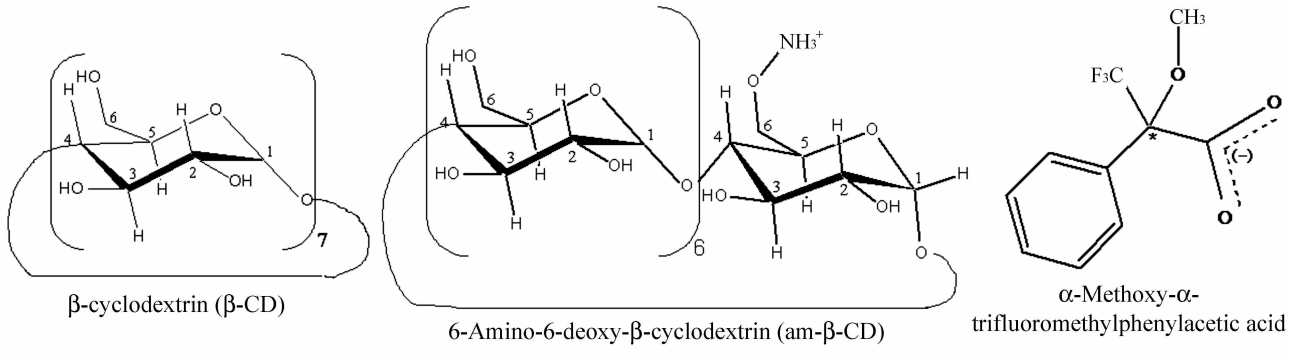

Chart 1

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through the attractive "long-range" Coulombic interaction.[9] In fact, it has been reported that cationic mono- and diamino-CDs exhibit higher/lower affinities toward negatively/positively charged guests than the corresponding native CDs.[6,10-13]

In this study, the chiral recognition of α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) by neutral or charged CD (Chart 1) was investigated. MTPA is used to determine the enantiomeric composition of alcohols or amines.[14,15] The inclusion complexes of β-CD and 6-amino-6-deoxy-β-CD (am-β-CD) with MTPA were modeled and refined by molecular modeling methods to correctly predict the elution order for enantiomeric separations. The intermolecular energies of the inclusion complexes of β-CD and am-β-CD with MTPA were compared.

Experimental Sections

Modeling host and guest molecules. Molecular mechanics and dynamics calculations were performed with the InsightII/Discover program (version 2000, Molecular Simulation Inc. San Diego, U.S.A.) using the consistent valence force field (CVFF)[16] on a SGI OCTANE 2 workstation (Silicon Graphics, U.S.A.). The β-CD structure was obtained by energy minimization of a crystallographic geometry.[17] The molecular structure of the am-β-CD was derived from the crystal structure of β-CD by modifying the substituents using the builder module within InsightII. The conformational search of (R)-, (S)-MTPA and am-β-CD were performed by simulated annealing molecular dynamics-full energy minimization strategy,[18,19] and the lowest energy conformation of each enantiomer was selected for further simulations. The conformations of these molecules are shown in Figure 1.

Monte Carlo docking minimization simulations. The host and guest molecules were positioned in the neighborhood with a distance of ~15 Å.[20] Monte Carlo docking simulations started by conjugate gradient energy minimization of this initial configuration for 100 iterations and accepted it as the first frame. In the course of trial to a new configuration, MTPA could take translational movement of maximum 3 Å to x, y and z axis and rotation of maximum 180° around x, y and z axis. For docking with guest molecule flexibly, the torsional angles of three single bonds could rotate upto 180°. Total 9 degrees of freedom was present for this system (3 translational, 3 rotational and 3 dihedral). Each cycle began with a random change of up to 5 degrees of freedom among them.[21] If the energy of the resulting configuration was within 1000 kcal/mol from the last accepted one, it was subjected to the 100 iterations of conjugated gradient energy minimization. The energy tolerance of 1000 kcal/mol was imposed to avoid significant overlap of van der Waals radii in the random search. After the energy minimization, the resulting structure was accepted based on criteria. (a) An energy check with the Metropolis criteria at 300 K,[22] and (b) a root-mean-squared displacement (RMSD) check, which compared the RMSD of the new configuration against those accepted so far. Configurations within 0.1 Å RMSD of pre-existing ones were discarded to avoid accepting similar configurations. The Monte Carlo simulations were performed until energy convergence. No cutoff was imposed on the calculation of non-bonded interactions, and the dielectric constant was set to 1. Boltzmann averages of energies were evaluated at 300 K.

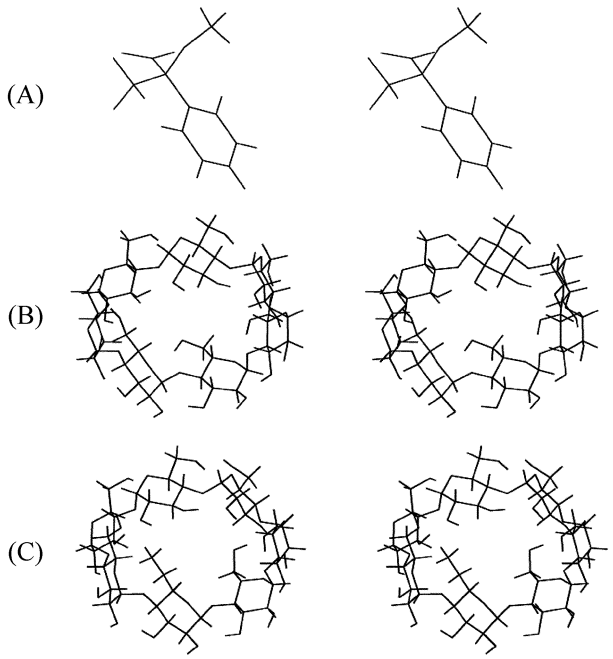

Figure 1. Stereoview of molecular models used in the MC simulation. (A) MTPA, (B) β-CD, and (C) am-β-CD.

Molecular dynamics simulations. We used the lowest-energy structures from the MC docking simulations as starting conformations for further molecular dynamics simulations.[23-25] No cutoff was imposed on the calculation of non-bonded interactions. Constant NVT molecular dynamics calculations were preformed using the leap-frog algorithm with a 1 fs time step. The initial atomic velocities were assigned from a Gaussian distribution corresponding to a temperature of 300 K. The system was equilibrated for 500 ps and production run was done for 20 ns. The temperature was controlled by velocity scaling in equilibration phase and by Berendsen algorithm[25] in production phases with a coupling constant of 0.2 ps. Intermediate structures were saved every 10 ps for analysis. The dielectric constant was set to r. The effects of the implicit solvent are approximated using a dielectric constant proportional to the distance ($\varepsilon = r$).[27]

Results and Discussion

Monte Carlo docking minimization simulations. In the process of Monte Carlo (MC) docking simulation, MTPA enantiomers were considered as guest-ligand and CDs as host-receptor molecules. During the simulations, the whole coordinates of CDs were flexible. The pathways of MC docking simulations showed a general tendency of inclusion complex formation and lowering interaction energy. The interaction energy was defined as the difference between the sum of the energy of individual host and guest molecule and

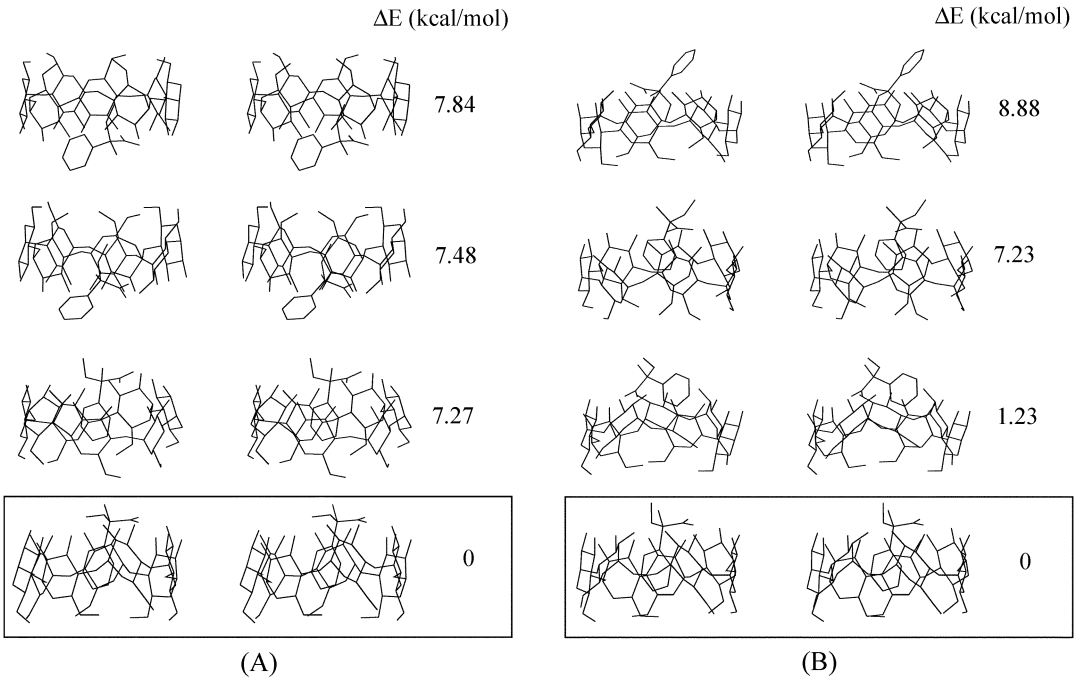

Figure 2. The conformations within 11 kcal/mol of the lowest interaction energy minimum obtained from 12,000 trials of the MC docking simulations. (A) (R)-MTPA:β-CD complexes and (B) (S)-MTPA:β-CD complexes. The lowest interaction energy configuration in the box was selected as the initial conformation for the following molecular dynamics (MD) simulations.

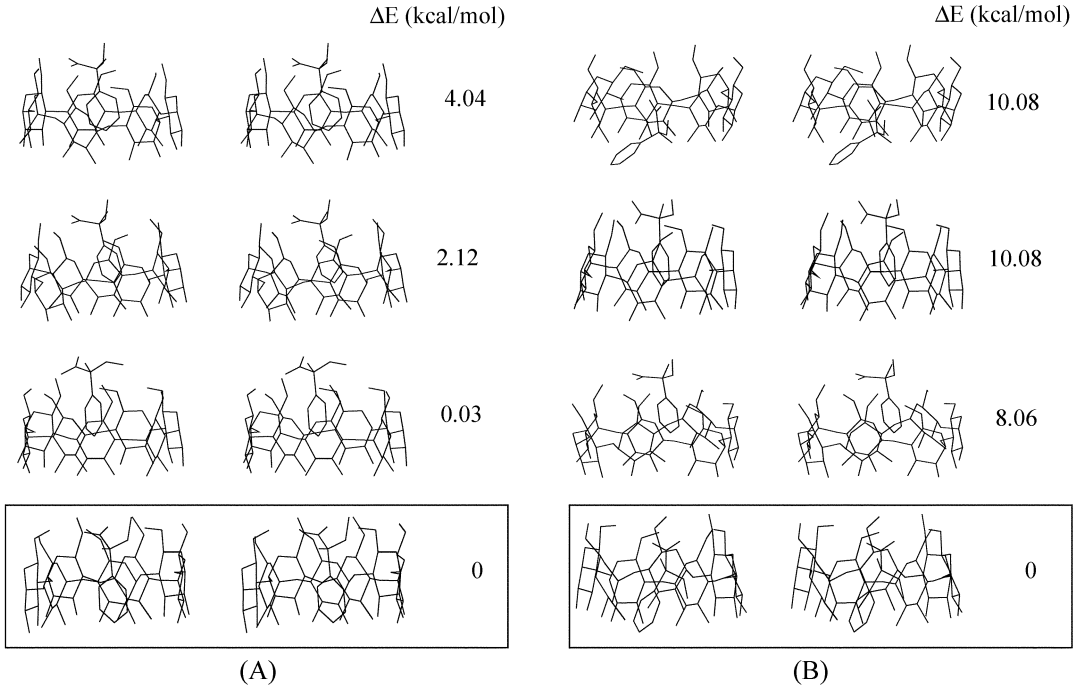

Figure 3. The conformations within 11 kcal/mol of the lowest interaction energy minima obtained from 12,000 trials of the MC docking simulations. (A) (R)-MTPA:am-β-CD complexes and (B) (S)-MTPA:am-β-CD complexes. The lowest interaction energy configuration in the box was selected as the initial conformation for the following molecular dynamics (MD) simulations.

the energy of the inclusion complex.[28]

We tried several MC runs for searching lowest energy configuration. Figure 2 and 3 show low energy configuration families among the inclusion complexes of β-CD and am-β-CD with MTPA. The lowest interaction energy configuration of each family in the box was selected as the initial configuration for the following molecular dynamics (MD) simulations. The orientation of guest was defined as being 'up' or 'down' meaning that the phenyl moiety was oriented toward the secondary rim or toward primary rim of CD,

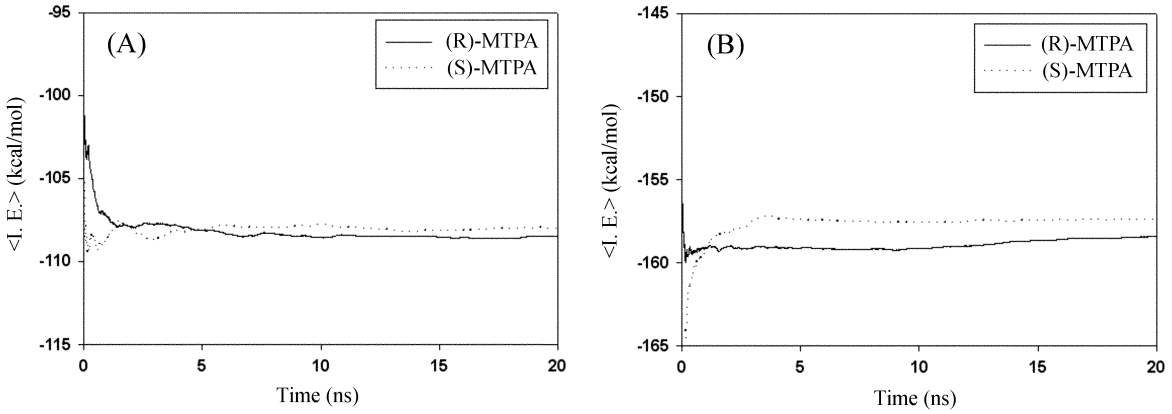

Figure 4. The average interaction energy in the MD simulations. The interaction energy was defined as nonbond energies between host and guest molecule. The average was evaluated using the relation, $\langle I \rangle$ $(1/N)\Sigma I_i$, where N is the number of frames and E_i is its energy. (A) MTPA:β-CD complex and (B) MTPA:am-β-CD complex. <I. E.> is average interaction energy.

respectively. The energetic analyses indicated the lowest energy configuration for the MTPA: β-CD complex was "down" and that for the MTPA:am-β-CD complex was "up".

Molecular dynamics simulations. The time change of the interaction energy during the MD simulations among the inclusion complexes of β-CD and am-β-CD with MTPA is shown in the Figure 4. The interaction energy was defined as nonbond energies between host and guest molecule. The interaction energy was evaluated using the relation, $\langle E \rangle = (1/N)\ \Sigma E_i$, where N is the number of frames and E_i is its energy. Figure 4 shows that the stability for complexation of the MTPA with am-β-CD is somewhat larger than those for the systems where no Coulombic binding exists. In other words, the $NH_3^+ \cdots CO_2^-$ interaction is the decisive factor in stabilizing the complex for complexation of the MTPA with

Table 1. Average intermolecular energies (kcal/mol) of competing enantiomeric MTPA-CDs complexes from the MD simulations

	β-CD			am-β-CD		
	R	S	ΔE	R	S	ΔE
Total	-108.50	-108.00	+0.50	-158.41	-157.35	−1.06
van der Waals	-9.41	-9.96	-0.55	-18.52	-19.17	-0.65
Coulombic	-99.09	-98.04	+1.05	-139.89	-138.18	−1.71

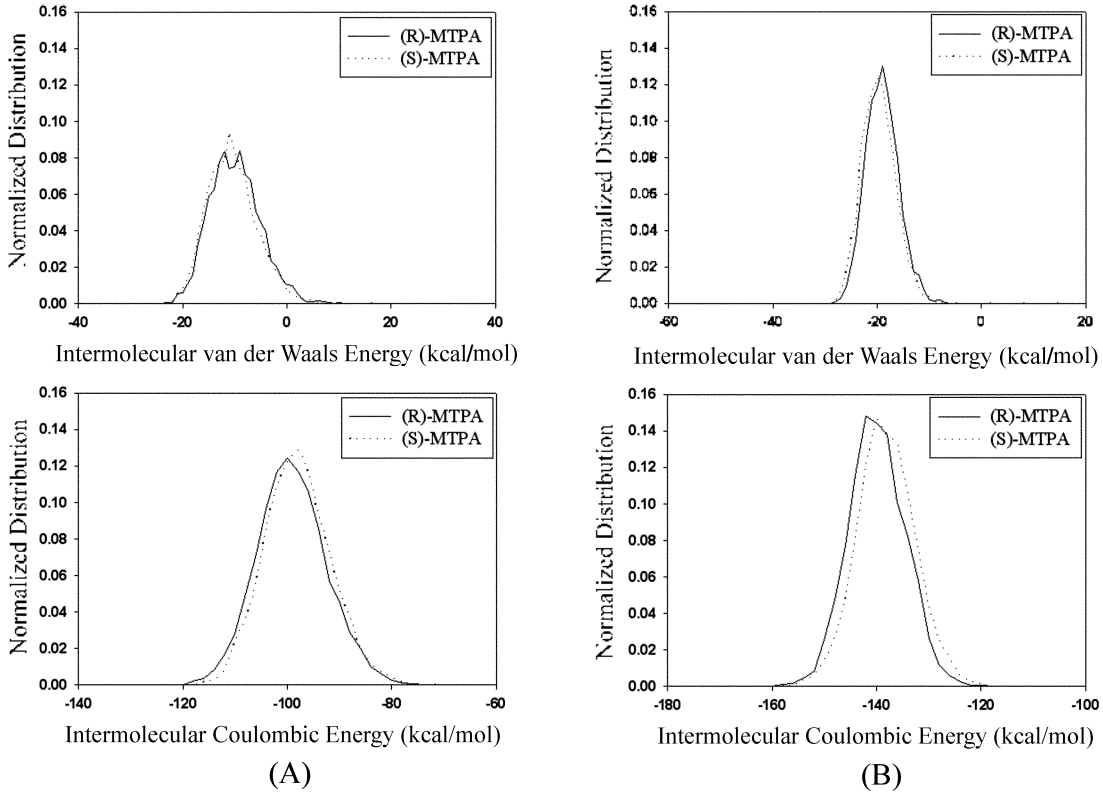

Figure 5. The normalized distribution of intermolecular energies in the MD simulations. (A) MTPA:β-CD complexes and (B) MTPA:am-β-CD complexes. Each intermolecular van der Waals and Coulombic energies was plotted in upper and lower panels, respectively.

am-β-CD. (R)-MTPA formed more stable inclusion complex by both β-CD and am-β-CD, where am-β-CD shows the more stable inclusion complex than β-CD. The difference of average interaction energy (ΔI.E.$_{S-R}$) during the MD simulations between the MTPA:β-CD complexes was 0.50 kcal/mol, and that of the MTPA:am-β-CD complexes was 1.06 kcal/mol as shown in Table 1. These results predicted the right sequence of thermodynamic stability; (R)-MTPA:am-β-CD. (S)-MTPA:am-β-CD. (R)-MTPA:β-CD and (S)-MTPA:β-CD.[9] They also indicated that enantio-discrimination power of am-β-CD was stronger than β-CD about 2.1 fold. which would be well correlated with the experimental data where am-β-CD had 1.9 fold more stable than β-CD in the free energy of chiral interaction.[9] It should be emphasized that the β-CD cavity exhibits consistent enantio-discrimination toward a series of structurally related guests[29] and that the amino substitution does not alter but rather enhances the original enantiodifferentiation obtained with native β-CD.

Figure 5 compares normalized distribution of intermolecular energies for MTPA:β-CD and MTPA:am-β-CD complexes during the MD simulations. The normalized distribution of intermolecular van der Waals energies for MTPA:β-CDs complexes is not much different between the two enantiomers comparing with that of intermolecular Coulombic energies. However, in case of MTPA:am-β-CD complexes. the distribution of intermolecular Coulombic energy differences for two enantiomers is relatively large. These results strongly indicate that Coulombic interaction is one of the important parts of chiral recognition process by am-β-CD.

Conclusion

Throughout this research. MC coupled to MD simulation was applied to the investigation for the MTPA complexation with two different CDs in terms of the relative distribution in the interaction energies. The calculated results are in good agreement with experimental observations in chiral recognition thermodynamics. We established that am-β-CD serves as a potentially better chiral discriminator than native β-CD. The ability of enantio-discrimination was enhanced by the Coulombic interaction which looks decisive for chiral recognition by am-β-CD. The prediction and understanding of chiral recognition at a molecular level will be valuable and challenging for the evaluation of chiral separation systems. Generalized molecular modeling methods for enantio-discrimination by cyclooligosaccharides are under investigation.

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References

1. Amstrong. D. W.; Ward. T. J.: Armstrong. R. D.; Beesley. T. E. *Science* **1986**. *232*, 1132.
2. Redondo. J.: Blazquez. M. A.: Torrens. A. *Chirality* **1999**. *11*. 694.
3. Hamilton. J. A.: Chen. L. *J. Am. Chem. Soc.* **1998**. *110*. 5833.
4. Berthod. A.; Jin. H. L.: Beesley, T. E.; Duncan, J. D.: Amstrong. D. W. *J. Pharm. Biomed. Anal.* **1990**. *8*, 123.
5. *Pergaman: Comprehensive Supramolecular Chemistry* in *Cyclodextrin*. Szejtli. J., Osa. T., Eds.: Oxford. U. K., 1996; Vol. 3.
6. Rekharsky. M. V.: Inoue. Y. *Chem. Rev.* **1998**. *98*. 1875.
7. Roseman. M.: Jencks. W. P. *J. Am. Chem. Soc.* **1975**. *97*. 631
8. Connors. K. A. *Chem. Rev.* **1997**. *97*, 1325.
9. Rekharsky. M. V.; Inoue, Y. *J. Am. Chem. Soc.* **2002**. *124*, 813.
10. Kitae. T.: Takashima, H.: Kano, K. *J. Inclusion Phenom. Macrocyclic Chem.* **1999**. *33*. 345.
11. Kitae. T.: Nakayama. T.: Kano. K. *J. Chem. Soc., Perkin Trans. 2* **1998**. 207.
12. Kano. T. *J. Phys. Org. Chem.* **1997**. *10*. 286.
13. Brown, S. E.; Coates. J. H.; Duckworth. P. A.: Lincoln. S. F.; Easton. C. J.: May. B. L. *J. Chem. Soc.* **1993**, *89*, 1035.
14. Dale. J. A.: Mosher. H. S. *J. Am. Chem. Soc.* **1973**. *95*. 512.
15. Kim. H.: Choi. J.: Kim. H.-W.: Jung. S. *Carbohydr. Res.* **2002**. *337*. 549.
16. Dauber-Osguthorpe. P.: Roberts, V. A.; Osguthorpe, D. J.: Wolff. J.; Genest, M.; Hagler. A. T. *Proteins* **1988**, *4*, 31.
17. Betzel, C.: Saenger. W.; Hingerty. B. E.: Brown, G. M. *J. Am. Chem. Soc.* **1984**. *106*. 7545.
18. Choi. Y.: Kang. S.: Yang. C.-H.: Kim. H.-W.: Jung. S. *Bull. Korean Chem. Soc.* **1999**. *20*. 753.
19. Choi. Y.: Yang, C.-H.; Kim, H.-W.: Jung, S. *Carbohydr. Res.* **2000**. *326*, 227.
20. Choi. Y.: Yang, C.-H.; Kim, H.-W.: Jung, S. *Carbohydr. Res.* **2000**. *328*. 393.
21. Hart. T. N.: Read. R. J. *Proteins* **1992**. *13*. 206.
22. Metropolis. N.: Rosenbluth. A. W.: Rosenbluth. M. N.: Teller. A. H.; Teller, E. *J. Chem. Phys.* **1953**. *21*. 1087.
23. Choi. Y.; Yang, C.-H.: Kim, H.-W.: Jung. S. *J. Incl. Phenom.* **2001**. *39*, 71.
24. Kim. H.: Kang. S.: Jung. S. *Bull. Korean Chem. Soc.* **2001**. *22*. 979.
25. Kim. H.: Jeong. K.: Lee. S.: Jung. S. *Bull. Korean Chem. Soc.* **2003**. *24*, 1.
26. Berendsen, H. J. C.; Postma, J. P. M.: van Gunsteren, W. F.; DiNola. A.: Haak, J. R. *J. Chem. Phys.* **1984**. *81*. 3684.
27. Luty. B. A.: Wasserman. Z. R.: Stouten. P. F. W.: Hodge. C. N.: Zacharias. M.: McCammon. J. A. *J. Comp. Chem.* **1995**. *16*. 454.
28. Shiona, F.; Kusaka, H.: Wada. K.; Azami. H.; Yasunami. M.; Suzuki. T.: Hagiwara, H.: Ando, M. *J. Org. Chem.* **1998**. *63*. 920.
29. Rekharsky. M. V.: Inoue. Y. *J. Am. Chem. Soc.* **2000**. *122*. 4418.