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Selective Acyl and Alkylation of Monobenzoyl *p*-*tert*-Butylcalix[4]arene

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Several calixarene derivatives of 5,11,17,23-tetra-*tert*-butyl-25-(3,5-dinitrobenzoyloxy)-26,27,28-trihydroxycalix[4]arene **2** were synthesized by the reaction of **2** with several acyl and alkylating agents in the presence of base such as pyridine and K_2CO_3 in THF. Acylation of monobenzoylated *p*-*tert*-butylcalix[4]arene **2** yielded their corresponding 1,3-diacylated calix[4]arenes **3a-3g**. On the other hand, alkylation of **2** produced a variety of calix[4]arene derivatives such as 1,2- and 1,3-disubstituted calix[4]arenes **4a-4c**, **4e-4f**, or 1,2,4-trisubstituted calix[4]arene **4d**. 1,2-Disubstituted calix[4]arenes are chiral. All derivatives exist as a cone conformation based on NMR studies.

Introduction

Gutsche and his coworkers^{1,2} discovered the selective esterification of *p*-*tert*-butylcalix[4]arene by the reaction of 3,5-dinitrobenzoyl chloride with calixarene in the presence of base. They reported that under the carefully controlled reaction conditions one monoester, two diesters (1,2- and 1,3-disubstituted) and one triester could be prepared selectively. By taking advantage of the reaction of the preparation of monoester, we recently published the synthetic procedure³ for the monoalkyl calix[4]arene and the selective acylation⁴ of calix[4]arene. To further extend the chemistry of selective functionalization of calix[4]arene we utilized the function of bulky group such as 3,5-dinitrobenzoyl at the lower rim and *p*-*tert*-butyl group at the upper rim of calixarene for the selective introduction of the different second and third, and possibly the fourth substituents at the lower rim of

calixarene.

For the introduction of second substituents, 5,11,17,23-tetra-*tert*-butyl-25-(3,5-dinitrobenzoyloxy)-26,27,28-trihydroxycalix[4]arene ("25-monoester **2**") was treated with several different acyl as well as alkyl halides in the presence of base, which produced ester and other substituents in one calixarene. For the reaction of acyl halides all 1,3-disubstituted calixarenes were obtained as expected, but a variety of products such as 1,2-(chiral), 1,3-disubstituted and 1,2,4-trisubstituted calix[4]arenes were obtained for the reaction of alkyl halides.

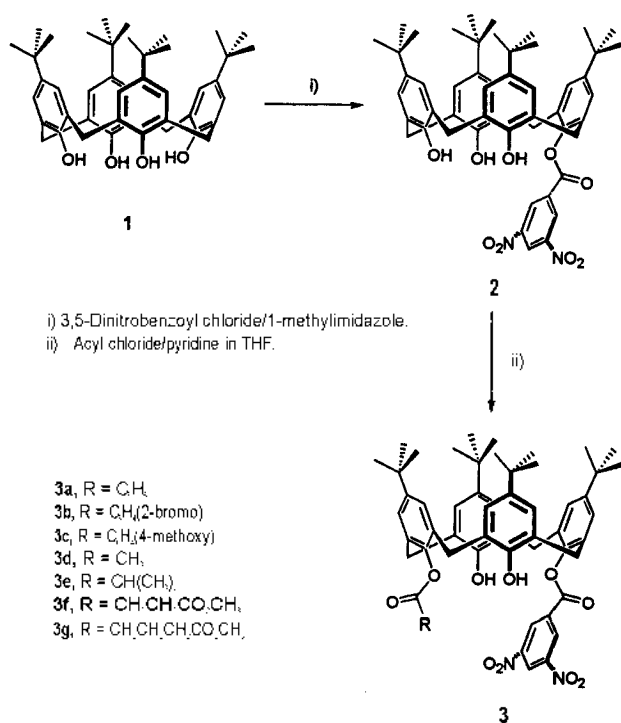
Results and Discussion

Acylation of Monobenzoylated Calix[4]arene **2**.

Since it is not possible to introduce^{5,6} directly two different acyl groups between the four hydroxy moieties at the lower

rim of calix[4]arene. we developed a method to put two different acyl substituents at the lower rim by the two step reactions. Previously, we reported³ that 25-(3,5-dinitrobenzyloxy)-26,27,28-trihydroxycalix[4]arene which do not have *tert*-butyl group at the para position reacted with several acyl chloride in the presence of pyridine to yield a various ABCB type calix[4]arenes. Following the previous procedure developed by our group, monobenzoylated *p*-*tert*-butylcalix[4]arene **2** treated with acyl halide such as benzoyl chloride, 2-bromobenzoyl chloride, 4-methoxybenzoyl chloride, acetyl chloride, isobutyryl chloride, 3-methoxycarbonylpropionyl chloride, and 4-methoxycarbonylbutanoyl chloride in the presence of pyridine. The various ABCB type calix[4]arenes **3a-3g** were prepared selectively as shown in Scheme 1. All reactions have been carried out in THF at room temperature without any difficulty. Substitution of acyl groups was occurred only at the opposite side of the existing 3,5-dinitrobenzoyl group with a cone conformation. In these reactions, 3,5-dinitrobenzoyl group obviously controlled the position of second acyl group presumably by the steric factor.

Substitution pattern and conformation of diacylated calix[4]arene were confirmed by the NMR spectra. The ¹H NMR spectrum of **3a** showed two pair of doublets at 3.52-3.94 ppm arising from the bridged methylene protons and a singlet at 4.94 ppm for the two hydroxy protons, indicating that second substitution was occurred at the opposite side of 3,5-dinitrobenzoyl group of calix[4]arene **2**. The IR absorption band of **3a** showed at 3500 cm⁻¹ as a sharp singlet for the OH and at 1740 cm⁻¹ for the C=O stretching band, indicating that two hydroxy groups are not hydrogen bonded each other. The ¹H NMR spectrum of **3b-3g** showed the similar pattern as described above such as two pair of doublets at 3.45-4.02 ppm for the methylene protons and a sin-

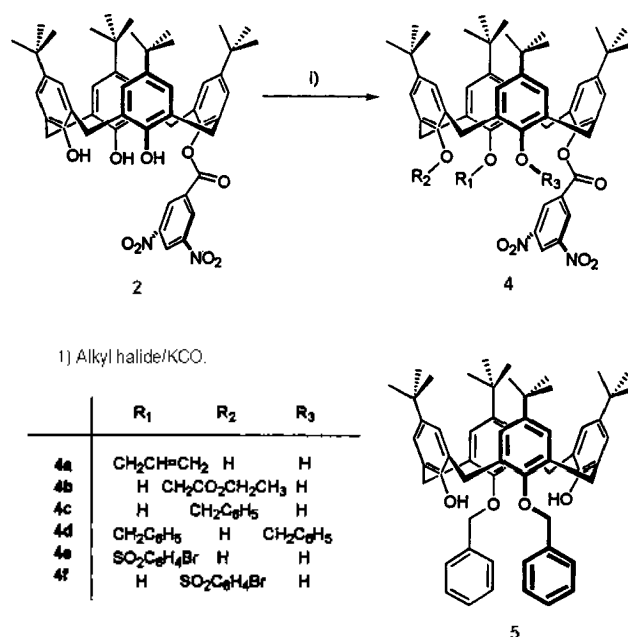


Scheme 1.

glet at 4.8-4.9 ppm for the two hydroxy protons. The IR absorption band of **3b-3g** also showed the similar pattern as observed for **3a**. The conformation of diacylated calix[4]arenes **3a-3g** was deduced from the ¹³C NMR chemical shifts of the bridge methylene carbons. In a syn orientation, the methylene signals appear around 31 ppm, whereas they appear around 37 ppm when both phenol rings are anti oriented.⁹ All of those diacylated calix[4]arenes show two peaks at about 31 ppm indicating that they exist as a cone conformation. The diacylated *p*-*tert*-butylcalix[4]arene **3** could be a useful starting material for further elaboration as synthetic route of ABCD type chiral calix[4]arenes.^{10,11}

Alkylation of Monobenzoylated Calix[4]arene 2; Synthesis of Chiral Calix[4]arenes. We have previously reported¹² that alkylation of monobenzoylated *p*-11-calix[4]arene with various alkyl halide results in the formation of 1,3-disubstituted calix[4]arene except methylation which end up 1,2-product. Under the similar reaction condition, on the other hand, monobenzoylated *p*-*tert*-butylcalix[4]arene **2** reacted with several alkylating agents such as benzyl bromide, allyl bromide, ethyl bromoacetate, and *p*-bromobenzenesulfonyl chloride (it is not alkyl halide, but react under the same condition) to produce a variety of substituted calixarenes such as 1,2-, 1,3-disubstituted, and 1,2,4-trisubstituted calix[4]arenes **4a-4f** depending on the alkylating agents as shown in Scheme 2. 1,2-Disubstituted calix[4]arenes are chiral regardless of conformation, but 1,3-disubstituted and 1,2,4-trisubstituted calix[4]arenes are not if they exist as a cone conformation.

Chiral 1,2-disubstituted calix[4]arene **4a** was obtained when **2** treated with allyl bromide in the presence of K₂CO₃. The ¹H NMR spectrum of **4a** shows four singlets at 0.8-1.3 ppm for the *t*-butyl protons, and four pair of doublets at 3.3-4.5 ppm for the eight bridge methylene protons, clearly indicating that **4a** is chiral as shown in Figure 1. One hydroxy proton peak appears at 9.88 ppm, on the other hand, the other appears at 6.49 ppm as confirmed by adding D₂O



Scheme 2.

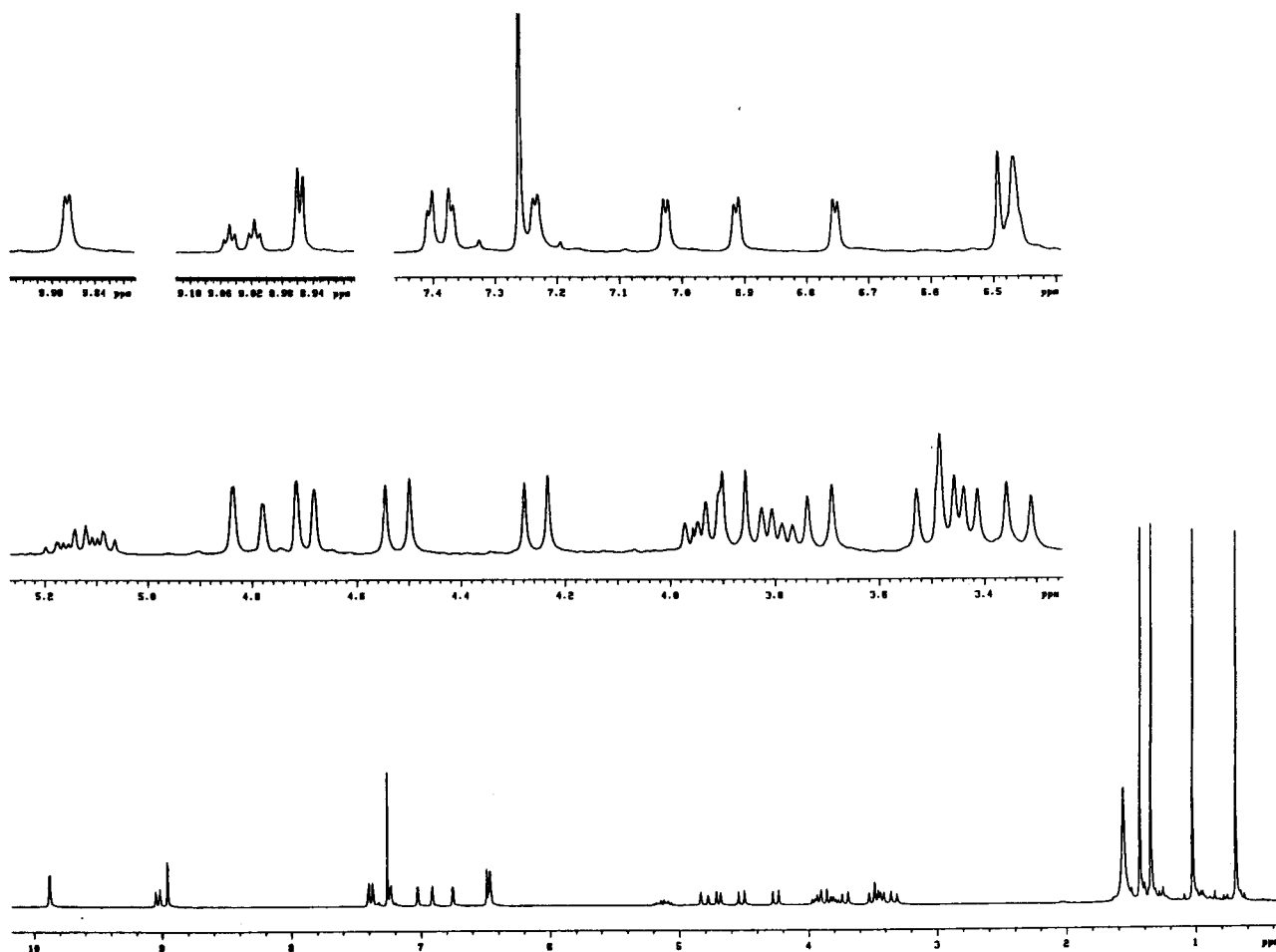


Figure 1. ^1H NMR spectrum of Chiral Calix[4]arene **4a**.

into the NMR solution. One hydroxy proton near the ester carbonyl group could form a hydrogen bond with carbonyl oxygen, which causes down field shift, but the other one could not able to form a hydrogen bond due to a relatively long distance toward carbonyl oxygen. The ^{13}C NMR spectrum shows four peaks at 30–32 ppm for the bridging carbons, indicating that **4a** exists as a cone conformation. Previously we reported¹ that the 1,2-disubstituted calix[4]arene existed as a partial cone conformation when two substituents are 3,5-dinitrobenzoyl and methyl and without *t*-butyl group at the para position. The ^{13}C NMR signals of the bridge methylene carbon showed four peaks at 37.89, 37.41, 31.47, and 31.12 ppm as expected for the two anti and two syn oriented carbons for the partial cone conformation. We do not have a good explanation at this moment for the different conformation, but *t*-butyl group at the para position might direct the cone conformation of **4a**.

The 1,3-disubstituted calix[4]arene **4b** was obtained when **2** was treated with ethyl bromoacetate under the same condition applied above. Reactivity between allyl bromide and ethyl bromoacetate toward calix[4]arene **2** in the presence of K_2CO_3 in THF under reflux might not much differ. Both reaction took about 5–7 hrs to complete. But the size of two alkyl halides could play a determining role for the reaction position. Large ethyl bromoacetate might prefer to approach as far away from the bulky ester group, which produce 1,3-

disubstituted calix[4]arene **4b**. But a relatively small allyl bromide could react to the hydroxy group next to ester without much difficulty to produce 1,2-disubstituted calix[4]arene **4a**. Similar 1,2-substitution was observed⁴ when *tert*-butylated calix[4]arene treated with small alkyl halide such as methyl iodide under the similar reaction condition.

The 1,3-disubstituted calix[4]arene **4c** was formed also at the beginning of the reaction when benzyl bromide was treated with **2**, but **4c** slowly changed to 1,2,4-trisubstituted **4d** when the reaction mixture refluxed more than 2 hours in the presence of excess benzyl bromide and K_2CO_3 . First benzylation at the opposite side of 3,5-dinitrobenzoyl group of **2** could be explained easily by the steric factor mentioned above, but the second benzylation might not be simple. Two benzyl groups at **4d** are located at the 2,4-position to the relative to the 3,5-dinitrobenzoyl group, which indicate that the migration of 3,5-dinitrobenzoyl group took place. Most likely explanation for the benzoyl shift is as followed. After the first benzylation, **4c** could form an anion in the presence of base, even though the anion formed could not attack benzyl bromide at this stage presumably due to the neighboring large benzyl and benzoyl groups. But the anion can attack benzoyl ester group near by to produce 1,2-disubstituted anion, which then attack benzyl bromide easily due to much favorable steric environment to produce 1,2,4-trisubstituted **4d** as shown in Figure 2. The 1,

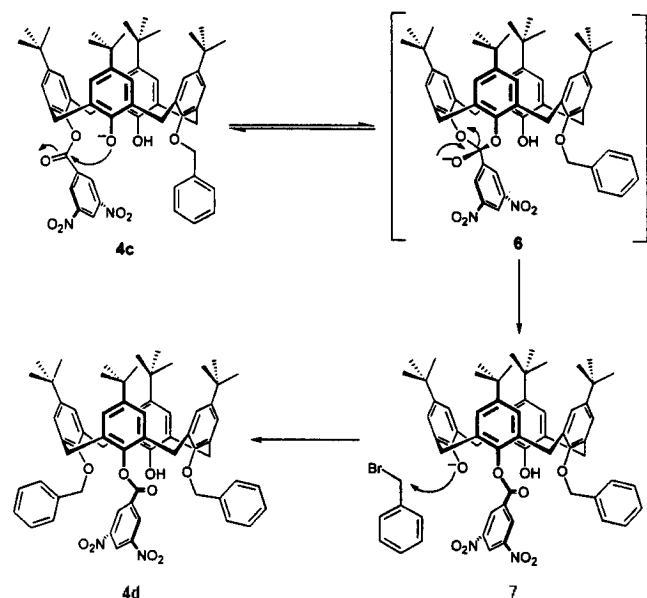


Figure 2. The Proposed Reaction Pathway for 1,2,4-Trisubstitution.

2-disubstituted intermediate **7** could not be isolated and not detected by TLC analysis. This suggests that the migration of benzoyl group is much slower than second benzylation. Dibenylation was confirmed by the hydrolysis of **4d** which produced 1,3-dibenzylated calix[4]arene **5**. Benzoyl migration was only observed when benzyl bromide treated with K_2CO_3 , suggesting that benzyl bromide happened to be a right alkylating agent for the benzoyl migration. When *p*-bromobenzoyl chloride treated with **2** in the presence of K_2CO_3 , a mixture of 1,2- (**4e**) and 1,3-disubstituted product (**4f**) were obtained. We do not have good explanation for this lack of selectivity. To understand the effect of size of alkyl group for the substitution pattern, **2** was treated with a number of alkyl halide such as methyl iodide, ethyl bromide etc, but unfortunately most ordinary alkyl halide do not react under the reaction condition applied. Even though methyl iodide do react with **2**, we failed to isolate any identifiable products.

Experimental

Melting points of all compounds were measured on a Mel-Temp apparatus without calibration. Infrared (IR) spectra were determined on a Nicolet 520 FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 300 AMX spectrometer. Chemical shifts are reported as δ values in parts per million relative to tetramethylsilane (δ 0.00) as an internal standard. Thin layer chromatography (TLC) analyses were carried out on silica gel plates. Column chromatography was carried out with E. Merck silica gel (230-400 mesh ASTM).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzoyloxy)-26,27,28-trihydroxycalix[4]arene 2. was prepared by the reaction of *p*-*tert*-butylcalix[4]arene with 3,5-dinitrobenzoyl chloride in the presence of 1-methylimidazole following the reported procedure²; mp 170 °C dec.

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzo-

xyloxy)-27-benzoyloxy-26,28-dihydroxycalix[4]arene 3a. To a solution of 0.3 g (0.36 mmol) of **2** in 30 mL of dry THF, 0.047 mL (0.45 mmol) of pyridine and then, 0.068 mL (0.45 mmol) of benzoyl chloride was added slowly. The mixture was stirred room temperature for 1 h. After removed the solvents by evaporation, the residue was triturated with methanol. After filtration, the crude product was recrystallized from $CHCl_3$ -MeOH to give 0.24 g (72%) pale yellow crystalline **3a**, mp 295-298 °C. ¹H NMR ($CDCl_3$) δ 9.60 (d, 2H, ArH of $ArNO_2$, $J=2.1$ Hz), 9.33 (t, 1H, ArH of $ArNO_2$), 8.21 (d, 2H, ArH), 7.61 (t, 1H, ArH), 7.36 (t, 2H, ArH), 7.06 (s, 4H, ArH), 6.90 (s, 2H, ArH), 6.88 (s, 2H, ArH), 4.94 (s, 2H, OH), 3.94, 3.95 and 3.47, 3.52 (two pairs of d, 8H, $ArCH_2Ar$, $J=13.8$ Hz and 14.1 Hz), 1.19 (s, 18H, $-C(CH_3)_3$), 1.00 (s, 9H, $-C(CH_3)_3$), 0.99 (s, 9H, $-C(CH_3)_3$). ¹³C NMR ($CDCl_3$) δ 165.15, 161.36 ($-CO_2-$), 150.10, 149.41, 149.02, 148.96, 142.95, 142.84, 142.81, 133.98, 133.61, 131.60, 131.42, 130.26, 130.19, 128.66, 127.94, 127.82, 126.11, 125.69, 125.64, and 122.79 (Ar), 34.08, 34.03, 33.83, 33.02, 32.72, 31.45, and 30.95 ($ArCH_2Ar$ and $-C(CH_3)_3$). IR (KBr) 3500 cm^{-1} (OH), 1740 cm^{-1} (C=O), 1540 and 1350 cm^{-1} (NO_2).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzoyloxy)-27-(2-bromobenzoyloxy)-26,28-dihydroxycalix[4]arene 3b. Following the procedure described for **3a** with 2-bromobenzoyl chloride, 0.17 g (47%) of **3b** was obtained after recrystallization from $CHCl_3$ -MeOH, mp 302-305 °C. ¹H NMR ($CDCl_3$) δ 9.49 (d, 2H, ArH of $ArNO_2$, $J=2.1$ Hz), 9.26 (t, 1H, ArH of $ArNO_2$), 8.15 (d, 1H, ArH, $J=7.8$ Hz), 7.75 (d, 1H, ArH), 7.36 (t, 1H, ArH), 7.21 (t, 1H, ArH), 7.11 (s, 2H, ArH), 7.04 (s, 2H, ArH), 6.87 (s, 4H, ArH), 4.84 (s, 2H, OH), 4.02, 3.90 and 3.54, 3.45 (two pairs of d, 8H, $ArCH_2Ar$, $J=13.8$ Hz and 14.1 Hz), 1.18 (s, 18H, $-C(CH_3)_3$), 0.99 (s, 9H, $-C(CH_3)_3$), 0.98 (s, 9H, $-C(CH_3)_3$). ¹³C NMR ($CDCl_3$) δ 164.60, 161.32 ($-CO_2-$), 150.04, 149.48, 149.17, 148.90, 143.08, 142.70, 142.68, 134.83, 133.45, 133.32, 131.42, 131.35, 131.06, 130.98, 130.13, 128.02, 127.82, 127.25, 126.18, 126.10, 125.85, 125.56, 122.74, and 122.64 (Ar), 34.09, 34.04, 33.82, 32.93, 32.67, 31.43, and 30.94 ($ArCH_2Ar$ and $-C(CH_3)_3$). IR (KBr) 3550 cm^{-1} (OH), 1745 cm^{-1} (C=O), 1540 and 1330 cm^{-1} (NO_2).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzoyloxy)-27-(4-methoxybenzoyloxy)-26,28-dihydroxycalix[4]arene 3c. Following the procedure described for **3a** with 4-methoxybenzoyl chloride, 0.28 g (80%) of **3c** was obtained after recrystallization from $CHCl_3$ -MeOH, mp 287-290 °C dec. ¹H NMR ($CDCl_3$) δ 9.60 (d, 2H, O_2NArH , $J=2.1$ Hz), 9.33 (t, 1H, O_2NArH), 8.16 and 6.83 (pair of d, 4H, ArH from 4-methoxybenzoyl, $J=9.1$ Hz), 7.05 (s, 4H, ArH), 6.89 (s, 2H, ArH), 6.88 (s, 2H, ArH), 4.95 (s, 2H, OH), 3.94, 3.93, 3.49, and 3.47 (two pairs of d, 8H, $ArCH_2Ar$, $J=14.4$ Hz and 14.1 Hz), 3.90 (s, 3H, $-OCH_3$), 1.18 (s, 18H, $-C(CH_3)_3$), 1.00 (s, 9H, $-C(CH_3)_3$), 0.99 (s, 9H, $-C(CH_3)_3$). ¹³C NMR ($CDCl_3$) δ 164.76, 164.17, 161.40 ($-CO_2-$), 150.13, 149.32, 148.98, 142.90, 142.82, 133.67, 132.44, 131.71, 131.41, 130.23, 127.96, 127.88, 126.11, 126.04, 125.71, 125.63, 122.73, 120.84, and 114.04 (Ar), 55.56 ($-OCH_3$), 34.08, 34.04, 33.83, 33.01, 32.78, 31.46, and 30.97 ($ArCH_2Ar$ and $-C(CH_3)_3$). IR (KBr) 3450 cm^{-1} (OH), 1740 cm^{-1} (C=O), 1540 and 1350 cm^{-1} (NO_2).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzo-

yoxy)-27-acetyloxy-26,28-dihydroxycalix[4]arene

3d. Following the procedure described for **3a** with acetyl chloride, 0.25 g (80%) of **3d** was obtained after recrystallization from CHCl_3 -MeOH. mp 262-264 °C. ^1H NMR (CDCl_3) δ 9.44 (d, 2H, ArH of ArNO_2 , $J=2.1$ Hz), 9.29 (t, 1H, ArH of ArNO_2), 7.07 (s, 2H, ArH), 6.97 (s, 2H, ArH), 6.95 (s, 4H, ArH), 4.87 (s, 2H, OH), 3.85, 3.75 and 3.56, 3.47 (two pair of d, 8H, ArCH_2Ar , $J=13.8$ Hz and 14.4 Hz), 2.41 (s, 3H, $-\text{CH}_3$), 1.17 (s, 18H, $-\text{C}(\text{CH}_3)_2$), 1.04 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 1.03 (s, 9H, $-\text{C}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3) δ 168.58, 161.09 ($-\text{CO}_2-$), 150.32, 149.38, 149.34, 148.95, 143.57, 142.60, 133.54, 131.69, 131.60, 130.24, 127.65, 127.14, 126.39, 126.29, 125.65, 125.53, and 122.79 (Ar), 34.12, 33.83, 33.75, 31.49, 31.02, and 30.98 (ArCH_2Ar and $-\text{C}(\text{CH}_3)_2$), 20.63 ($-\text{CH}_3$). IR (KBr) 3450 cm^{-1} (OH), 1730 cm^{-1} (C=O), 1540 and 1340 cm^{-1} (NO_2).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzo-yloxy)-27-isobutyryloxy-26,28-dihydroxycalix[4]arene 3e.

Following the procedure described for **3a** with isobutyryl chloride, 0.18 g (64%) of **3e** was obtained after recrystallization from CHCl_3 -MeOH. mp 290 °C dec. ^1H NMR (CDCl_3) δ 9.48 (d, 2H, ArH of ArNO_2 , $J=2.1$ Hz), 9.32 (t, 1H, ArH of ArNO_2), 7.09 (s, 2H, ArH), 7.04 (s, 2H, ArH), 6.85 (s, 4H, ArH), 5.00 (s, 2H, OH), 3.89, 3.80 and 3.47, 3.43 (two pairs of d, 8H, ArCH_2Ar , $J=13.8$ Hz and 14.4 Hz), 3.06 (septet, 1H, $-\text{CH}-$), 1.42 (d, 6H, CH_3 , $J=6.9$ Hz), 1.24 (s, 18H, $-\text{C}(\text{CH}_3)_2$), 0.98 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 0.95 (s, 9H, $-\text{C}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3) δ 175.24, 161.39 ($-\text{CO}_2-$), 150.13, 149.20, 149.01, 148.97, 142.91, 142.84, 142.26, 133.42, 131.29, 130.07, 128.01, 127.55, 126.10, 126.03, 125.55, and 122.89 (Ar), 34.01, 33.98, 33.86, 32.86, 32.74, 31.50, and 30.93 (ArCH_2Ar and $-\text{C}(\text{CH}_3)_2$), 30.89, 19.33 ($-\text{CH}(\text{CH}_3)_2$). IR (KBr) 3450 cm^{-1} (OH), 1740 cm^{-1} (C=O), 1540 and 1350 cm^{-1} (NO_2).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzo-yloxy)-27-(3-methoxycarbonylpropionyloxy)-26,28-dihydroxycalix[4]arene 3f. Following the procedure described for **3a** with 3-methoxycarbonylpropionyl chloride, 0.21 g (62%) of **3f** was obtained after recrystallization from CHCl_3 -MeOH. mp 276-279 °C. ^1H NMR (CDCl_3) δ 9.45 (d, 2H, ArH of ArNO_2 , $J=2.1$ Hz), 9.30 (t, 1H, ArH of ArNO_2), 7.13 (s, 2H, ArH), 6.99 (s, 2H, ArH), 6.94 (s, 4H, ArH), 4.89 (s, 2H, OH), 3.87, 3.83 and 3.54, 3.46 (two pairs of d, 8H, ArCH_2Ar , $J=14.1$ Hz and 14.4 Hz), 3.70 (s, 3H, $-\text{CH}_3$), 3.01 (t, 2H, $-\text{CH}_2-$, $J=6.9$ Hz), 2.81 (t, 2H, $-\text{CH}_2-$), 1.18 (s, 18H, $-\text{C}(\text{CH}_3)_2$), 1.03 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 1.01 (s, 9H, $-\text{C}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3) δ 172.31, 170.68, 161.13 ($-\text{CO}_2-$), 150.26, 149.36, 149.21, 148.90, 143.39, 142.58, 133.46, 131.63, 131.61, 130.24, 127.65, 127.26, 126.29, 126.26, 125.60, 125.51, and 122.82 (Ar), 52.02 ($-\text{OCH}_3$), 34.86, 33.82, 33.66, 33.45, 30.98, 31.46, and 30.95 (ArCH_2Ar and $-\text{C}(\text{CH}_3)_2$), 28.67, 28.59 ($-\text{CH}_2\text{CH}_2-$). IR (KBr) 3450 cm^{-1} (OH), 1730 and 1740 cm^{-1} (C=O), 1540 and 1340 cm^{-1} (NO_2).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzo-yloxy)-27-(4-methoxycarbonylbutanoyloxy)-26,28-dihydroxycalix[4]arene 3g. Following the procedure described for **3a** with 4-methoxycarbonylbutanoyl chloride, 0.33 g (93%) of **3g** was obtained after recrystallization from CHCl_3 -MeOH. mp 269-273 °C dec. ^1H NMR (CDCl_3) δ 9.47 (d, 2H, O_2NArH , $J=2.1$ Hz), 9.30 (t, 1H, O_2NArH),

7.07 (s, 2H, ArH), 6.99 (s, 2H, ArH), 6.91 (s, 4H, ArH), 4.89 (s, 2H, OH), 3.85, 3.76, 3.53, and 3.45 (two pairs of d, 8H, ArCH_2Ar , $J=14.4$ Hz and 14.1 Hz), 3.68 (s, 3H, $-\text{CH}_3$), 2.80 (t, 2H, $-\text{CH}_2-$, $J=7.5$ Hz), 2.49 (t, 2H, $-\text{CH}_2-$, $J=7.5$ Hz), 2.14 (quintet, 2H, $-\text{CH}_2-$, $J=7.5$ Hz), 1.19 (s, 18H, $-\text{C}(\text{CH}_3)_2$), 1.01 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 1.00 (s, 9H, $-\text{C}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3) δ 173.18, 170.99, 161.17 ($-\text{CO}_2-$), 150.22, 149.31, 149.26, 148.98, 143.26, 142.71, 142.50, 133.44, 131.49, 130.25, 127.74, 127.36, 126.26, 126.20, 125.59, 125.52, and 122.83 (Ar), 51.65 ($-\text{OCH}_3$), 34.09, 33.84, 33.44, 32.70, 32.44, 31.50, and 30.98 (ArCH_2Ar and $-\text{C}(\text{CH}_3)_2$), 30.96 and 19.88 ($-\text{CH}_2\text{CH}_2\text{CH}_2-$). IR (KBr) 3470 cm^{-1} (OH), 1745 and 1740 cm^{-1} (C=O).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzo-yloxy)-26-allyloxy-27,28-dihydroxy-calix[4]arene

4a. To a solution of 0.3 g (0.36 mmol) of **2**, 0.15 g (1.08 mmol) of K_2CO_3 and 0.62 mL (7.20 mmol) of allyl bromide in 60 mL of THF was added and then refluxed for 5 hrs. The solvents were evaporated and the residue was dissolved with 30 mL of CHCl_3 and washed with 0.1 N HCl. The organic layer was separated, dried over anhydrous Na_2SO_4 , evaporated the solvents, and the residue triturated with methanol. Recrystallization of crude products from CHCl_3 -MeOH to give 0.15 g (49%) of fine yellow flaky crystal **4a**. mp 243-246 °C dec. ^1H NMR (CDCl_3) δ 9.88 (s, 1H, OH), 9.05 and 9.02 (two t, 1H, ArNO_2), 8.95 (d, 2H, ArNO_2 , $J=2.1$ Hz), 7.40 (d, 1H, ArH, $J=2.4$ Hz), 7.37 (d, 1H, ArH), 7.23 (d, 1H, ArH), 7.02 (d, 1H, ArH), 6.91 (d, 1H, ArH), 6.75 (d, 1H, ArH), 6.47 (s, 2H, ArH), 6.49 (s, 1H, OH), 5.12 (m, 1H, $-\text{CH}=\text{from allyl}$), 4.84-4.68 (q, 2H, $-\text{CH}_2=\text{from allyl}$), 3.93 and 3.74 (two pairs of d, 2H, $-\text{CH}_2-\text{from allyl}$, $J=11.7$ Hz), 4.50, 4.23, 3.86, 3.69, 3.48, 3.44, 3.41, and 3.31 (four pairs of d, 8H, ArCH_2Ar , $J=13.5$ Hz and 14.1 Hz), 1.43 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 1.35 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 1.02 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 0.69 (s, 9H, $-\text{C}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3) δ 163.14 ($-\text{CO}_2-$), 161.76, 150.35, 149.74, 149.15, 148.84, 148.27, 147.95, 147.82, 144.04, 142.67, 142.09, 134.88, 134.21, 133.95, 133.90, 133.78, 131.94, 131.43, 131.09, 130.94, 129.12, 128.97, 128.85, 126.75, 126.68, 126.42, 126.08, 126.02, 125.72, 125.65, 125.13, 124.84, 122.42, 122.38, and 119.34 (Ar), 78.18 ($-\text{CH}_2-$ from allyl), 34.53, 34.38, 33.95, 33.79, 32.32, and 31.38 ($-\text{C}(\text{CH}_3)_2$), 31.73, 31.60, 30.99, and 30.91 (ArCH_2Ar). IR (KBr) 3450 cm^{-1} (OH), 1735 cm^{-1} (C=O), 1625 cm^{-1} (C=C), 1540 and 1340 cm^{-1} (NO_2).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzo-yloxy)-27-(ethoxycarbonylmethoxy)-26,28-dihydroxycalix[4]arene 4b.

A mixture of 0.30 g (0.36 mmol) of **2**, 0.15 g (1.08 mmol) of K_2CO_3 and 0.48 mL (4.68 mmol) of ethyl bromoacetate in 60 mL of THF was refluxed for 7 hrs and worked up as described for **4a**. Column chromatography (eluent : CHCl_3) gave a 0.16 g (47%) of fine pale yellow crystal **4b**. mp 213-217 °C. ^1H NMR (CDCl_3) δ 9.74 (d, 2H, O_2NArH , $J=2.1$ Hz), 9.27 (t, 1H, O_2NArH), 7.10 (s, 2H, ArH), 7.04 (s, 2H, ArH), 6.94 (s, 2H, OH), 6.88 (s, 2H, ArH), 6.84 (s, 2H, ArH), 4.78 (s, 2H, $-\text{CH}_2\text{CO}_2-$), 4.20 (quartet, 2H, $-\text{CH}_2-$ of ethyl), 4.06, 3.47 and 3.30 (two pairs of d, 8H, ArCH_2Ar , $J=13.9$ Hz and 14.1 Hz), 1.30 (t, 3H, $-\text{CH}_3$ of ethyl), 1.27 (s, 18H, $-\text{C}(\text{CH}_3)_2$), 1.03 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 0.93 (s, 9H, $-\text{C}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3) δ 168.78, 161.92 ($-\text{CO}_2-$), 150.18, 149.06, 148.84,

148.78, 147.84, 142.84, 142.10, 134.39, 133.89, 132.18, 131.50, 130.88, 130.74, 128.59, 126.28, 125.61, 125.56, 125.32, 124.67, and 122.36 (Ar), 72.49 (-CH₂-), 61.87 (-CH₂-CH₃), 34.11, 33.93, 33.82, 32.17, 31.98, 31.61, 31.40, 31.23, 31.08, 30.89, and 30.87 (ArCH₂Ar and -C(CH₃)₃), 14.03 (-CH₃). IR (KBr) 3450 cm⁻¹(OH), 1735 and 1740 cm⁻¹(C=O), 1550 and 1350 cm⁻¹(NO₂).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzyloxy)-27-benzyloxy-26,28-dihydroxycalix[4]arene 4c.

A mixture of 0.3 g (0.36 mmol) of **2**, 0.15 g (1.08 mmol) of K₂CO₃ and 0.85 mL (7.20 mmol) of benzyl bromide in 60 mL THF was refluxed for 2 hrs and worked up as described for **4a** to give 0.20 g (60%) of fine yellow needles **4c**. mp 231-233 °C. ¹H NMR (CDCl₃) δ 9.68 (d, 2H, O₂NArH, *J*=2.1 Hz), 9.34 (t, 1H, O₂NArH), 7.50-7.20 (m, 5H, ArH from benzyl), 7.08 (d, 2H, ArH), 7.00 (d, 2H, ArH), 6.88 (s, 2H, ArH), 6.74 (s, 2H, ArH), 6.46 (s, 2H, OH), 5.42 (s, 2H, -CH₂- from benzyl), 3.98, 3.78, 3.28, and 3.26 (two pairs of d, 8H, ArCH₂Ar), 1.26 (s, 18H, -C(CH₃)₃), 1.01 (s, 9H, -C(CH₃)₃), 0.87 (s, 9H, -C(CH₃)₃). ¹³C NMR (CDCl₃) δ 161.83 (-CO₂-), 150.14, 148.86, 148.54, 147.87, 142.99, 142.21, 134.88, 134.29, 132.32, 131.28, 130.58, 129.69, 129.13, 128.77, 126.28, 126.22, 125.49, 124.71, and 122.53 (Ar), 79.95 (-CH₂- from benzyl), 34.05, 33.87, 33.83, 32.10, 31.60, 30.92, and 30.83 (ArCH₂Ar and *t*-butyl). IR (KBr) 3500 cm⁻¹(OH), 1740 cm⁻¹(C=O), 1540 and 1350 cm⁻¹(NO₂).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzyloxy)-26,28-bisbenzyloxy-27-hydroxycalix[4]arene 4d.

Procedure A: A mixture of 0.3 g (0.36 mmol) of **2**, 0.15 g (1.08 mmol) of K₂CO₃ and 0.85 mL (7.20 mmol) of benzyl bromide in 50 mL of THF was refluxed for 12 hrs and worked up as described for **4a** to give 0.18 g (54%) of light yellow crystalline **4d**. Procedure B: A mixture of 0.5 g (0.54 mmol) of **4c**, 0.22 g of K₂CO₃ and 1.28 mL of benzyl bromide in 50 mL of THF was refluxed for 28 hrs and worked up as described for **4a** to give 0.40 g (73%) of **4d**. mp 310 °C dec. ¹H NMR (CDCl₃) δ 9.36 (d, 2H, ArH of ArNO₂, *J*=2.1 Hz), 8.43 (t, 1H, ArH of ArNO₂), 7.35 (s, 2H, ArH), 7.16 (s, 2H, ArH), 6.56 (s, 1H, OH), 6.97-6.87 (m, 5H, ArH), 4.50 and 4.36 (pair of d, 4H, -CH₂-, *J*=9.6 Hz and 10.5 Hz), 4.53, 4.13, 3.39, and 3.38 (two pairs of d, 8H, ArCH₂Ar, *J*=12.9 Hz and 14.4 Hz), 1.42 (s, 9H, -C(CH₃)₃), 1.38 (s, 9H, -C(CH₃)₃), 0.88 (s, 18H, -C(CH₃)₃). ¹³C NMR (CDCl₃) δ 164.00 (-CO₂-), 150.97, 150.20, 149.18, 147.38, 146.57, 145.32, 144.22, 141.31, 135.70, 134.83, 132.83, 132.18, 131.54, 131.29, 130.63, 129.18, 128.08, 128.03, 127.45, 125.89, 125.79, 125.29, 125.11, and 122.90 (Ar), 78.76 (-CH₂- from benzyl), 31.81, 31.61 (ArCH₂Ar), 34.43, 33.93, 33.85, 31.41, 30.99, and 30.61 (-C(CH₃)₃). IR (KBr) 3450 cm⁻¹(OH), 1740 cm⁻¹(C=O), 1550 and 1340 cm⁻¹(NO₂).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzyloxy)-26-(*p*-bromobenzenesulfonyloxy)-27,28-dihydroxycalix[4]arene 4e.

A mixture of 0.30 g (0.36 mmol) of **2**, 0.15 g (1.08 mmol) of K₂CO₃ and 0.93 g (3.60 mmol) of *p*-bromobenzenesulfonyl chloride in 60 mL of THF was refluxed for 6 hrs and worked up as described for **4a**. Separation by column chromatography (eluent: CHCl₃) gave 0.08 g (21%) of light yellow needles **4e** and 0.10 g (27%) of pale yellow crystalline **4f**. Compound **4e**: mp 274-

276 °C. ¹H NMR (CDCl₃) δ 9.78 (s, 1H, OH), 9.13 and 9.04 (two t, 1H, ArNO₂), 8.87 (d, 2H, ArNO₂, *J*=2.1 Hz), 7.61 and 7.22 (pair of d, 4H, ArH from brosyl, *J*=8.7 Hz), 7.40 (d, 1H, ArH, *J*=2.1 Hz), 7.32 (d, 1H, ArH, *J*=2.4 Hz), 7.16 (d, 1H, ArH, *J*=2.1 Hz), 7.00 (d, 1H, ArH, *J*=2.4 Hz), 6.80 (d, 1H, ArH, *J*=2.1 Hz), 6.71 (d, 1H, ArH, *J*=2.4 Hz), 6.62 (d, 1H, ArH, *J*=2.1 Hz), 6.60 (d, 1H, ArH, *J*=2.4 Hz), 4.11 (s, 1H, -OH), 4.21, 3.92, 3.71, 3.70, 3.55, 3.40, 3.14, and 2.67 (four pairs of d, 8H, ArCH₂Ar, *J*=14.1 Hz, 13.5 Hz, 14.4 Hz, and 14.7 Hz), 1.42 (s, 9H, -C(CH₃)₃), 1.33 (s, 9H, -C(CH₃)₃), 0.93 (s, 9H, -C(CH₃)₃), 0.85 (s, 9H, -C(CH₃)₃). ¹³C NMR (CDCl₃) δ 162.99, and 161.76 (-CO₂-), 150.10, 150.06, 149.98, 149.08, 148.93, 148.39, 143.98, 143.81, 142.00, 141.08, 134.58, 134.00, 133.28, 133.03, 132.81, 132.65, 132.12, 130.99, 130.54, 130.23, 130.07, 129.41, 128.88, 128.44, 126.79, 126.64, 126.17, 126.00, 125.55, 125.49, 122.83, and 122.47 (Ar), 34.56, 34.21, 34.10, 34.01, 31.82, 31.74, 31.38, 31.66, 31.57, 30.92, and 30.86 (ArCH₂Ar and -C(CH₃)₃). IR (KBr) 3500 cm⁻¹(OH), 1735 cm⁻¹(C=O), 1550 and 1345 cm⁻¹(NO₂), 1355 and 1140 cm⁻¹(O=S=O).

5,11,17,23-Tetra-tert-butyl-25-(3,5-dinitrobenzyloxy)-27-(*p*-bromobenzenesulfonyloxy)-26,28-dihydroxycalix[4]arene 4f.

mp 217-220 °C. ¹H NMR (CDCl₃) δ 9.50 (d, 2H, ArNO₂, *J*=2.1 Hz), 9.32 (t, 1H, ArNO₂), 7.93 and 7.74 (pair of d, 4H, ArH from brosyl, *J*=8.7 Hz), 7.11, 6.70, 6.90, and 6.78 (four s, 8H, ArH), 4.64 (s, 2H, OH), 3.86, 3.82, 3.42, and 3.16 (two pairs of d, 8H, ArCH₂Ar, *J*=13.5 Hz and 14.1 Hz), 1.22 (s, 18H, -C(CH₃)₃), 1.00 (s, 9H, -C(CH₃)₃), 0.91 (s, 9H, -C(CH₃)₃). ¹³C NMR (CDCl₃) δ 161.02 (-CO₂-), 149.88, 149.81, 149.65, 149.03, 142.98, 142.52, 142.46, 135.03, 133.10, 133.06, 132.88, 131.34, 130.37, 130.32, 129.87, 127.71, 127.23, 126.95, 126.00, 125.44, and 122.96 (Ar), 34.11, 34.04, 33.87, 32.98, 32.69, 31.47, 30.79, 30.91, 29.68 (ArCH₂Ar and -C(CH₃)₃). IR (KBr) 3500 cm⁻¹(OH), 1735 cm⁻¹(C=O), 1545 and 1360 cm⁻¹(NO₂), 1355 and 1140 cm⁻¹(O=S=O).

5,11,17,23-Tetra-tert-butyl-25,27-bisbenzyloxy-26,28-dihydroxycalix[4]arene 5.

A mixture of 0.50 g (0.49 mmol) of **4d**, and 1.5 g of NaOH in 100 mL THF, 30 mL EtOH and 60 mL of H₂O was refluxed for 1 hr. Acidified and extracted with CHCl₃, evaporated the solvents, and the crude product was recrystallized from methanol to yielded 0.23 g (57%) of white crystal **5**. mp 195-198 °C. ¹H NMR (CDCl₃) δ 7.63 (m, 4H, ArH of benzyl), 7.36 (m, 6H, ArH of benzyl), 7.28 (s, 2H, OH), 7.04 (s, 4H, ArH), 6.78 (s, 4H, ArH), 5.05 (s, 4H, -CH₂- of benzyl), 4.26 and 3.24 (pair of d, 8H, ArCH₂Ar, *J*=12.9 Hz), 1.28 (s, 18H, -C(CH₃)₃), 0.94 (s, 18H, -C(CH₃)₃). ¹³C NMR (CDCl₃) δ 150.73, 149.75, 146.93, 141.26, 137.16, 132.57, 128.59, 127.76, 127.63, 127.32, 125.47, and 124.95 (Ar), 77.92 (-CH₂Ar), 33.91, 33.79, 31.69, 30.99, and 29.70 (-C(CH₃)₃ and ArCH₂Ar). IR (KBr) 3500 cm⁻¹(OH).

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Motional Properties in the Structure of GlcNAc(β 1,3)Gal(β)OMe Studied by NMR Spectroscopy and Molecular Modeling

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Conformational flexibilities of the GlcNAc(β 1,3)Gal(β)OMe are investigated through NMR spectroscopy and molecular modeling. Adiabatic energy map generated with a dielectric constant of 50 contains three local minima. All of the molecular dynamics simulations on three local minimum energy structures show fluctuations between two low energy structures, N2 at $\phi=80^\circ$ and $\psi=60^\circ$ and N3 at $\phi=60^\circ$ and $\psi=-40^\circ$. We have presented adequate evidences to state that GlcNAc(β 1,3)Gal(β)OMe exists in two conformationally discrete forms. Two state model of N2 and N3 conformers with a population ratio of 40 : 60 is used to calculate the effective cross relaxation rate and reproduces the experimental nOes very well. Molecular dynamics simulation in conjunction with two state model proves successfully the dynamic equilibrium existed in GlcNAc(β 1,3)Gal(β)OMe and can be considered as a powerful method to analyze the motional properties in the structure of carbohydrate. This observation also cautions against the indiscriminate use of a rigid model to analyze NMR data.

Introduction

Cell membranes must interact with their external environment as a part of important biological processes such as cell recognition, intercellular adhesion, and regulation of growth and defence against invading organisms. The interactions are usually achieved by binding of a protein to a carbohydrate receptor anchored to the cell membrane as a part of glycoprotein or glycolipid. These carbohydrates have the biological functions which control the several intracellular reactions as recognition signals.^{1,2} Accordingly, knowledge of structural details of such protein-carbohydrate interactions is fundamental to understand the interaction of cell and its environment.^{3,4}

N-acetylglucosamine residue occurs in glycoproteins, glycolipids, and proteoglycans with a variety of structures. One of the linkages commonly found in all three classes of glycoconjugates is GlcNAc(β 1,3)Gal. Repeating units of this disaccharide are present in certain mucins, membrane glycoproteins, and polyglycosylceramides where they are associated with the li-antigenic structures and serve as precursors of the ABH, Lewis, and P, blood group antigens.^{5,7} Specially, lectins such as Wheat Germ Agglutinin (WGA) and Limulus polyphemus agglutinin (LPA) recognize the carbohydrates which contain GlcNAc as a terminal sugar

residue in the membrane. Study of interactions between the WGA and various carbohydrate containing GlcNAc is our on going project.

According to the our previous NMR study it is found that free lactose and free melibiose exist with a variety of conformational flexibility and there are considerable conformational changes of melibiose induced by binding to ricin.^{8,9} It shows tendency of protein binding to restrict conformational freedom about glycosidic bond. In order to understand the structural details of such protein-carbohydrate interactions it is necessary to study the structural changes of oligosaccharide as a recognition signal. Here, structure of β-D-GlcNAc-[1-3]-β-D-Gal-1-OMe (GlcNAc(β 1,3)Gal(β)OMe) which can be a receptor for proteins having GlcNAc specificity is studied using nuclear magnetic resonance spectroscopy, adiabatic energy map, and molecular dynamics simulation.¹⁰⁻¹²

NMR is the best method to provide structural data in solution where motional variations are less restricted than in crystals. The nuclear Overhauser effects used to evaluate interproton distance constraints are also susceptible to large variations because of the unusual way that motional averaging can affect the measured parameters. It is important to consider the possibility that the assumption of a static structure, implicit in most structure determination protocols,