Unambiguous Identification of Regioisomers in Selectively Modified β-Cyclodextrins

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Introduction

Cyclodextrins have gained prominence in the last few decades in fields ranging from industrial applications to enzyme mechanisms because of their ability to complex small molecules.[1] However, limitations of structure and available functional groups have inhibited this activity and this has spurred intense investigative efforts in methods for modification of cyclodextrins.[2] Among these, monomodification, where a single hydroxyl group is converted to a desired functionality, has been found to be particularly attractive. Several well-defined protocols for cyclodextrin monosubstitution are now available. However, one of the major problems with these methods is that the hydroxyl group at the 2-, 3-, or 6-position can undergo the chemical transformation. Therefore, it is essential to identify the exact position at which this change has taken place. Although X-ray crystallography is the most definitive method for structure determination, it is not always possible to grow crystals of modified cyclodextrins that are suitable for such endeavors and, thus, very few modified-cyclodextrin crystal structures are published.[3−6] NMR spectroscopy is the method of choice for identification of regioisomers because it can provide experiments for unambiguous assignment of structures.

Selectively substituted cyclodextrins have very complicated NMR spectra since the high symmetry of the parent cyclodextrin is broken by the substituent. The spectral dispersion is very low in both ^{1}H and ^{13}C spectra as a result of the repeating monomer (α-glucose) unit. One-dimensional NMR spectra give little information for unambiguous identification of these isomers. Although advanced two-dimensional[7−10] as well as selective experiments[11,12] are widely used in the solution structure determination, they have not been used for identification of regioisomers in substituted cyclodextrins. The reason for this is that the high spectral overlap makes this identification tedious and unattractive.

We now present a method for identification of modified β-cyclodextrin regioisomers and demonstrate its utility using three regioisomers (**1**−**3**, Chart 1)[13−15] in which the same group (4-methylamino-3-nitrobenzyl) is attached to the 2-, 3-, or 6-position. Although, the 4-methylamino-3-nitro derivative[16] is used for this study, this procedure is applicable to modified cyclodextrins in which the substituent is attached through a −CH− or −CH$_2$− group. The applied procedure uses two sets of experiments, a selective long-range INEPT experiment[17] followed by a two-dimensional heteronuclear multiple bond correlation (HMBC) experiment.[18]

Results and Discussions

NMR Spectra of Cyclodextrin Regioisomers. The NMR spectra of the three regioisomers appear to be similar (Figure 1 and the bottom row in Figure 2). In the ^{1}H NMR spectra, only the three aromatic signals (2′, 5′, and 6′) are well resolved at the low-field region in all three regioisomers (Figure 1). The seven anomeric doublets are in a very narrow chemical shift region around 5.1 ppm. The benzyl methylene protons have a quartet structure around 4.70 ppm in **1**, two doublets at 5.25 and 4.96 ppm in **2**, and two doublets at 4.65 and 4.41 ppm in **3**. The rest of the skeletal protons (approximately 80%) are in a 0.7 ppm wide, crowded area around 3.8 ppm. One-dimensional ^{1}H NMR spectra do not give information about the substitution pattern. Even though the spectral dispersion of the carbon spectra is higher, only the aromatic signals are well resolved (bottom row in Figure 2). Seven anomeric carbon signals are around 105 ppm in all three cases. The C-4 signals in the seven α-glucose units are around 85 ppm, and this region of the cyclodextrin moiety is the most resolved. C-2, C-3, and C-5 in the glucose units are in a crowded area around 75 ppm.

The substitution at different positions usually increases the chemical shift of the substituted carbon signal. However, the chemical shift change is not the same for all three regioisomers. The substitution with the 4-methylamino-3-nitrobenzyl group at C-2 (in **1**), C-3 (in **2**), and C-6 (in **3**) increases the chemical shift by 7.3, 1.5, and 9.8 ppm, respectively, relative to the one in the native β-cyclodextrin. There is no empirical rule to identify different regioisomers on the basis of the chemical shift change, and furthermore these changes may vary with different substituents. The only way to distinguish these regioisomers is to find heteronuclear long-range correlations between the nuclei in the substituent

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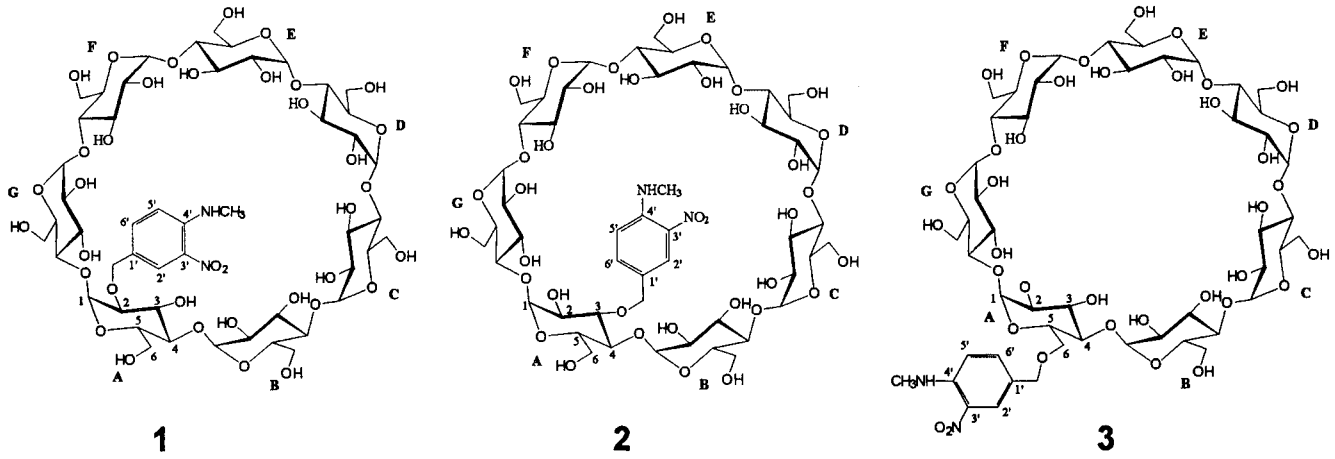
Chart 1



1 **2** **3**

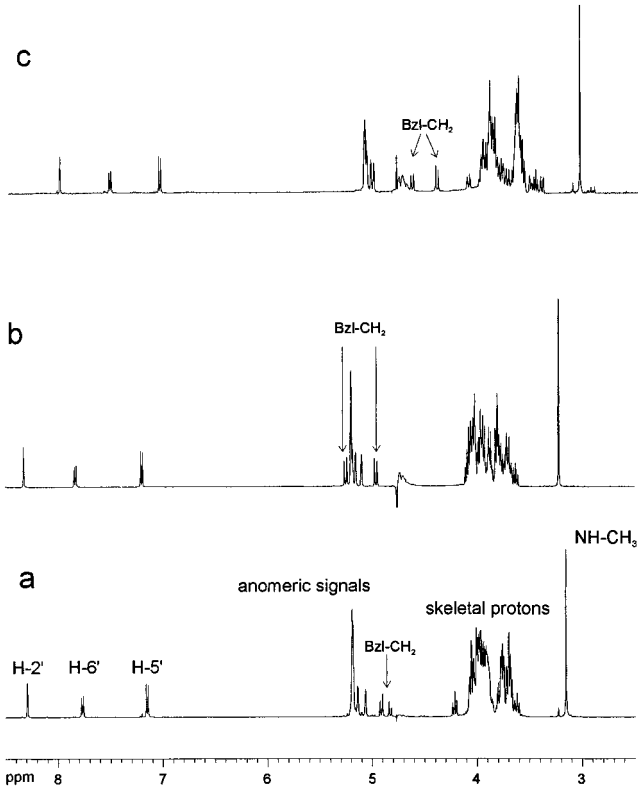


Figure 1. One-dimensional ^{1}H NMR spectra of three regioisomers: (a) 2^A-(4-methylamino-3-nitrobenzyl)-β-cyclodextrin (**1**); (b) 3^A-(4-methylamino-3-nitrobenzyl)-β-cyclodextrin (**2**); (c) 6^A-(4-methylamino-3-nitrobenzyl)-β-cyclodextrin (**3**).

and in the cyclodextrin. Long-range correlation experiments, however, are very insensitive, and the poor solubility of cyclodextrin derivatives makes this investigation very difficult to carry out.

Identification of Cyclodextrin Regioisomers. One possibility for identification of a regioisomer is to look for a long-range correlation between the *carbon* of the methylene group in the 4-methylamino-3-nitrobenzyl moiety and the corresponding *proton* of the substituted site in β-cyclodextrin. Since the cyclodextrin skeletal protons are in a very narrow and crowded region, the observation of this long-range coupling is very difficult.

The other possibility is to find correlations between the methylene *protons* in the 4-methylamino-3-nitrobenzyl group and the corresponding substituted *carbon* in the α-glucose ring. Since the benzyl methylene signals in the ^{1}H NMR spectrum are well separated from both skeletal and aromatic signals in all three regioisomers, it is possible to investigate long-range correlations to the β-cyclodextrin moiety. These proton signals (at 4.70 ppm in **1**, at 4.96 ppm in **2**, and at 4.41 ppm in **3**) were excited selectively in a heteronuclear long-range INEPT experiment. Correlations to the cyclodextrin carbons at 80.6 ppm in **1**, at 75.8 ppm in **2**, and at 71.3 ppm in **3** (besides the expected correlations to the aromatic ring carbons C-1′, C-2′, and C-6′) were observed (top row in Figure 2). These signals are assigned to the carbon atom of the glucose unit at the substituted site. However, at this point an unambiguous assignment of the exact substitution pattern cannot be made.

A routine DEPT experiment is used to assign the signal at 71.3 ppm to the methylene group at the 6-position in **3** because of its anti-phase appearance relative to methyne signals.[19] The distinction between the 2- and 3-substituted isomers (**1** and **2**) is made through a standard HMBC experiment in conjunction with the data obtained with the carbon-detected experiment. HMBC is a proton-detected two-dimensional experiment in which one-bond correlations are suppressed by a low-pass J filter.[20] The heteronuclear long-range magnetization is labeled with the carbon frequency during the incremented delay (t_1), and this magnetization is transferred to detectable proton magnetization by a carbon-read pulse. While the long-range INEPT experiment gives information on the long-range interactions of the benzyl protons in the 4-methylamino-3-nitrobenzyl group to the glucose carbons, the HMBC sequence is used to monitor the long-range interactions of these carbon atoms in the substituted positions to near protons. The HMBC sequence has the advantage of being more sensitive than the carbon-detected heteronuclear long-range INEPT.

The HMBC spectrum of **1** (a in Figure 3) shows a correlation of the substituted carbon (at 80.6 ppm) to the benzyl methylene protons, and there is an additional important correlation to another proton (triplet at 4.1 ppm). This proton has two large axial couplings to its neighbors, and thus, this signal can be assigned to H-3 in the substituted α-glucose ring. The carbon signal at 80.6 ppm and the substitution in **1** are therefore assigned

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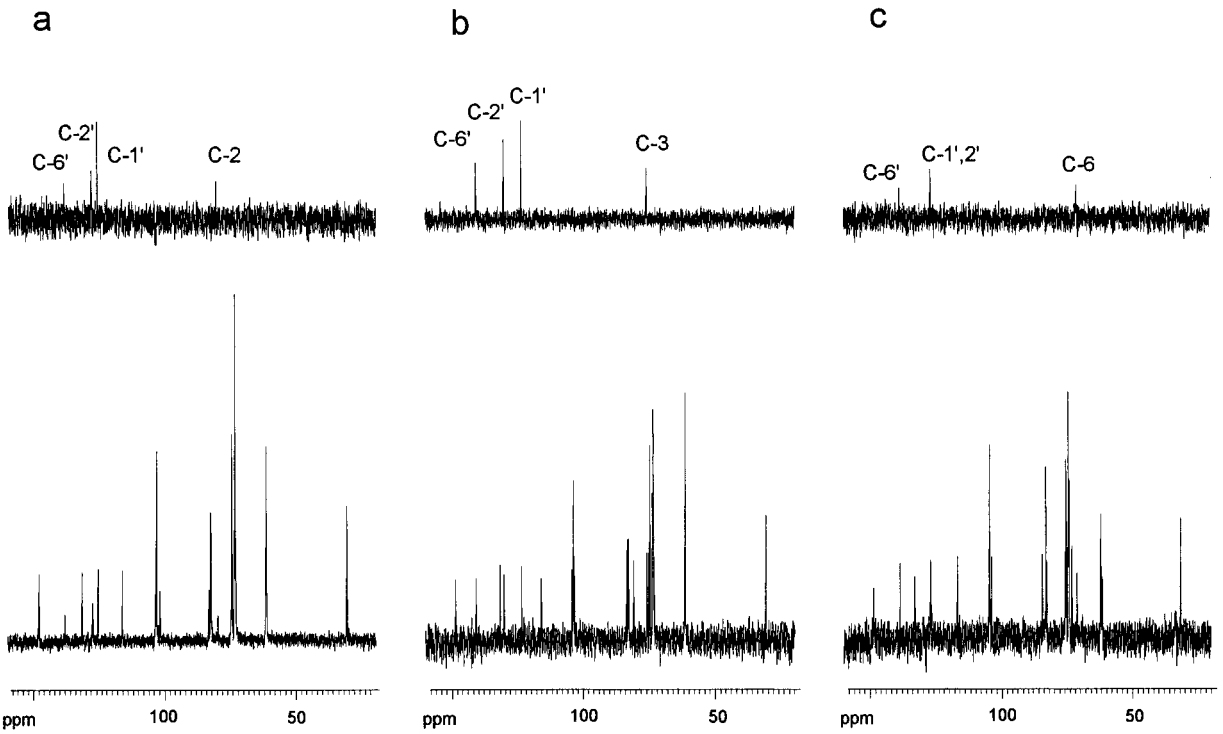

Figure 2. ^{13}C NMR spectra of different regioisomers: (a) 2^A-(4-methylamino-3-nitrobenzyl)-β-cyclodextrin (**1**); (b) 3^A-(4-methylamino-3-nitrobenzyl)-β-cyclodextrin (**2**); (c) 6^A-(4-methylamino-3-nitrobenzyl)-β-cyclodextrin (**3**). The bottom row shows normal ^{13}C spectra; the top row shows the results of the selective long-range INEPT experiment. The ppm scale is the same for both top and bottom spectra.

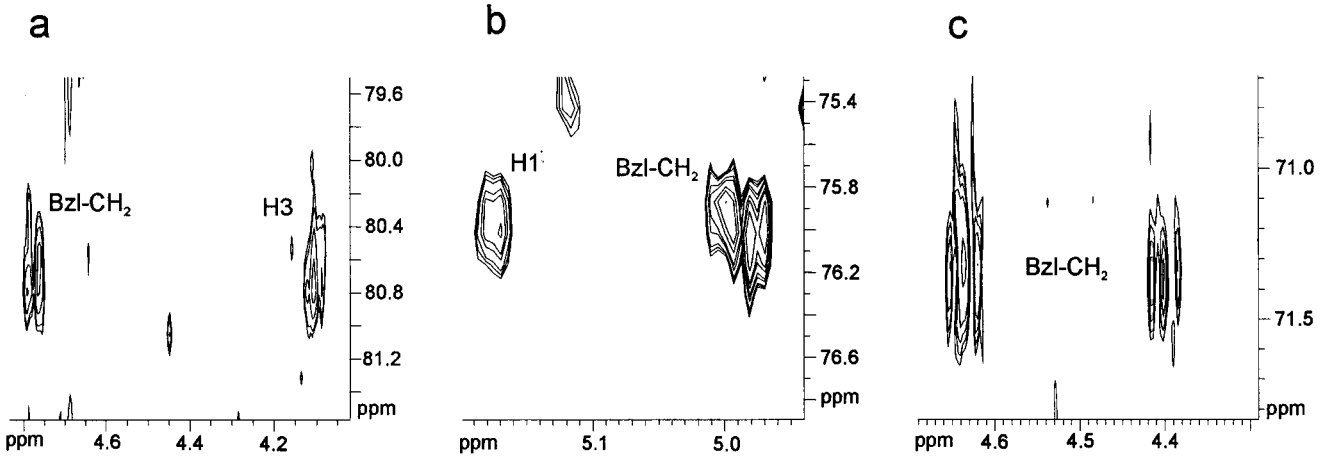

Figure 3. Expanded regions of the HMBC spectra on the substituted carbon: (a) HMBC spectrum of **1**; (b) HMBC spectrum of **2**; (c) HMBC spectrum of **3**.

to C-2 in this case. The HMBC spectrum of **2** (b in Figure 3) also shows two important correlations of the substituted carbon (at 75.8 ppm). The first one is a correlation to the benzyl protons (indicating that this position was substituted), and the second one is a correlation to one of the anomeric doublets (at 5.18 ppm). In this case the substitution is assigned to C-3. Thus, with this set of two experiments, it is possible to unambiguously assign the identity of three regioisomers in monosubstituted cyclodextrins.

Conclusions

Unambiguous identification of monosubstituted cyclodextrin regioisomers can be made by investigation of the carbon signal of the glucose unit at the site of modification. If this carbon signal has an anti-phase appearance in a routine DEPT experiment, the substitution is at the 6-position. If this carbon signal has a correlation to a proton with two large axial−axial couplings to its neighbors in an HMBC experiment, the substitution is at the 2-position. If this carbon signal shows a correlation to an anomeric proton (around 5.1 ppm) in an HMBC experiment, the substitution is at the 3-position.

Experimental Section

All NMR experiments were recorded on a Bruker ARX-500 instrument. Samples were prepared using 5−10 mg of each compound dissolved in 0.7 mL of D_2O. The data were acquired at room temperature without temperature control. The long-range INEPT experiment was run using a 5 mm broadband probe with the same pulse sequence as described in ref 17

without any modifications. Proton pulses were made selective by attenuating the decoupler channel. The length of the selective 90° proton pulse was 10 ms. Delays of 20 ms each were applied for both defocusing and refocusing ($\Delta_1/2$ and $\Delta_2/2$ in ref 17). The hard ^{13}C 90° pulse length was 12 μs. A total of 16k transients were collected in all cases in overnight experiments. The proton-detected heteronuclear experiment was run using a 5 mm inverse-constructed probe. The HMBC pulse sequence was set up with a low-pass filter, and a 60 ms long-range evolution time was applied in all cases. The 90° proton and carbon pulse lengths were 13 and 12 μs, respectively. The proton transmitter was placed on the water signal, and the sweep with was 7.0 ppm in the F_2 dimension. A total of 256 increments with 64 transients in each were collected, and the sine-bell window function was applied in both dimensions prior to Fourier transformation.

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Supporting Information Available: ^{13}C-DEPT spectra of the three regioisomers (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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