  Bull. Chem. Soc. Jpn., 60, 3705—3711 (1987)

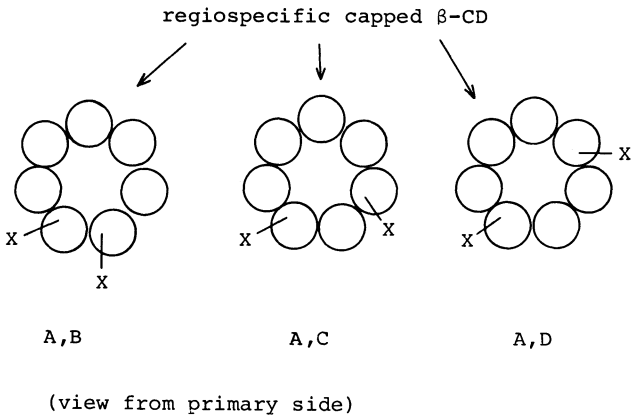
400 MHz Two-Dimensional NMR Studies of Cyclodextrin Derivatives for ^{1}H and ^{13}C Chemical Shift Determination

Iwao TABUSHI, Tatsuya NABESHIMA, Kazuo YAMAMURA,* and Haruo FUJITA
Department of Synthetic Chemistry, Kyoto University, Sakyo-ku, Kyoto 606
(Received May 2, 1987)

Two-dimensional (2D) NMR spectroscopies, proton (400 MHZ)- proton (400 MHz) and carbon (100 MHz)-proton (400 MHz) chemical shift correlation spectroscopy, were developed as a reliable technique for the strict assignment of crowded and complicated NMR absorptions of substituted cyclodextrin derivatives, pentakis[2,6-*O*-(*t*-butyldimethylsilyl)]-*A,B*-bis-[2-*O*-(*t*-butyldimethylsilyl)-6-deoxy-6-(methylthio)]-β-cyclodextrin (**2**) and *A,B*-bis[6-deoxy-6-(methylthio)]-β-cyclodextrin (**1**).

Cyclodextrins bind a large variety of guest molecules of appropriate shapes and sizes in their "hydrophobic" cavities in aqueous solution.[1] This concept of molecular recognition in water has led the authors to a new concept of artificial enzymes or artificial receptors[2,3] and the approaches to realize these have been attempted via attachment of appropriate functional groups onto the "rim" of cyclodextrins. For specific derivatization of cyclodextrins, the explicit determination of the product structures is necessary and important. For the purpose, ^{13}C and ^{1}H NMR[4-16] have been especially useful.

More important, however, is the macrocyclic regiochemistry (see Scheme 1), since it seems very crucial to the development of artificial enzymes or receptors.[17,18] Nevertheless, this regiochemistry on cyclodextrins is not explicitly determined by simple spectroscopies[16,19] other than NMR (high magnetic field) spectroscopy. But instead, a great deal of effort of synthetic[19] or enzymatic[20,21] approach has been made to overcome the drawback.



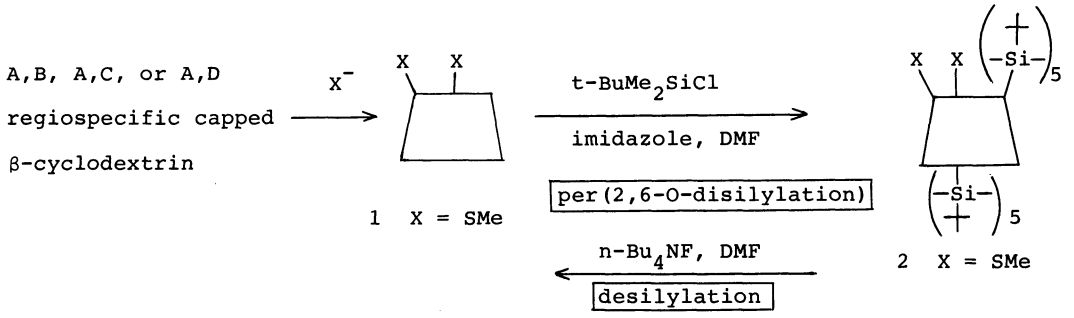
Scheme 1. Regiochemistry of prim-disubstituted β-cyclodextrin.

Recently two-dimensional (2D) NMR spectroscopy[22] has been developed rapidly for the structure determinations of complicated molecules such as natural products,[23] proteins,[24] or carbohydrates.[25] 2D NMR gives much more reliable and well defined informations of chemical shifts or coupling constants than the convensional one-dimensional NMR. Parent α- and β-cyclodextrins were studied by 250 or 270 MHz 2D NMR, and the assignments of individual ^{13}C absorptions of C_1 to C_6[25e] have been successfully made. Substituted cyclodextrins, however, usually show finely separated and delicately overlapped complex absorptions of ^{13}C (or ^{1}H), probably due to appreciable remote substituent effects on chemical shifts and also due to minor but often important conformational changes.

Now we wish to report a complete study of pentakis[2,6-*O*-(*t*-butyldimethylsilyl)]-*A,B*-bis[2-*O*-(*t*-butyldimethylsilyl)-6-deoxy-6-(methylthio)]-β-cyclodextrin (**2**) by using 400 MHz 2D NMR by taking proton-proton[27] (400 MHz) and proton-carbon chemical shift correlation[28] (400—100 MHz). Explicit assignments of C_1-C_6 carbons are presented herein.

Results and Discussion

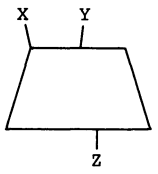
Per(2,6-*O*-disilylation) of disubstituted cyclodextrin derivative **1** with *t*-$BuMe_2SiCl$[14] was carried out (see



Scheme 2.

Scheme 2). This persilylation is important because of the following reasons; (a) separation and isolation of the silylated derivatives are easily achieved by a usual silica-gel column chromatography, (b) the silylated compounds are satisfactorily stable under weak acidic or from weak to strong basic conditions, (c) an appropriate substituent can be conveniently introduced into the unsilylated C_3-OH groups, and (d) desilylation is readily carried out by treatment with n-Bu_4NF in DMF at room temperature (Scheme 2). By use of this silylation-desilylation technique, a promising possibility of constructing sophisticated enzyme models or receptor models through preparation of regiospecific prim,prim-disubstituted cyclodextrins with or without additional appropriate functional group at the secondary position can be done without serious difficulty (Fig. 1).

Pentakis[2,6-*O*-(*t*-butyldimethylsilyl)]-*A*,*B*-bis[2-*O*-(*t*-butyldimethylsilyl)-6-deoxy-6-(methylthio)]-β-cyclodextrin (**2**), which was prepared according to Scheme 2, gave a very crowded conventional ^1H NMR spectrum (400 MHz) (Fig. 2) like other cyclodextrin derivatives. Direct analysis of the spectrum does not afford enough informations to determine the structure of **2** explicitly. Only ^1H absorptions for C_1-H bond appeared at δ 4.80—4.89 separately from other ^1H absorptions. The assignment is based on the comparison with the known ^1H chemical shift for C_1-H protons of the parent cyclodextrin. The ^1H absorptions at δ 2.73—3.04 may be ascribed to H_{6A} ($H_{6A'}$) and H_{6B} ($H_{6B'}$) protons tentatively (Fig. 3), since these are reasonable as CH_2 protons α to the SMe substituent. For other ^1H absorptions, however, explicit assignments were difficult, particularly for other protons attached



X, Y = substituent at primary rim of cyclodextrin

Z = substituent at C_3

Fig. 1. Regiospecific prim-disubstituted β-cyclodextrin with an appropriate functional groups at secondary C_3 carbon.

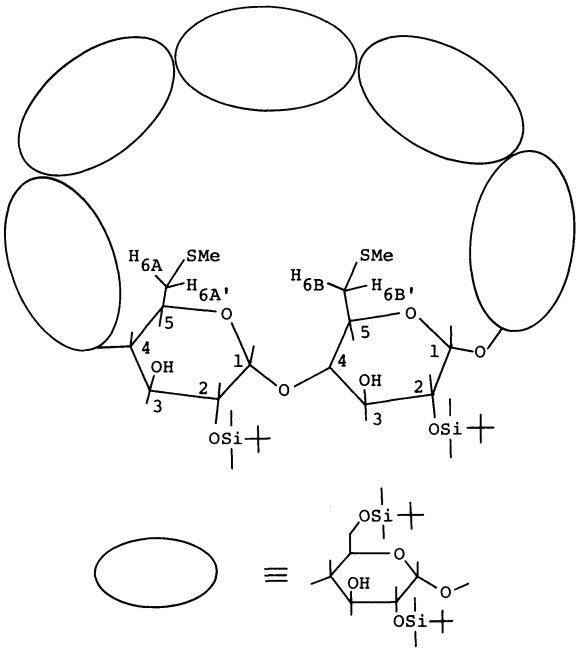


Fig. 3. Structure of **2**. Two H_6 protons on the same ring are interchangeable in the NMR assignment.

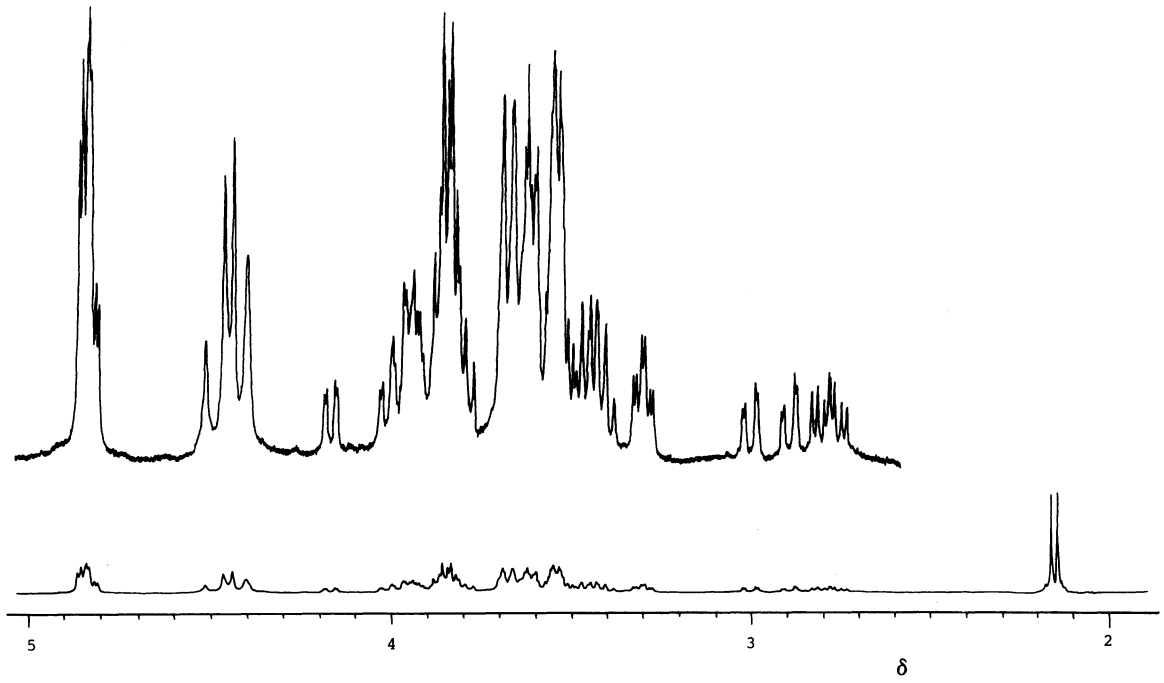


Fig. 2. 400 MHz ^1H NMR spectrum of **2**.

to carbons of cyclodextrin skeleton, because of the extreme complexity of the spectrum.

^{13}C NMR, satisfactorily well-separated absorptions were obtained at 100 MHz NMR (Fig. 4), in which fine structures of the ^{13}C absorptions provide a useful information not only as to the skeletal structure but also as to the possible conformation. Clearly, groups of the ^{13}C absorptions which appeared at well-separated magnetic fields centered at δ 103.0, 82.1, and 62.0 are to be assigned to C_1, C_4, and C_6, respectively, by comparison with ^{13}C absorptions of parent β-cyclodextrin. Thus, $C_{4A,B}$ and $C_{6A,B}$ carbons are tentatively ascribed to the carbon absorption peaks centered at δ 86.4 and 35.9 respectively, by considering the substituent effect of MeS group, while unequivocal determinations of C_2, C_3, C_5, and $C_{5A,B}$ are not feasible from the present one-dimensional NMR spectrum. The lack of NMR data of related compounds suitable for mutual comparison makes the further assignment difficult. Therefore, it is important and necessary to obtain a series of different types of well resolved NMR spectra and to combine information of ^{13}C and

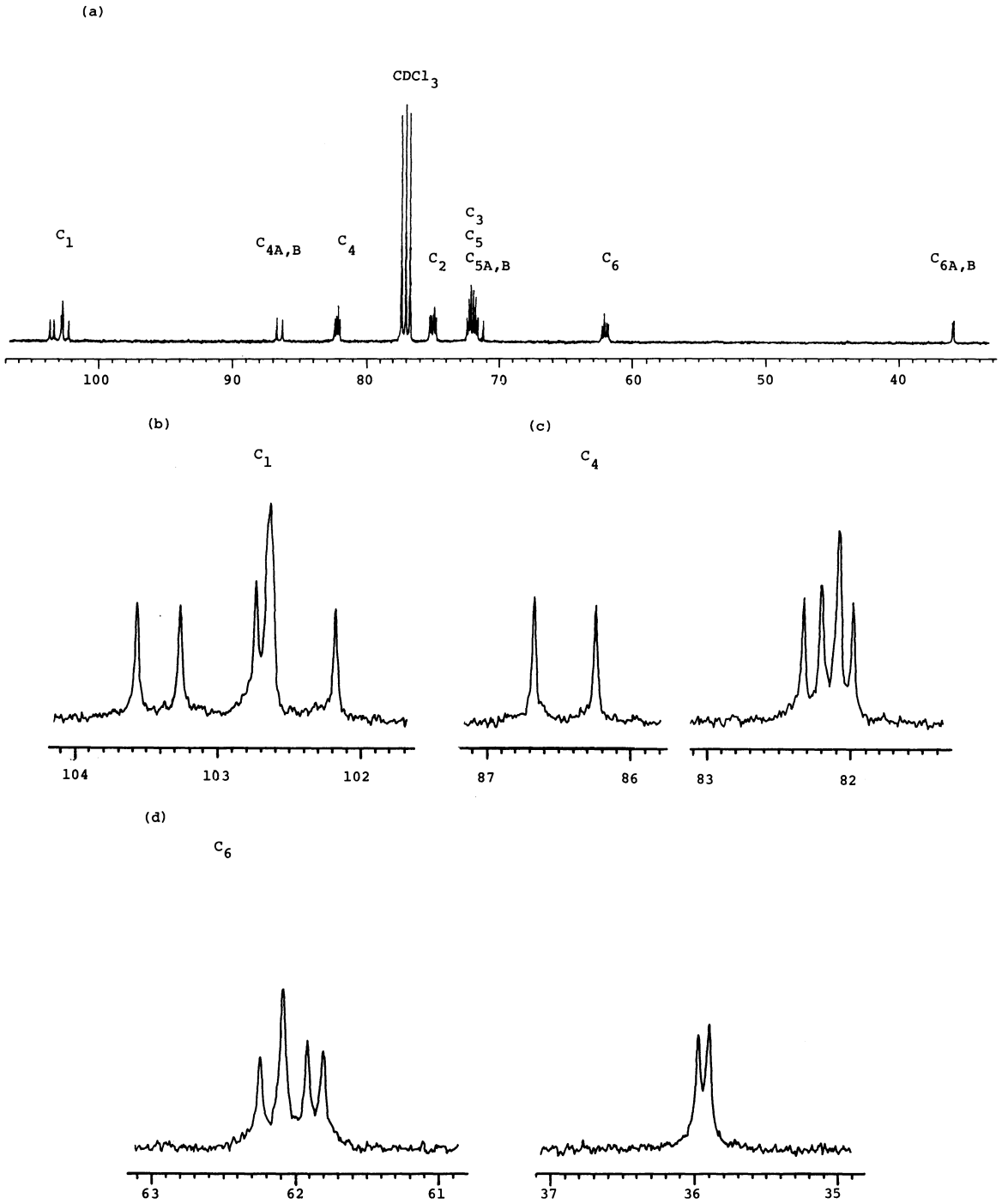


Fig. 4. 100 MHz ^{13}C NMR spectrum of **2**.

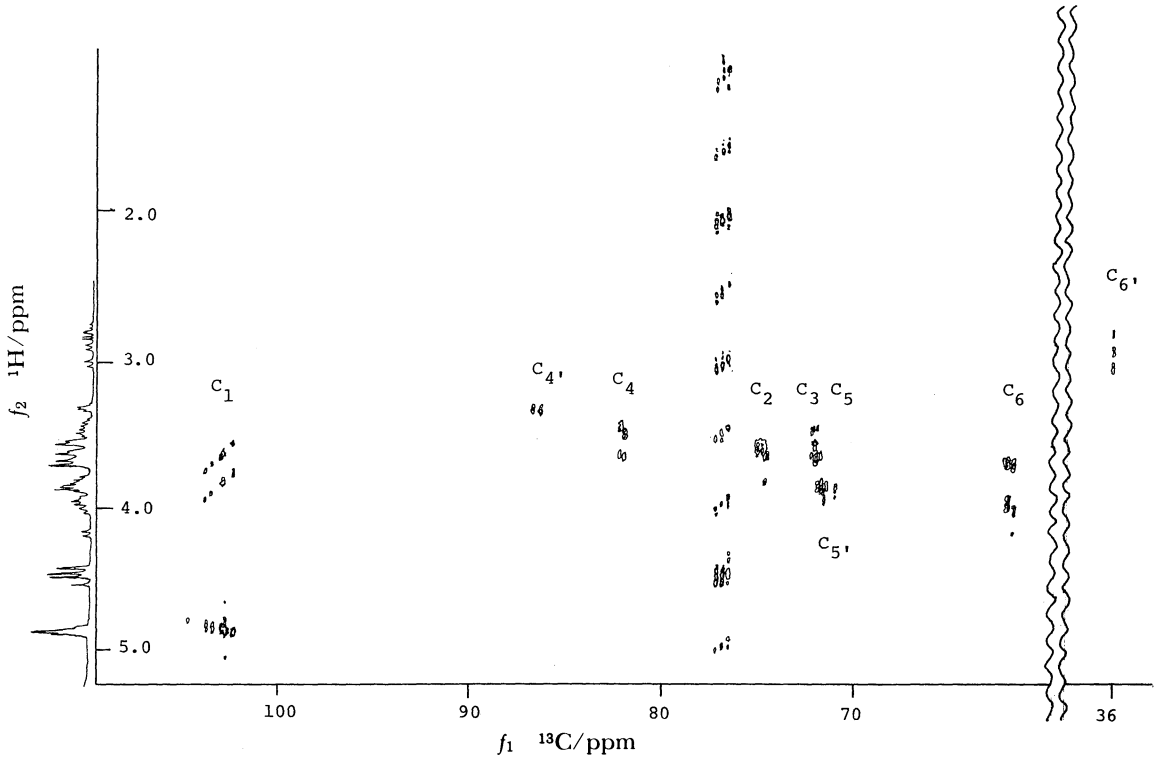


Fig. 5. Contour plot of the two-dimensional (2D) ^{1}H–^{13}C shift correlation of **2** in $CDCl_3$, 30°C.

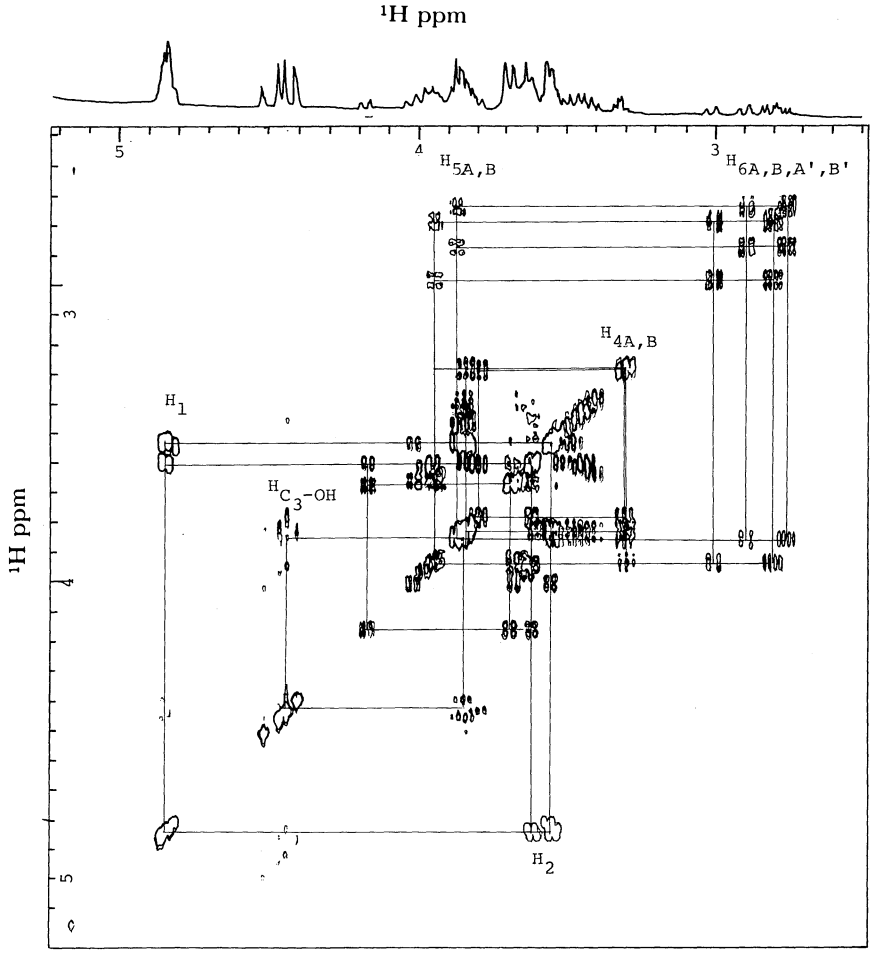


Fig. 6. Contour plot obtained in the 400 MHz ^{1}H COSY of **2**.

Table 1. ^{13}C NMR Chemical Shift (δ from TMS) of Pentakis[2,6-*O*-(*t*-butyldimethylsilyl)]-*A*,*B*-bis[2-*O*-(*t*-butyldimethylsilyl)-6-deoxy-6-(methylthio)]-β-cyclodextrin (**2**) and *A*,*B*-Bis[6-deoxy-6-(methylthio)]-β-cyclodextrin (**1**)

	C_1 $C_{1A,B}$	C_4	C_{4A} C_{4B}	C_2 $C_{2A,B}$	C_3 C_5 $C_{3A,B}$ $C_{5A,B}$	C_6	C_{6A} C_{6B}
2	103.57	82.32	86.67	75.22	72.42	62.24	35.98
	103.27	82.20	86.24	75.17	72.28	62.08	35.90
	102.74	82.08		75.08	72.14	61.91	
	102.64	81.97		74.95	71.93	61.80	
	102.18			74.86	71.77		
				74.75	71.63		
					71.20		
1	103.40	82.65	86.29	74.09		60.98	36.28
	103.22	82.57	86.20	74.04			36.12
	103.02			73.99			
	102.75			73.94			
				73.70			
				73.47			
				73.19			
				73.10			
				72.33			
				71.87			

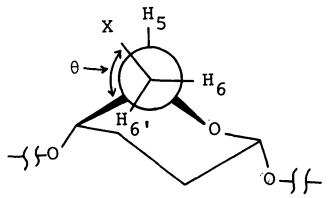


Fig. 7. Dihedral angle between C_4-C_5 axis and C_6-X axis.

Table 2. The Observed Coupling Constants (Hz) between H_6 and $H_{5(6)}$ of the MeS-Substituted Glucose Rings of **2**[a)]

	$H_{6A',B}$	$H_{6B',B}$	H_{5A}	H_{5B}
$H_{6A,A'}$	14	—	6.0	—
			2.5	
$H_{6B,B'}$	—	14	—	6.5
				2.5

a) Assignments A and B are tentative and interchangeable.

^1H NMR spectra for strict structure and conformation determination of cyclodextrin derivatives.

Figures 5 and 6 depict the 2D ^1H-^{13}C chemical shift correlation spectrum and 400 MHz ^1H COSY spectrum of **2**, respectively. Figure 5 displays proton chemical shift information in f_1 (vertical axis) and carbon shifts in f_2 (horizontal axis), one signal appearing for each directly bonded carbon-proton pair. Thus, in the ^1H-^{13}C shift correlation, H_{4A}-C_{4A} correlation and H_{6A}-C_{6A} correlation are clearly seen (Fig. 5), suggesting that the ^1H absorptions at δ 3.28—3.34 and 2.73—3.04 (see also Fig. 2) are assigned unambiguously to H_{4A} (H_{4B}) and H_{6A} (H_{6B}), respectively. Similarly, ^1H COSY spectrum clearly depicts the H_6-H_5 correlation (see Fig. 6), strongly indicating that H_{5A} (H_{5B}) proton absorbs at δ 3.88 (3.95) though hidden by a very complicated ^1H absorptions. Finally the H_{5A}-C_{5A} correlation found in Fig. 5 strongly indicates that C_{5A} and C_{5B} carbons absorb at δ 71.2 and 71.8, one of which is overlapped by the ^{13}C absorptions of C_3 carbons. The presence of H_{5A}-H_{4A} correlation observed (Fig. 6) also supports the mutual consistency of the present assignment. It is clear that the H_4-H_5-H_6 network on A and B rings of **2** has been successfully solved by the 2D NMR technique, providing considerable increase in resolving power and information content over that obtainable using conventional methods.

The 2D NMR technique was found also effective in the assignment of H_1-H_2-C_2 network of **2**. Thus, from ^1H COSY spectrum, H_1-H_2 correlation has led to the clear assignment of H_2 protons (Fig. 6). Therefore, a decisive assignment, ^{13}C absorption at δ 74.8—75.2 due to C_2 carbons, was made clearly. The complete ^{13}C absorptions together with their assignments are summarized in Table 1.

The absorptions due to C_3-OH protons were concluded to have appeared at δ 4.3—4.6 region (see Fig. 2), since a clear correlation was seen between H-C_3 and C_3-O-H in the ^1H COSY spectrum.

H_{6A} (H_{6B}) (see Fig. 7) are coupled to $H_{6A'}$ ($H_{6B'}$) with the coupling constant of J=14 Hz and the two $H_{6A,A'}$ ($H_{6B,B'}$) protons coupled again to H_{5A} (H_{5B}) but with different coupling constants of 6 and 2.5 Hz (6.5 and 2.5 Hz) (see Table 2), suggesting the preference of rotamers around the C_5-C_6 axis (most populated position in the freely or restrictedly rotating substituted methylene) at certain angle (ca. 40° or ca. 200°) twisted from C_5-C_4. One H_6 proton absorbing at δ 4.14—4.23 obviously belongs to the unsubstituted glucose ring. Therefore, H_6 protons may be used as the rotamer probe for the dihedral angle change.

In conclusion, the 2D NMR technique was very effective, particularly for the explicit assignment of ^{13}C NMR absorptions of the glucose ring carrying the substituent (SMe) at C_6 carbons of *A,B*-disubstituted cyclodextrin derivative, **2**. The technique was found also powerful for the desilylated compound, *A,B*-bis[6-deoxy-6-(methylthio)]-β-cyclodextrin (**1**), which was obtained via the desilylation of **2** (Scheme 2). The ^{13}C NMR absorptions of **1** together with their assignments are given in Table 1. The correlation of ^{13}C NMR assignments between **1** and **2** was satisfactory as shown in Table 1.

Experimental

Instruments and Apparatus. ^1H (400 MHz), ^{13}C (100 MHz), and 2D correlation NMR spectra were recorded on a JEOL JNM-GX 400 spectrometer. Chemical shifts in $CDCl_3$ are given in δ values from tetramethylsilane (TMS) used as an internal standard. ^{13}C and 2D NMR spectra were obtained with a coaxial dual cell. The inner tube (5 mmφ) contained 1% TMS in $CDCl_3$, which was used as an external standard by taking the chemical shift of the central peak of $CDCl_3$ from TMS as δ 77.02. The outer tube (10 mmφ) contained a DMSO-d_6 solution of a cyclodextrin derivative to be measured. Both tubes were purchased from Wilmad Glass Co. 2D homonuclear chemical shift correlation (COSY) spectrum (512×1024 matrix after Fourier transformation) was obtained from 512 spectra of 2048 points. The spectral width was 2200 Hz, and 90° pulse of ^1H was 9.1 μs. In carbon-proton chemical shift correlation spectra, spectral widths of two axes were 2200 Hz (^1H axis) with 512 data points and 12000 Hz (^{13}C axis) with 4096 data points. 90° pulse widths of ^{13}C and ^1H were 19.4 and 26.5 μs, respectively. All measurements were carried out at 30±1 °C.

IR spectra were obtained using a Hitachi model 260-50 spectrophotometer. $CDCl_3$ (99.8%), DMSO-d_6 (99.9%), and TMS were purchased from Aldrich or E. Merck.

Materials. Commercially available *N,N*-dimethylformamide was kept standing over CaH_2 overnight at room temperature, and then distilled under reduced pressure (ca. 20 mmHg (1 mmHg=133.322Pa)) before use. Imidazole was freshly recrystallized from benzene, and then dried in vacuo at room temperature before use. Methanethiol sodium salt (15% aqueous solution, Tokyo Kasei) was used without further purification.

Per(2,6-*O*-disilylation) of cyclodextrin derivatives were carried out by treatment of the corresponding unsilylated cyclodextrin derivatives with a mixture of *t*-butyldimethylchlorosilane and dry imidazole in dry DMF according to the reported procedure.[14] The persilylated compounds were purified by silica-gel column chromatography (3×30 cm) at least twice using $CHCl_3$ as an eluent. The purity was checked by TLC using $CHCl_3$ or benzene-AcOEt (100 : 1, v/v).

A typical procedure for desilylation of silylated cyclodextrins—A silylated cyclodextrin derivative (0.5 g) was treated with tetrabutylammonium fluoride (1.0 g) in 30 mL of DMF at room temperature overnight. After the mixture was evaporated to dryness in vacuo, 30 mL of water and 1 mL of tetrachloroethylene were added to the residue. The mixture was stirred for 1 h at 0 °C to afford white precipitates, which were collected by suction filtration. The precipitates were dissolved in ca. 100 mL of 30—40% aqueous EtOH, and then tetrachloroethylene and the solvent were evaporated to dryness at 30 °C, finally at 80 °C at 20 mmHg. This procedure was repeated at least three times for the complete removal of tetrachloroethylene included in the cyclodextrin cavity.

***A,B*-Bis[6-deoxy-6-(methylthio)]-β-cyclodextrin (1).** *m*-Benzenedisulfonyl-capped β-cyclodextrin[20] (2.8 g, 2.1 mmol) was treated with 20 g (43 mmol) of a 15% aqueous MeSNa solution at 80 °C for 14 h under Ar. The mixture was acidified to pH 4 by the addition of 3M hydrochloric acid (1M=1 mol dm^{-3}) to give a pale yellow precipitates, which were collected by filtration. The filtrate was stirred with 1 mL of tetrachloroethylene at 0 °C for 1 h. The precipitates thus formed (containing tetrachloroethylene) were collected. Tetrachloroethylene included in the precipitates was removed as described above. The removal of tetrachloroethylene was ascertained by IR, viz., by the disappearance of the 775, 910 cm^{-1} absorptions. The precipitates were combined and dried in vacuo at 90 °C overnight, giving 0.9 g of **2**: IR (KBr) 3350 (br), 2910, 1150, 1030, 935, 755 cm-1; ^1H NMR (DMSO-d_6) δ=2.06 (s, Me-S, 3H), 2.10 (s, Me-S, 3H), 2.63 (dd, S-C(6)-H, *J*=8, 14 Hz, 1H), 2.75 (dd, S-C(6)-H, *J*=8, 14 Hz, 1H), 3.01 (d, S-C(6)-H, *J*=14 Hz, 1H), 3.13 (d, S-C(6)-H, *J*=14 Hz, 1H), 3.23—3.86 (m, other H), 4.40—4.56 (m, C(6)-OH, 5H), 4.80—4.91 (m, C(1)-H, 7H), 5.67—5.93 (m, C(2)-OH and C(3)-OH, 14H).

Pentakis[2,6-*O*-(*t*-butyldimethylsilyl)]-*A,B*-bis[2-*O*-(*t*-butyl dimethylsilyl)-6-deoxy-6-(methylthio)]-β-cyclodextrin (2). *A,B*-Bis[6-deoxy-6-(methylthio)]-β-cyclodexttrin (0.9 g, 0.75 mmol) was treated with *t*-butyldimethylchlorosilane (4.5 g, 30 mmol) and dry imidazole (4.3 g, 63 mmol) in 40 mL of dry DMF in a reported way,[14] giving 250 mg of the corresponding per(2,6-*O*-disilylated) compound **2** after the chromatographic purification as described above. **2**: IR (KBr) 3455 (br), 2960, 2940, 2900, 2865, 1470, 1365, 1260 (Si-CH_3), 1160, 1140, 1095, 1045, 1010, 860, 840, 785 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.01—0.07 (m, Me_2Si-O-C(6), 30H), 0.12—0.19 (m, Me_2 Si-O-C(2), 42H), 0.85—0.90 (m, *t*-BuSi-O-C(6), 45H), 0.90—0.96 (m, *t*-Bu-O-C(2), 63H), 2.15 (s, Me-S-C(6), 3H), 2.17 (s, Me-S-C(6), 3H), 2.76 (dd, S-C(6)-H, *J*=6, 14 Hz, 1H), 2.81(dd, S-C(6)-H, *J*=6.5, 14 Hz, 1H), 2.89 (dd, S-C(6)-H, *J*=2.5, 14 Hz, 1H), 3.00 (dd, S-C(6)-H, *J*=2.5, 14 Hz, 1H), 3.25—4.21 (m, other H, 49 H), 4.39—4.67 (m, C(3)-OH, 7H), 4.80—4.89 (m, C(1)-H, 7H); Found : C, 53.76 ; H, 9.54%. Calcd for $C_{116}H_{242}O_{33}S_2Si_{12}$: C, 54.29 ; H, 9.51%.

References

1) E.g., F. Cramer, *Angew. Chem.*, **5**, 115 (1956).

2) For reviews see a) I. Tabushi, *Tetrahedron*, **40**, 269 (1984); b) I. Tabushi and K. Yamamura, "Cyclophanes. I," ed by F. Voegtle, Springer Verlag, New York (1983), p. 145; c) "Host Guest Complex Chemistry, I and II," ed by F. Voegtle, Springer Verlag, New York (1981) and (1982).

3) For reviews see I. Tabushi and Y. Kuroda, *Adv. Catal.*, **32**, 417 (1983); M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry," Springer Verlag, New York (1978); R. Breslow, *Acc. Chem. Res.*, **13**, 170 (1980); I. Tabushi, *ibid.*, **15**, 66 (1982); R. Breslow, *Science*, **218**, 532 (1982); W. Saenger, *Angew. Chem., Int. Ed. Engl.*, **19**, 344 (1980); J. Szejtli, "Cyclodextrins and Their Inclusion Complexes," Akademiai Kiado, Budapest (1982).

4) K. Takeo, K. Hirose, and T. Kuge, *Chem. Lett.*, **1973**, 1233.

5) R. J. Bergeron, M. P. Meeley, and Y. Machida, *Bioorg. Chem.*, **5**, 121 (1976).
6) A. Harada, M. Furue, and S. Nozakura, *Macromolecules*, **9**, 701 (1976).
7) T. Ogawa and M. Matsui, *Carbohydr. Res.*, **56**, C1-6 (1977).
8) B. Siegel, A. Pinter, and R. Breslow, *J. Am. Chem. Soc.*, **99**, 2309 (1977).
9) D. Gagnaire, D. Mancier, and M. Vincendon, *Org. Magn. Reson.*, **11**, 344 (1978).
10) J. Boger, R. J. Corcoran, and J.-M. Lehn, *Helv. Chim. Acta*, **61**, 2190 (1978).
11) I. Tabushi, Y. Kuroda, and K. Shimokawa, *J. Am. Chem. Soc.*, **101**, 1614 (1979).
12) J. Boger, D. G. Brenner, J. Knowles, *J. Am. Chem. Soc.*, **101**, 7630 (1979).
13) I. Tabushi, L.C. Yuan, K. Shimokawa, K. Yokota, T. Mizutani, and Y. Kuroda, *Tetrahedron Lett.*, **24**, 2273 (1981).
14) R. L. Wife, D. E. Reed, D. P. Leworthy, D. M. Barnett, P. D. Regen, and H. C. Volger, *Int. Symp. on Cyclodextrins, Budapest*, **1981**, 301.
15) A. Ueno and R. Breslow, *Tetrahedron Lett.*, **23**, 3451 (1982).
16) H.-R. Schulten, T. Komori, K. Fujita, A. Shinoda, T. Imoto, and T. Kawasaki, *Carbohydr. Res.*, **107**, 177 (1982).
17) K. Hirotsu, T. Higuchi, K. Fujita, T. Ueda, A. Shinoda, and T. Imoto, *J. Org. Chem.*, **47**, 1143 (1982).
18) Y. Iwakura, K. Ueno, F. Toda, S. Onozuka, K. Hattori, and M. L. Bender, *J. Am. Chem. Soc.*, **97**, 4432 (1975); S. Onozuka, M. Kojima, K. Hattori, and F. Toda, *Bull. Chem. Soc. Jpn.*, **53**, 3225 (1980).
19) I. Tabushi, Y. Kuroda, K. Yokota, and L.C. Yuan, *J. Am. Chem. Soc.*, **103**, 711 (1981).
20) I. Tabushi, T. Nabeshima, K. Fujita, A. Matsunaga, and T. Imoto, *J. Org. Chem.*, **50**, 2638 (1985).
21) K. Fujita, A. Matsunaga, and T. Imoto, *J. Am. Chem. Soc.*, **106**, 5740 (1984).
22) A. Bax, "Two-Dimensional Nuclear Magnetic Resonance in Liquids," Reidel Publishing Co., Boston (1982).
23) E.g., a) A. Pardi, R. Walker, H. Rappoport, G. Wider, and K. Wuethrich, *J. Am. Chem. Soc.*, **105**, 1652 (1983); b) N. S. Bhacca, M. F. Balandrin, A. D. Kinghorn, T. A. Frenkiel, R. Freeman, and G. A. Morris, *ibid.*, **105**, 2538 (1983); c) M. A. Weiss, D. J. Patel, R. T. Sauer, and M. Karplus, *Proc. Natl. Acad. Sci., U.S.A.*, **81**, 130 (1984).
24) For a review see G. Wider, S. Macura, A. Kumar, R. Ernest, and K. Wuethrich, *J. Magn. Reson.*, **56**, 207 (1984).
25) a) L. D. Hall and G. A. Morris, *Carbohydr. Res.*, **82**, 175 (1980); b) G. A. Morris, *J. Magn. Reson.*, **44**, 277 (1981); c) G. A. Morris and L.D. Hall, *J. Am. Chem. Soc.*, **103**, 4703 (1981); d) D. Y. Gagnaire, F.R. Taravel, and M. R. Vignon, *Macromolecules*, **15**, 126 (1982); e) G. A. Morris and L. D. Hall, *Can. J. Chem.*, **60**, 2431 (1982); f) W. Curatolo, L. J. Neuringer, D. Ruben, and R. Haberkorn, *Carbohydr. Res.*, **112**, 297 (1983); g) P. J. Card and G.S. Reddy, *J. Org. Chem.*, **48**, 4734 (1983); h) B, Coxon, *Anal. Chem.*, **55**, 2361 (1983); i) G. Batta and A. J. Liptak, *J. Am. Chem. Soc.*, **106**, 248 (1984).
26) F. R. Travel and M. R. Vignon, *Polym. Bull. (Berlin)*, **7**, 153 (1982).
27) a) K. Nagayama, K. Wuethrich, and R. R. Ernst, *Biochem. Biophys. Res. Commun.*, **90**, 305 (1979); b) K. Nagayama, A. Kumar, K. Wuethrich, and R. R. Ernst, *J. Magn. Reson.*, **40**, 321 (1980); c) A. Kumar, G. Wagner, R. R. Ernst, and K. Wuethrich, *Biochem. Biophys. Res. Commun.*, **96**, 1156 (1980); d) A. Bax, R. Freeman, and G. A. Morris, *J. Magn. Reson.*, **42**, 164 (1981).
28) a) A. A. Mandsley and R. R. Ernst, *Chem. Phys. Lett.*, **50**, 368 (1977); b) A. A. Mandsley, L. Mueller, and R. R. Ernst, *J. Magn. Reson.*, **28**, 463 (1977); c) G. Bodenhausen and R. Freeman, *ibid.*, **28**, 471 (1977); d) G. Bosenhausen and R. Freeman, *J. Am. Chem. Soc.*, **100**, 320 (1978); e) R. Freeman and G. A. Morris, *J. Chem. Soc., Chem. Commun.*, **1978**, 684; f) L. Mueller and R. R. Ernst, *Mol. Phys.*, **38**, 963 (1979); g) L. D. Hall, G. A. Morris, and S. Sukumar, *J. Am. Chem. Soc.*, **102**, 1745 (1980); h) A. Bax and G. A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).