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An NMR approach for the determination of the substitution pattern in mono-modified cyclodextrins

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Abstract

One of the problems in cyclodextrin chemistry is the unequivocal determination of structures of systems under investigation. A simple procedure that can be used for the determination of the position of the substitution in these systems is presented here. The main requirement of this procedure is that the proton attached to the substituted carbon has a magnetic environment that is different from other likely sites of attachment.

Keywords: cyclodextrins; modified-cyclodextrins; NMR; selective long-range INEPT; HSQC.

Cyclodextrins have arguably led the way in the investigation of systems in supramolecular[1] and molecular recognition[2] chemistry.[3] In recent years, modified cyclodextrins have increasingly allowed one to probe this area to a depth which has not been possible with other systems.[4] However, a major obstacle in this endeavor has been the unequivocal determination of structures in these new systems without which all subsequent investigations become suspect. Several elegant NMR techniques have been introduced and have become popular in this quest.[5] Among these, ROESY and its variants[6] have been particularly useful because of the awkward size of these molecules.

The heart of the problem is that there are multiple modifiable sites[7] in these molecules, and it is difficult to determine the regioisomer that is formed in a reaction. For example, if modification of a single hydroxyl group (mono-modification) is carried out on β-cyclodextrin, it is important and difficult to assign the position (2-, 3- or 6-) at which the substitution has taken place. We have synthesized[8] three regioisomers of cyclodextrins (**1**, **2** and **3**) in which the same group is attached to each of these positions. This provides us with an opportunity to develop an NMR method to distinguish between regioisomers of cyclodextrin without the ambiguity of variations in the chemical shift of the signals due to change in the substituent. We now report a simple procedure which can be used for the determination of the position of a substitution in mono-substituted cyclodextrins.

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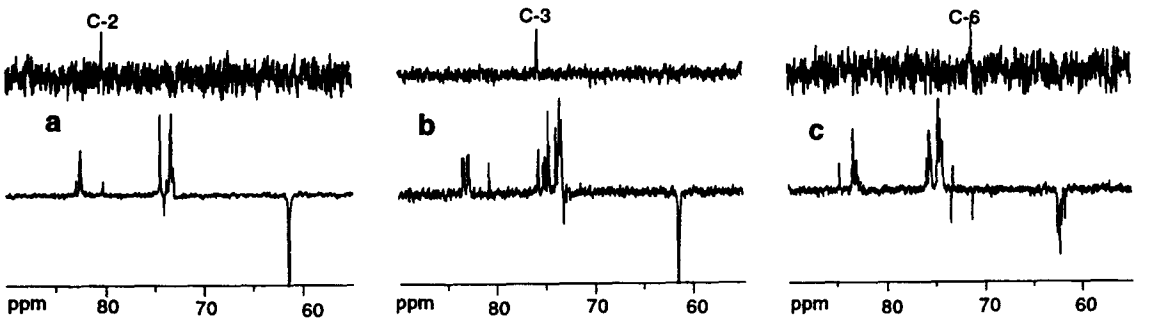
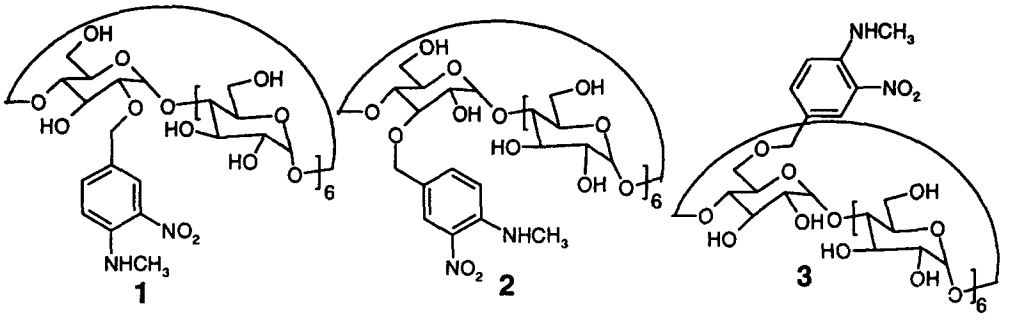


Figure 1. ^{13}C NMR spectra of three regioisomers. a: **1**; b: **2**; c: **3**. The bottom row shows the expanded regions of the DEPT experiment on the skeletal signals, the top row shows the results of the selective long range INEPT experiment. The ppm scale is the same for both top and bottom spectra



We recently reported a method[9] to identify regioisomers of β-cyclodextrin with a set of selective long-range INEPT[10] and HMBC[11] experiments. However, the low sensitivity of these long-range experiments and the difficulty[12] in the analysis of the HMBC spectrum limits the usefulness of this procedure. The technique presented here utilizes an HSQC experiment which is more sensitive and, as can be seen later, is very simple to analyze.[13]

Starting from an absence of any information on the regiochemistry of **1**, **2** and **3**, a selective INEPT experiment[14] is carried out on these compounds. The INEPT spectra (Fig. 1, top) indicate that the substituted carbon has a chemical shift of 80.6 ppm, 75.8 ppm and 71.3 ppm for compounds **1**, **2** and **3**, respectively. The chemical shift differences of these signals from those of the native cyclodextrin are given in Table 1. In the past,[8,15] these differences have been used as supporting evidence for the position of the substitution in modified cyclodextrins. An analysis of Table 1 exposes the danger in making such inferences from these data. For example, the chemical shift of the substituted carbon in **1** is +7.3 ppm and +6.3 ppm downfield from C-2 and C-3 of native cyclodextrin, respectively. Thus, it is not possible to unambiguously determine whether the substitution in **1** is at the 2- or the 3-position from this data. Similar arguments can be made about the regiochemistry of **2**.[16] Another problem in using chemical shift differences to establish the substitution pattern is that these values are dependent on the nature of the substituent. Furthermore, very few cyclodextrins mono-modified at each of these position are available thus far to establish a trend. At this point, if an (hypothetical) unknown cyclodextrin derivative is observed to have a signal for the substituted carbon at 78 ppm ($\Delta 2$=+4.7, $\Delta 3$=+3.7), it would not be possible to assign its regiochemistry with any confidence.

Table 1 also indicates that it is relatively more reliable to assign the structure of **3** from this data. The chemical shift difference for this compound is consistent with substitution at the 6-position since this signal is downfield from that of C-6 of the native cyclodextrin ($\Delta 6$=+11.9). The identity of **3** can be

Table 1
The chemical shift differences of the substituted carbon relative to those of native cyclodextrin

Cpd	SC	$\Delta 2$	$\Delta 3$	$\Delta 6$
1	80.6	+7.3	+6.3	+19.1
2	75.8	+2.5	+1.5	+14.3
3	71.3	-2.0	-3.0	+11.9

All values are in ppm; Cpd = Compound; SC = Chemical shift of the Substituted Carbon; $\Delta 2$ = SC-CS2; $\Delta 3$=SC-CS3; $\Delta 6$=CS-CS6 where CS2, CS3 and CS6 are chemical shifts of C-2, C-3 and C-6 of the native cyclodextrin, respectively. + = downfield; - = upfield.

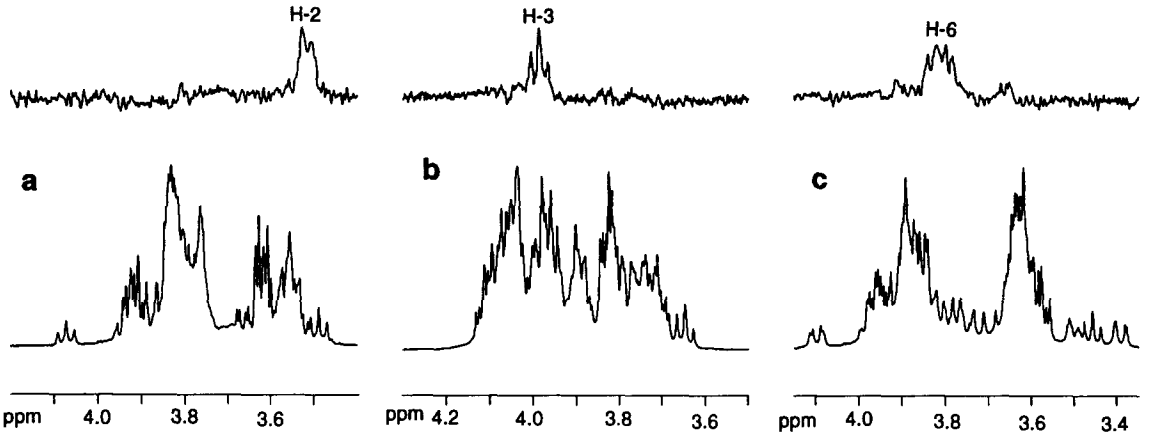


Figure 2. ^{1}H NMR spectra of the three regioisomers. **a**: **1**; **b**: **2**; **c**: **3**. The bottom row shows the skeletal regions of the ^{1}H NMR spectra. The top row shows three projections extracted (at the chemical shift of the substituted carbon at ppms 80.6 for **1**, 75.8 for **2** and 71.3 for **3** as shown in Fig. 1) from the HSQC experiment. The ppm scale is the same for both top and bottom spectra

assigned with confidence from a DEPT spectrum (Fig. 1, bottom spectra). This set of spectra provides low intensity signals for the substituted sugar unit. Among these, the signal for the substituted carbon in compounds **1** (80.6 ppm) and **2** (75.8 ppm) are in-phase, whereas, that for **3** (71.3 ppm) is anti-phase relative to methyne (-CH-) signals, indicating that the substituent in **3** is attached to a methylene group. Thus, **3** can be assigned to the regioisomer in which the substituent is attached to the 6-position. However, the substitution at the 2- and 3-positions cannot be unambiguously distinguished from these DEPT or INEPT spectra.

Conclusive evidence for the substitution pattern in **1** and **2** can be obtained from the multiplicity of the proton signals extracted as one-dimensional projections from two-dimensional HSQC experiments. Each fine structure of the proton signals (hydrogens attached to the substituted carbon in **1**, **2** and **3**, respectively) extracted as a one dimensional projection of HSQC experiment is distinct (Fig. 2, top spectra).[17]

The HSQC projection for **1** gives a doublet-like proton signal at 3.52 ppm (**a** in Fig. 2). This compound can now be assigned to the one in which the substituent is at the 2-position for the following reasons. In **1**, the H-2 is in the axial position of a $^4C_1$ conformation glucose ring of β-cyclodextrin. This proton has two coupling partners, the axial H-3 and the equatorial H-1.[18] The axial–axial homonuclear coupling

constant (between H-2 and H-3) is large (around 7.5 Hz). However, the axial–equatorial coupling constant (between H-2 and H-1) has a smaller magnitude (around 3.5 Hz). These two couplings result in a double doublet structure on the detected proton signal. Since the signal is extracted from a two-dimensional heteronuclear spectrum, the low resolution washes away the smaller coupling, resulting in a single doublet-like structure.

The HSQC projection for **2** gives a triplet proton signal at 3.99 ppm (**b** in Fig. 2). This compound can now be assigned as the one in which the substituent is at the 3-position for the following reasons. In **2**, the axial H-3 proton also has two coupling partners, the axial H-4 and the axial H-2. These two protons contribute equally (two large axial–axial couplings) to the resultant multiplet structure. The overall appearance of the detected signal, therefore, is a triplet.

In the third case, where the substituent is at the 6-position (**3**), the HSQC projection for **3** is a quartet at 3.79 ppm (**c** in Fig. 2). This compound can now be assigned as the one in which the substituent is at the 6-position for the following reasons. This signal accommodates two proton signals of the methylene group. There is a large geminal coupling (around 9 Hz) between these signals. The coupling constant to H-5 is very small on both H-6 signals, and cannot be measured from the proton spectrum. The overall appearance of the detected signal, therefore, is a quartet.

Thus, it can be seen that if the proton signal is a doublet, triplet, or a quartet, then it can be concluded that the substitution is at the 2-, 3-, or the 6-position, respectively.

Although the INEPT part of this procedure requires at least one proton on the carbon adjacent to the substituted oxygen to unambiguously assign the chemical shift of the substituted carbon, in cases where such protons are not available, (e.g., sulfonate and carboxylate esters) one would need to rely on the comparison of the spectra of the modified and native cyclodextrins to determine the chemical shift of the substituted carbon. Once this information is derived, then the HSQC projection can be used to determine the position of the substitution from the multiplicity of the proton attached to that carbon.

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12. HMBC signals have dispersive components which hinder the extraction of projections from this spectrum. HMBC row at the substituted carbon has many correlations to protons in the same glucose unit which makes the assignment difficult.
13. The HSQC spectrum provides absorptive signals which facilitate extraction of a projection at the substituted carbon which has only one signal in the row.
14. The benzyl methylene signals were excited selectively at 4.70 ppm in **1**, at 4.96 ppm in **2**, and at 4.41 ppm in **3**.
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16. Accurate assignment of regiochemistry have been made in the past (Refs. 8 and 13) based on these chemical shift differences in conjunction with information available from the synthetic methodology.
17. The sevenfold symmetry of the β-cyclodextrin is broken by the selective substitution in all three cases resulting in a crowded approximately 0.7 ppm wide chemical shift region around 3.8 ppm (bottom row in Fig. 2).
18. The anomeric proton H-1 is equatorial because of the α-conformation of the sugar ring.