Journal of Inclusion Phenomena and Macrocyclic Chemistry **45:** 211–216, 2003.

Preparation of β-Cyclodextrin Derivatives Possessing Two Trimethylammonio Groups on Their Primary Hydroxy Sides as Chiral Guest Selectors

HATSUO YAMAMURA[1,*], YUICHI YAMADA[1], RIE MIYAGI[1], KAZUAKI KANO[2], SHUKI ARAKI[1] and MASAO KAWAI[1]

[1]*Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan;* [2]*Central Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Inashiki-gun, Ibaraki 300-1192, Japan*

(Received: 22 April 2002; in final form: 13 February 2003)

Key words: cyclodextrin, trimethylammonio group, hydrophobic interaction, Coulomb interaction, chiral discrimination

Abstract

Three isomers of β-cyclodextrin derivatives **2a–c** possessing two trimethylammonio groups on their C(6) atoms were prepared by reaction of the corresponding regioisomers of diamino compounds with methyl iodide. These derivatives recognize chiral guests through three-point interactions composed of both Coulomb interactions by the two cationic moieties and hydrophobic interaction by the molecular cavity.

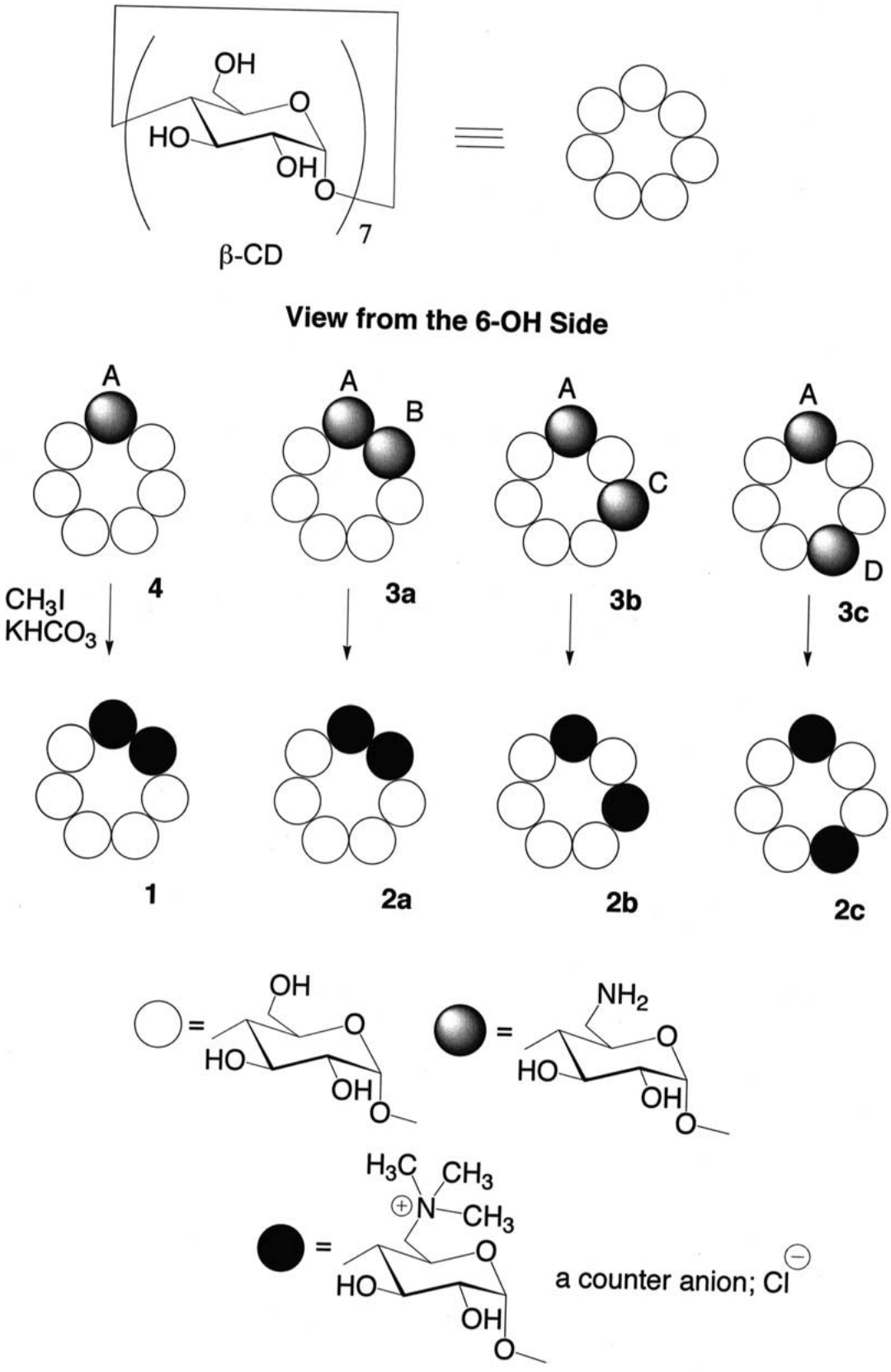
Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucose residues. The most common containing six, seven, and eight glucose units are known as α-, β-, and γ-CD, respectively. The molecule can include each enantiomer of certain chiral guest compounds in its chiral molecular cavity to form diastereomeric complexes. Differences in the properties of the two diastereomeric complexes enable discrimination of each enantiomer. Recently, the significance of the CDs in chiral selective chromatography has been increasing [1]. For example, in capillary zone electrophoresis (CZE), enantiomeric separation is effectively attained by addition of CD to a mobile phase [2, 3]. However, the enantioselectivity displayed by an unmodified CD is modest [4]. In order to attain more clear discrimination of enantiomers, modified cyclodextrins have been studied [5–12]. In particular, Kano and his co-workers have studied charged (anionic and cationic) CD derivatives and their guest inclusion [6–12]. They have established the significance of a cooperative behaviour between inclusion interactions and Coulomb interactions of the host with the guest. Armstrong demonstrated that a quaternary ammonium-functionalized CD worked as an effective chiral selector in CZE [13]. The positive charge of a quaternary ammonio group is independent of the pH and also possess a strong affinity for an anionic guest, providing more potential utility compared to the primary amine-containing CDs studied previously [6, 7, 9, 12]. We have previously reported the synthesis of a quaternary-ammonio CD, namely β-CD derivative **1** possessing a trimethylammonio group on a single C(6), which acts as a novel chiral selector on CZE analysis for D/L-acetylphenylalanine [14]. The observed chiral discrimination by **1** is obviously due to cooperative recognition of the guest through an electrostatic interaction between the trimethylammonio group of **1** and the carboxylate group of the amino acid, and also inclusion of the amino acid in the hydrophobic molecular cavity of **1**. Introduction of an additional interaction to the molecular system is expected to allow more sophisticated recognition of guest molecules. Therefore, we have studied novel CD derivatives **2a–c**; each of these regioisomers possesses quaternary ammonio groups on two of primary hydroxy positions, in AB, AC and AD positions respectively (Scheme 1). And we quantified their chiral guest recognition by calorimetric studies [15, 16], and found that the two cationic groups interact with a guest stereoselectively as we expected. Here we would like to present a practical method for preparation of the valuable bis(trimethylammonio) derivatives **2a–c**.

Experimental

General

^{1}H NMR (600 MHz) spectra were recorded at 25 °C on a Varian AVANCE 600. Proton signals were assigned using COSY and TOCSY (mixing time, 120 m s) experiments. FAB mass measurements were carried out with a Shimadzu-Kratos CONCEPT 32IH spectrometer. Thin layer chromatography (TLC) was run on precoated silica-gel plates (Art 5554, Merck) with the following solvent systems; 1-PrOH-AcOEt-water-acetic acid [5 : 1 : 5 : 1(v/v/v/v)]. Spot detection was carried out by an UV light and/or staining with 0.1% 1,3-naphthalenediol in EtOH-water-H_2SO_4 [200 : 157 : 43 (v/v/v)]. A gel permeation chromatography was performed using Sephadex LH-20 (Pharmacia Biotech). A trimethylammoniomethyl-modified resin (AG1-X8, BIO-RAD) was used for an anion exchange chromatography.


OH
O
HO
OH
O
7
β-CD
View from the 6-OH Side
A
A
B
A
C
A
D
4
3a
3b
3c
CH3I
KHCO3
1
2a
2b
2c
OH
O
HO
OH
O
NH2
O
HO
OH
O
H3C
CH3
N
CH3
O
HO
OH
O
a counter anion; Cl

Scheme 1.

6^A, 6^X,-Bis(trimethylammonio)-6^A, 6^X-dideoxy-β-cyclodextrin dichlorides **2a–c**[1]. A solution of AB-diamine **3a** [18, 19] (102 mg, 8.42×10^{-5} mol) in 60% aq. MeOH (50 cm^3) was treated with $KHCO_3$ (85.0 mg, 8.50×10^{-4} mol) and methyl iodide (955 mg, 6.73×10^{-3} mol) were added and the mixture was stirred in the dark for 25 h. The reaction mixture was concentrated *in vacuo* and applied to a gel permeation chromatography (eluent, water) followed by anion exchange chromatography (eluent, water) giving a crude product (110 mg). Further gel permeation chromatography gave the desired **2a** (73 mg, 68%). The AC isomer **2b** and the AD isomer **2c** were prepared similarly from the corresponding diamino compounds [19] (in the yields of 78% and 68%, respectively).

2a R_f 0.13. (Found: C, 39.66; H, 7.25; N, 1.86. Calc. for $C_{49}H_{86}N_2O_{33}Cl_2 \cdot 9H_2O$: C, 39.70; H, 7.22; N, 1.93%.) ^{1}H NMR (600 MHz, D_2O) δ 3.25 and 3.26 (18H, Me), 4.10 (1H, t, J = 8.75, H3 of trimethylammonioglucose (TMAglucose)], 4.13 (1H, t, J = 8.75, H3 of TMAglucose), 4.52 (1H, t, J = 9.05, H5 of TMAglucose), 4.55 (1H, t, J = 9.05, H5 of TMAglucose), 5.06 (1H, d, J = 3.60, H1 of glucose), 5.07 (1H, d, J = 4.25, H1 of glucose), 5.08 (1H, d, J = 4.00, H1 of glucose), 5.11 (1H, d, J = 3.40, H1 of glucose) and 5.15 (1H, d, J = 3.60, H1 of glucose), 5.21 (1H, d, J = 2.90, H1 of TMAglucose), 5.35 (1H, d, J = 2.95, H1 of TMAglucose). *m/z* (+FAB, HR) 1253.47981 [$(M - Cl)^+$ $C_{48}H_{86}N_2I_{33}Cl$ requires *m/z* 1253.48014], 1203.48807 [$(M - CH_3 - 2Cl)^+$ $C_{47}H_{83}N_2O_{33}$ requires *m/z* 1203.48781], 1158.42969 [$(M - NH(CH_3)_2 - 2Cl)^+$ $C_{45}H_{76}O_{33}$ requires *m/z* 1158.42996], (−FAB, HR) 1287.44038 [$(M - H)^-$ $C_{48}H_{85}N_2O_{33}Cl_2$ requires *m/z* 1287.44116].

2b R_f 0.13. (Found: C, 39.73; H, 7.18; N, 1.88. Calc. for $C_{48}H_{86}N_2O_{33}Cl_2 \cdot 9H_2O$: C, 39.70; H, 7.22; N, 1.93%.) ^{1}H NMR (600 MHz, D_2O) δ 3.23 and 3.24 (27H, Me), 4.07 (1H, t, H3 of TMAglucose), 4.10 (1H, t, H3 of TMAglucose), 4.52 (2H, m, H5 of TMAglucoses), 5.04, 5.08, 5.09, 5.11, 5.12, 5.16 and 5.17 (7H, seven of d, J = 3.70, 3.65, 3.30, 3.55, 3.60, 4.10 and 3.65, respectively, H1 of glucoses and TMAglucoses). *m/z* (+FAB, HR) 1253.48335 [$(M - Cl^+$ $C_{48}H_{86}N_2O_{33}Cl$ requires *m/z* 1253.48014], 1203.48928 [$(M - CH_3 - 2Cl)^+$ $C_{47}H_{83}N_2O_{33}$ requires *m/z* 1203.48781], 1158.42983 [$(M - NH(CH_3)_2 - 2Cl)^+$ $C_{45}H_{76}O_{33}$ requires *m/z* 1158.42996], (−FAB, HR) 1287.44232 [$(M - H)^-$ $C_{48}H_{85}N_2O_{33}Cl_2$ requires *m/z* 1287.44116].

2c R_f 0.13. (Found: C, 39.29; H, 7.02; N, 1.82. Calc. for $C_{48}H_{86}N_2O_{33}Cl_2 \cdot 10H_2O$: C, 39.21; H, 7.27; N, 1.91%). ^{1}H NMR (600 MHz, D_2O) δ 3.23 and 3.24 (27H, Me), 4.08 (1H, t, J = 8.75, H3 of TMAglucose), 4.09 (1H, t, J = 8.90, H3 of TMAglucose), 4.46–4.51 (2H, m, H5 of TMAglucoses), 5.06–5.14 (7H, H1 of glucoses and TMAglucoses). *m/z* (+FAB, HR) 1253.47821 [$(M - Cl)^+$ $C_{48}H_{86}N_2O_{33}Cl$ requires *m/z* 1253.48014], 1203.48801 [$(M - CH_3 - 2Cl)^+$ $C_{47}H_{83}N_2O_{33}$ requires *m/z* 1203.48781], 1158.42884 [$(M - NH(CH_3)_2 - 2Cl)^+$ $C_{45}H_{76}NO_{33}$ requires *m/z* 1158.42996], (−FAB, HR) 1287.44180 [$(M - H)^-$ $C_{48}H_{85}N_2O_{33}Cl_2$ requires *m/z* 1287.44116].

[1] The AD isomer **2c** was reported previously: Ref. [17].

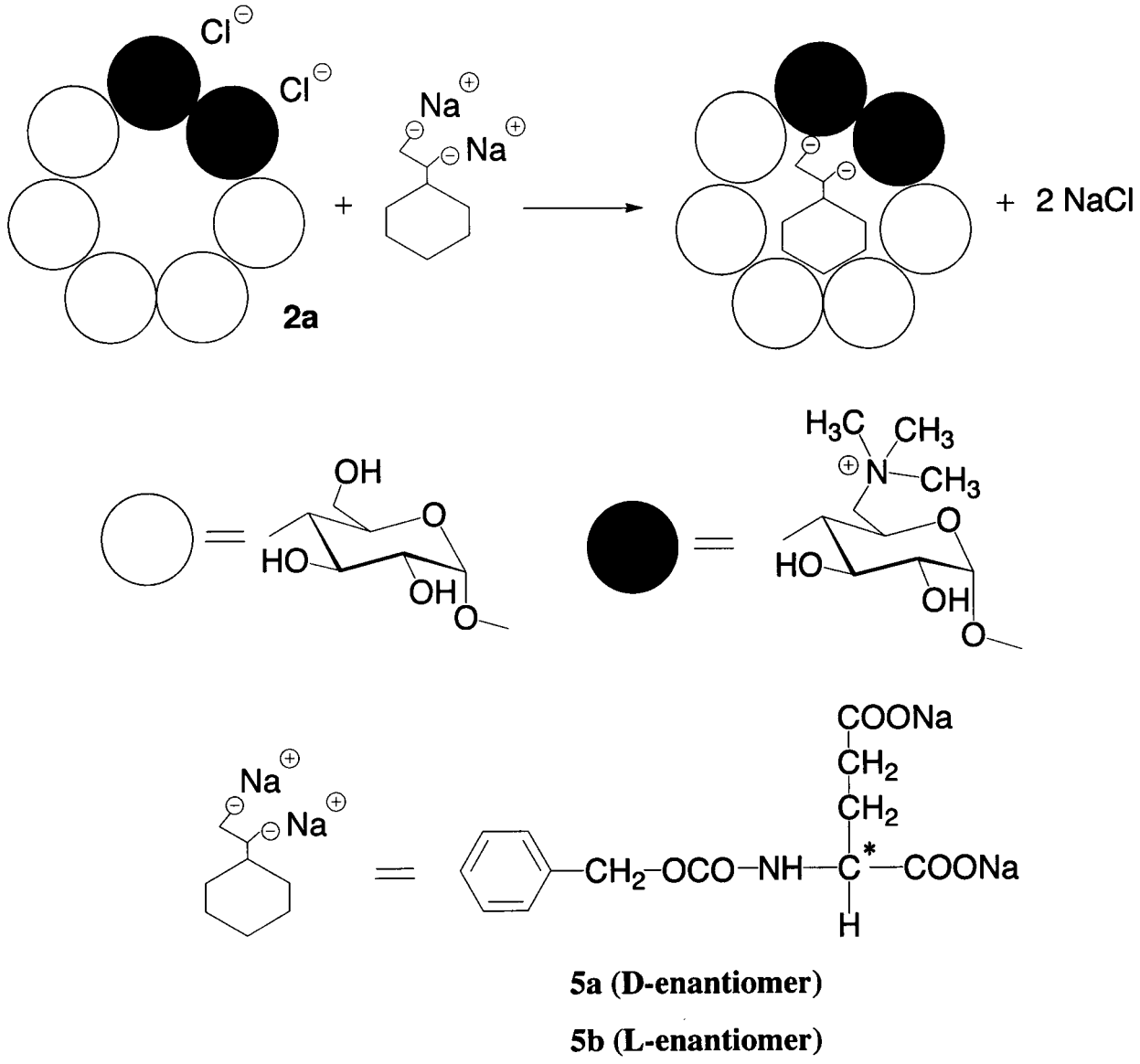
Results and discussion

Syntheses

There are a few ways to obtain the CD derivatives possessing two trimethylammonio groups. One is a direct substitution of disulfonylated CDs with trimethylamine. The monotrimethylammonio β-CD derivative **1** has been prepared by the reaction of mono-tosyl CD with trimethylamine [20]. However, this method requires relatively drastic reaction conditions including the use of gaseous amine which may cause undesired side reactions such as hydrolysis of sulfonate and/or intramolecular dehydration [21]. In place of the substitution above, we established an alternative method to get **1** (Scheme 1) [14], namely methylation of an amino β-CD derivative **4** whose convenient synthesis had already been described [22]. Then we applied this methodology to the methylation of the known diamino β-CDs **3a–c** [18, 19] in order to give the desired bis(trimethylammonio) derivatives **2a–c** (Scheme 1). Thus, the AB-diamine **3a** was treated with methyl iodide in the presence of $KHCO_3$ in aqueous methanol. There were five primary hydroxy groups and fourteen secondary hydroxy groups together with the two amino groups on **3a**. In order to avoid undesirable *O*-methylation, very mild reaction conditions comprising use of a weak base and aqueous methanol were adopted. The trimethylation of the two amino groups needed excess amount of methyl iodide to complete the reaction in a reasonable reaction time. TLC demonstrated the gradual conversion of **3a** (R_f 0.43) to the desired product **2a** (R_f 0.13) through an intermediate product (R_f 0.27). After 25 h passed, it seemed that all of **3a** was converted to **2a** with a trace of by-products that showed a little larger R_f values than that of **2a** and may be the additionally *O*-methylated derivatives. The reaction mixture was concentrated *in vacuo* and was subjected to gel permeation chromatography in order to remove impurities such as inorganic salts. The obtained crude product was treated with anion exchange chromatography to convert the counter anion to Cl^- ion and was purified again by gel permeation chromatography in order to remove small amount of by-products, giving the desired bis(trimethylammonio)-β-CD dichloride **2a** in 68%. The AC- and AD-bis(trimethylammonio) isomers, **2b** and **2c**, were prepared similarly from the corresponding diamino derivatives. Their structures were determined by their elemental analyses, FAB-MS spectra and ^{1}H-NMR spectra. They showed that undesired methylation of hydroxy groups was effectively avoided and that the two trimethylammonio groups had been regiospecifically introduced on C(6) carbons of β-CD molecule.

Characteristic inclusion complex observed in ^{1}H-NMR spectra

Chiral recognition of carbobenzyloxy-D- and L-glutamic acids (Cbz-D- and L-Glu) **5a,b** by bis(trimethylammonio) derivatives **2** have been studied by calorimetric and ROESYNMR studies [15, 16]. The two pairs of Coulombic interactions (—N^+Me_3- - -^-O_2C—) and also the hydrophobic



Scheme 2.

interaction of the CD cavity with the Cbz group of the amino acid seemed cooperative leading to chiral recognition. In order to understand the guest recognition, it seems noteworthy to describe here unique 1D-NMR spectra of the complex of AB-bis(trimethylammonio) β-CD dichloride **2a** with each of Cbz-D- and L-Glu disodium salts **5a,b** (Scheme 2). Concentration of a host–guest complex in the solution depends on the concentration of the host **2a** and the guest **5** and also its association constant. In addition, an observed proton signal in a spectrum of the host–guest mixture was an averaged image of that of the complex and the free species, which indicated the exchange between the complex and the free species is fast on the NMR time scale. Therefore, when we compare structures of two kinds of host–guest complexes based on each NMR spectrum, it should be noted that the spectra really reflect the structures of the complexes and they are comparable. However, it was not possible to fully complex the CD **2a** and Cbz-Glu **5** in the NMR sample solution because of their solubility and the association constant. Here, equimolar amounts of the AB-isomer dichloride **2a** and Cbz-D-Glu disodium salt **5a** were mixed at a concentration[2] such that [complexed **2a**]/[total **2a**] = [complexed **5a**]/[total **5a**] = 0.61 based on the association constant obtained from the calorimetric study [15]. In a similar way, the L-isomer **5b** was also mixed with **2a** at a concentration[3] such that [complexed **2a**]/[total **2a**] = [complexed **5b**]/[total **5b**] = 0.61. The solutions were applied to 1D-NMR experiments.

Complexation of the amino acids by the AB-isomer **2a** caused splitting of phenyl signals of the amino acid (Figure 1). In the spectrum of the mixture of Cbz-D-Glu **5a** and **2a**, the Cbz aromatic protons appeared at 7.40 $\sim$ 7.55 ppm and the H*ortho*, H*meta* and H*para* were differentiated. Similar phenomena were observed in the case of the L-enantiomer **5b**. The aliphatic proton signals (α-CH, β- and γ-CH_2) of Cbz-Glu also showed chemical shift changes although they were very small. In contrast, signals of the

[2] 2.04×10^{-2} M.
[3] 1.42×10^{-2} M.

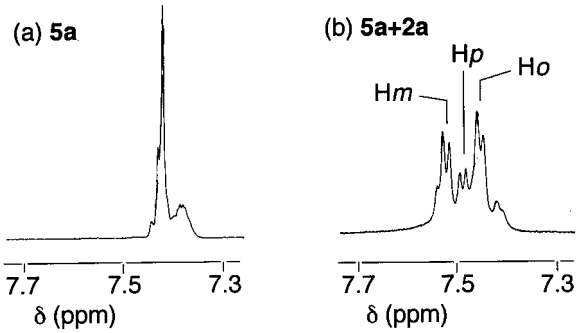


Figure 1. ^{1}H-NMR spectra showing change in aromatic proton signals of Cbz-D-Glu **5a** due to complexation by AB-bis(trimethylammonio)-CD **2a**. (a) Free **5a**, (b) $[\mathbf{5a}]_0 = [\mathbf{2a}]_0 = 2.04 \times 10^{-2}$ M; [complex **5a**]/[total **5a**] = 0.61, which was calculated from the corresponding association constant [15].

CD **2a** showed a dramatic change in the H2–H6 region of glucose units (3.3–4.8 ppm) (Figure 2). In particular, the H5 signals of the trimethylammonioglucoses were shifted upfield. This is probably due to the anisotropic effect of the included Cbz-Glu in the CD cavity. Complexation of Cbz-D-Glu **5a** afforded two clearly-discriminated signals due to the H5 protons of the two trimethylammonioglucoses in **2a**, H5A at 4.44 ppm and H5B at 4.30 ppm, which originally had almost identical chemical shifts (4.52 and 4.55 ppm) in the free **2a**. In the case of the complex with L-enantiomer **5b**, H5B moved to 4.27 ppm while H5A appeared at 4.50 ppm. The induced shifts on 1D ^{1}H-NMR spectra suggested small but clear differences in the structures of the two inclusion complexes. Conformation of the phenyl ring of the Cbz-D/L-Glu **5a,b** in the complexes may be restricted to afford the observed change of chemical shifts, which contributed to the unique NOEs observed in ROESY experiments and the thermodynamic parameters obtained by calorimetric titration experiments [15].

Conclusion

The β-CD derivatives possessing two cationic functional groups have been prepared, which discriminated the chiral amino acids Cbz-Glu. The CD derivatives are promising as chiral discriminator on chromatographic separation such as CZE. Also, these derivatives may provide ideal models to study cooperation of weak interactions, namely Coulomb interactions and hydrophobic interaction, and also the relationship between guest recognition and stereochemistry of inclusion complexes. Experiments using several kinds of enantiomeric and diastereomeric guests are underway.

Acknowledgements

We wish to thank Japan Maize Products Co. Ltd. for a generous gift of β-CD. We greatly thank Dr. J.E. Harvey of University of York, UK, for helpful comments on this paper.

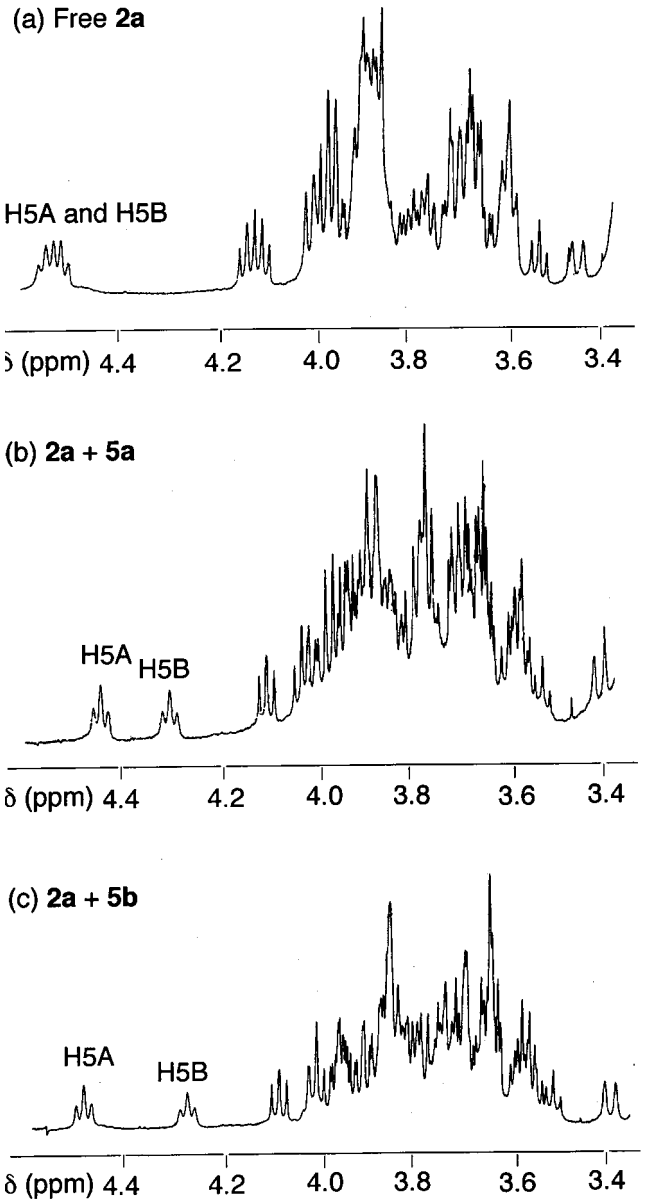


Figure 2. ^{1}H-NMR spectra showing change in glucose signals of AB-bis(trimethylammonio)-CD **2a** caused by complexing with Cbz-D and L-Glu **5a** and **5b**. (a) Free **2a**, (b) $[\mathbf{2a}]_0 = [\mathbf{5a}]_0 = 2.04 \times 10^{-2}$ M, (c) $[\mathbf{2a}]_0 = [\mathbf{5b}]_0 = 1.42 \times 10^{-2}$ M; [complexed **2a**]/[total **2a**] = 0.61 for both amino acid enantiomers, which were calculated from the corresponding association constants [15]. The H5 signals of the two trimethylammonioglucose units (A and B) in **2a** were shown as H5A and H5B.

References

1. Z. Juvancz and J. Szejtli: *Trends in Anal. Chem.* **21**, 379 (2002).
2. S. Fanali: *Chromatographic Science Series Volume 64, Capillary Electrophoresis Technology*, Marcel Dekker, New York (1993), pp. 731–751.
3. S.A. Wren: *J. Chromatogr.* **636**, 57 (1993).
4. K. Kano: *J. Phys. Org. Chem.* **10**, 286 (1997).
5. S.E. Brown, J.H. Coates, P.A. Duckworth, S.F. Lincoln, C.J. Easton, and B.L. May: *J. Chem. Soc., Faraday Trans.* **89**, 1035 (1993).
6. T. Kitae, T. Nakayama, and K. Kano: *J. Chem. Soc., Perkin Trans. 2* 207 (1998).
7. T. Kitae, H. Takashima, and K. Kano: *J. Incl. Phenom. Macrocyclic Chem.* **33**, 345 (1999).
8. K. Kano and H. Hasegawa: *Chem. Lett.* 698 (2000).

9. K. Kano, T. Kitae, Y. Shimofuri, N. Tanaka, and Y. Mineta: *Chemistry-A Eur. J.* **6**, 2765 (2000).
10. K. Kano, H. Hasegawa, and M. Muneki: *Chirality* **13**, 474 (2001).
11. K. Kano and H. Hasegawa: *J. Am. Chem. Soc.* **123**, 10616 (2001).
12. K. Kano and H. Hasegawa: *J. Incl. Phenom. Macrocyclic. Chem.* **41**, 41 (2001).
13. U.B. Nair and D.W. Armstrong: *Microchemical J.* **57**, 199 (1997).
14. H. Yamamura, A. Akasaki, Y. Yamada, K. Kano, T. Katsuhara, S. Araki, M. Kawai, and T. Tsuda: *Electrophoresis* **22**, 478 (2001).
15. M. Rekharsky, H. Yamamura, M. Kawai, and Y. Inoue: *J. Am. Chem. Soc.* **123**, 5360 (2001).
16. H. Yamamura, M. Rekharsky, A. Akasaki, S. Araki, M. Kawai, and Y. Inoue: *J. Phys. Org. Chem.* **14**, 416 (2001).
17. A.V. Eliseev and A.K. Yatsimirskii: *J. Org. Chem.* **59**, 264 (1994).
18. B.D. Blasio, S. Galdiero, M. Saviano, C. Pedone, E. Benedetti, E. Rizzarelli, S. Pedotti, G. Vecchio, and W.A. Gibbons: *Carbohydr. Res.* **282**, 41 (1996).
19. R.P. Bonomo, S. Pedotti, G. Veccchio, and E. Rizaqrelli: *Inorg, Chem.* **35**, 6873 (1996).
20. Y. Matsui and A. Okimoto: *Bull. Chem. Soc. Jpn.* **51**, 3030 (1978).
21. K. Fujita, H. Yamamura T. Imoto, and I. Tabushi: *Chem. Lett.* 543 (1988).
22. K. Hamasaki, H. Ikeda, A. Nakamura, A. Ueno, F. Toda, I. Suzuki, and T. Osa: *J. Am. Chem. Soc.* **115**, 5035 (1993).