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Short communication

Synthesis of phenyl-adducted cyclodextrin through the click reaction ☆

Atanu Biswas [a,*], H.N. Cheng [b], G.W. Selling [a], J.L. Willett [a], D.F. Kendra [c]

[a] Plant Polymer Research Unit, National Center for Agricultural Utilization Research, USDA/Agricultural Research Services, 1815 N. University Street, Peoria, IL 61604, USA
[b] Ashland Hercules Water Technologies, 500 Hercules Road, Wilmington, DE 19808-1599, USA
[c] Mycotoxin Research Unit, National Center for Agricultural Utilization Research, USDA/Agricultural Research Services, 1815 N. University Street, Peoria, IL 61604, USA

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ABSTRACT

A new derivative of β-cyclodextrin (CD) has been made incorporating the phenyl group through the use of click reaction. The resulting product suggests a self-association phenomenon through the formation of inclusion compound between the phenyl group and CD. The product has been characterized by ^{1}H and ^{13}C NMR spectroscopy.

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1. Introduction

Cyclodextrins (CDs) are well known materials, consisting of α-D-glucopyranoside units linked 1-4 in a cyclic arrangement (Dodziuk, 2006; Szejtli, 2004). Typical CDs contain 6–8 glucose monomers in a ring, called α-CD, β-CD, and γ-CD, respectively, and are produced enzymatically from starch. They are used in pharmaceutical, agricultural, specialty chemicals, and many other applications.

Among its properties, the ability to form host–guest complexes with hydrophobic molecules is probably the most commercially valuable (Szejtli, 2004). Thus, the literature is replete with CD complexes with a variety of substances (Del Valle, 2004; Uekama, Hirayama, & Irie, 1998; Zhang and Rees, 1999), e.g., drug molecules for improved solubility or controlled release, fragrance molecules for controlled release, cholesterol removal in food industry, and removal of toxic substances from the environment. Recently the use of CD for detection of mycotoxin for food safety application has also been reported (Maragos et al., 2008). CD may also enhance the antifungal activity of antifungal agents (Chow, Chen, & Timmins, 1989; Hostetler, Hanson, & Stevens, 1992).

We have been fascinated with the use of CD because it comes from a renewable raw material and is amenable to chemical reactions. In particular, we are interested in derivatizing it in order to produce more customized properties. Previously only one earlier report has appeared on phenyl-modified CD (Zhao & Liu, 2006). We have now shown that the click reaction (e.g., Rostovtsev, Green, Fokin, & Sharpless, 2002; Tornøe, Christensen, & Meldal, 2002; Wu & Fokin, 2007) is an alternative (and convenient) method to place a phenyl derivative on CD. As far as we know, this is the first report of the click reaction on CD. The click reaction product obtained in this way exhibits possible self-assembly behavior in polar solvents.

2. Experimental

2.1. Materials

β-Cyclodextrin, hydrate and phenyl acetylene, 98+% were purchased from Alfa Aesar. Ascorbic acid, sodium L-ascorbate and copper(II)sulfate were obtained from Sigma Aldrich. The solvents were purchased from Aldrich and sodium azide, 99% from Acros.

2.2. Synthesis of bromoacetylated CD

The solvents used were N,N-dimethylacetamide (DMAc) and pyridine. Pyridine to acetyl bromide mole ratio was 1:1. In a 250 mL Erlenmeyer flask 10 grams of β-cyclodextrin (8.8 mmol) and 60 ml of DMAc were stirred with a stir bar until the solution became clear. Pyridine, 16 g (202 mmol), was added to this solution and the mixture was cooled by an ice water bath. To this mix-

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* Corresponding author. Tel.: +1 309 692 6407.
E-mail address: atanu.biswas@ars.usda.gov (A. Biswas).

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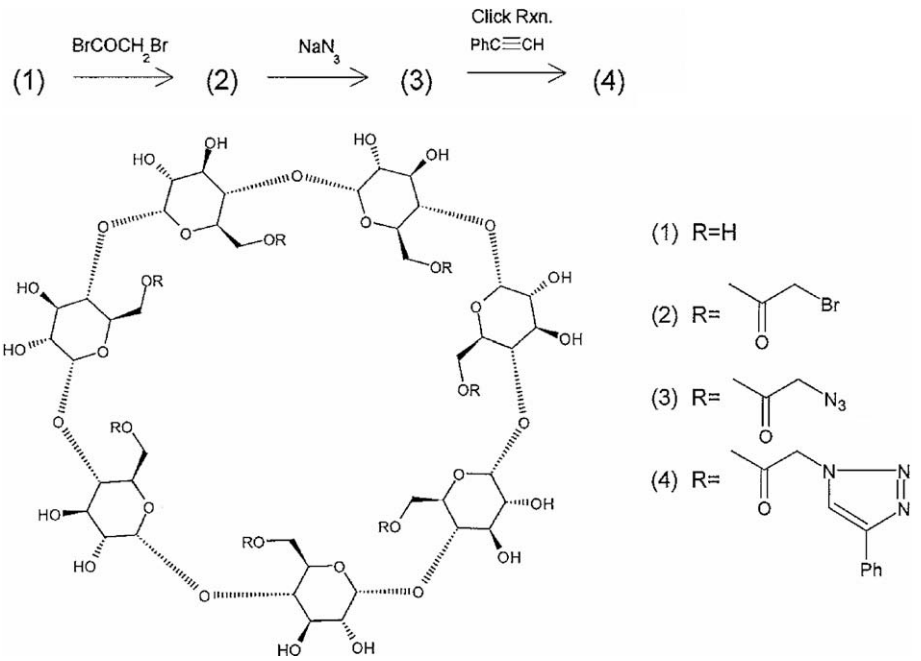

Scheme 1. Reaction sequence used in this work. For convenience, the β-CD shown for structures (2), (3), and (4) has a DS of 1.0 and is substituted entirely at the 6 position. The real DS is 0.7.

ture were added 40.2 g of bromoacetyl bromide (200 mmol) in 0.1 ml increments to obviate any exotherm. After stirring the mixture for 2 days at room temperature it was poured into 300 ml of 1 N HCl solution, stirred for 20 min. The mixture was divided into several 50 mL centrifuge tubes and centrifuged at 3000 rpm at room temperature for 20 min. The supernatant liquid was decanted and the residue was washed with water and centrifuged. This process was repeated a few times. The product was dried in a vacuum oven overnight at 50 °C to give 15.8 g of cyclodextrin bromoacetate (2) as beige colored powder. DS was calculated from the bromine content analysis.

2.3. Synthesis of CD azide

The following materials were placed in a 100 mL round bottomed flask with a stir bar: 1.81 g brominated CD, 15 mL DMAc, 0.652 g sodium azide in 5 mL DMAc. They were stirred at room temperature for 24 h and then placed in a heating mantle with a Therm-o-watch set at 60 °C for 2 h. When done, it was cooled to room temperature.

2.4. Click reaction

To the above reaction mixture, we added 1.0 g phenyl acetylene, 0.162 g sodium ascorbate, 0.064 g copper (II) sulfate, and 3 ml water. The mixture was stirred at room temperature for 3 days. The reaction mixture was poured into a beaker with 150 mL of 1 N HCl and stirred for 30 min, transferred to four 50 mL plastic centrifuge tubes, and centrifuged at 3200 rpm for 20 min. The water layer was decanted off. De-ionized water was then added and centrifuged as before. The product was subjected to a repeated water wash and then an ethanol wash. The product was then placed in a vacuum oven at 50 °C overnight. The total solids recovered was 1.25 g.

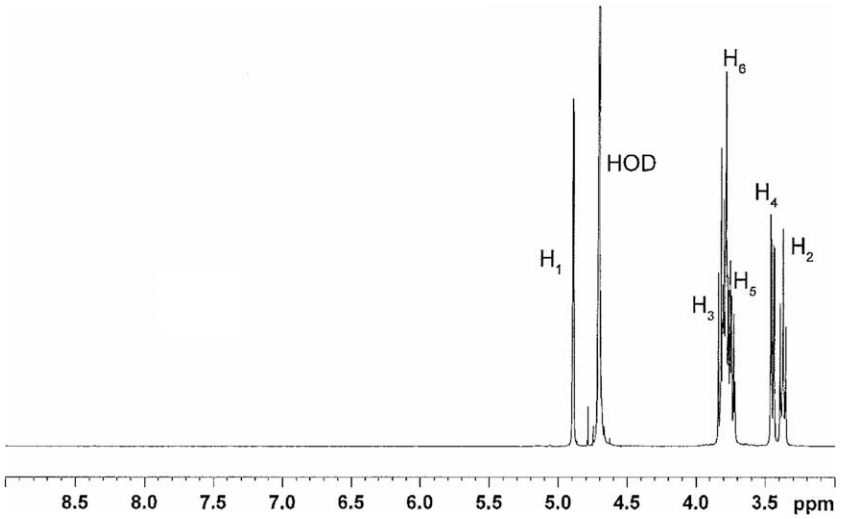

Fig. 1. ^{1}H NMR spectrum of β-cyclodextrin in D_2O.

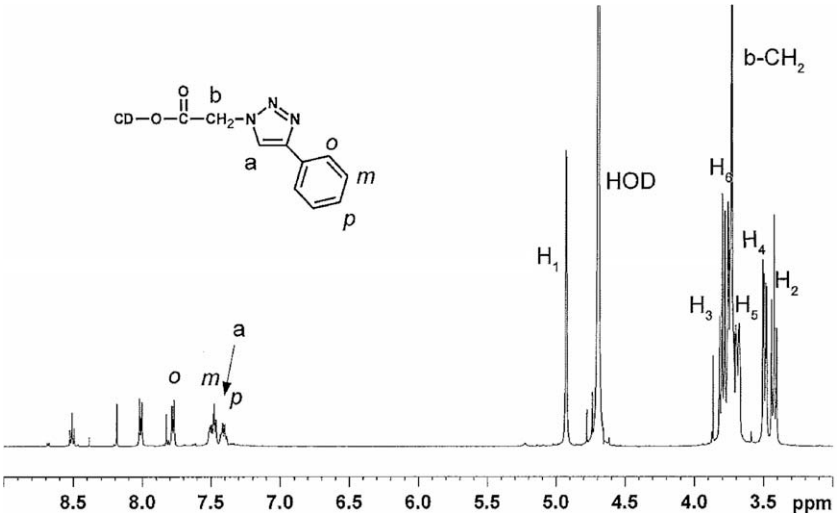

Fig. 2. ^{1}H NMR spectrum of the click reaction product (phenyl-adducted β-cyclodextrin) in D_2O.

2.5. NMR characterization

NMR characterization was performed on a Bruker (Boston, MA) Avance 500 NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Bruker Icon NMR software was used with a HP x1100 Pentium 4 workstation. All NMR peaks were referenced to tetramethylsilane at 0.0000 ppm.

3. Results and discussion

In this work, the synthesis of phenyl-adducted CD entails a three-step reaction sequence (Scheme 1). In the first step, β-CD is reacted with bromoacetyl bromide in a solvent mixture of DMAc and pyridine. In order to ensure that we can get an adequate amount of observed effects, we target a relatively high average degree of substitution (DS) of 0.7 on each glucopyranose moiety. Thus, there are roughly 5 bromine units, on the average, on each β-CD molecule.

In the second step, the sodium azide is added to the brominated CD, using either dimethylsulfoxide (DMSO) or DMAc as solvent and with moderate heat. The azide replaces the bromine essentially quantitatively. The last step is the click reaction, where the azide undergoes a cycloaddition with phenylacetylene in the presence of copper sulfate and sodium ascorbate with moderate heat to give the phenyl-triazole. Again the solvent is either DMSO or DMAc, although DMSO is found to give a better yield.

The reaction product has been characterized by NMR. The ^{1}H NMR spectra of the starting β-CD and the final click product in D_2O are shown in Figs. 1 and 2. The unreacted CD gives a relatively simple spectrum in D_2O, with known assignments shown in Table 1. After the click reaction, an extra peak occurs at 3.72 ppm corresponding to the methylene next to the ester, and several peaks in the 7.3–7.9 ppm range corresponding to the 1,2,3-triazole and phe-

Table 1
^{1}H NMR chemical shifts for unreacted β-CD and phenyl derived β-CD in D_2O.

Proton number	δ (ppm), unreacted	δ (ppm), phenyl	Difference
H-1	4.89	4.90	0.01
H-3	3.82	3.79	−0.03
H-6,6′′	3.79	3.77	−0.02
H-5	3.74	3.67	−0.07
H-4	3.45	3.47	0.02
H-2	3.38	3.42	0.04

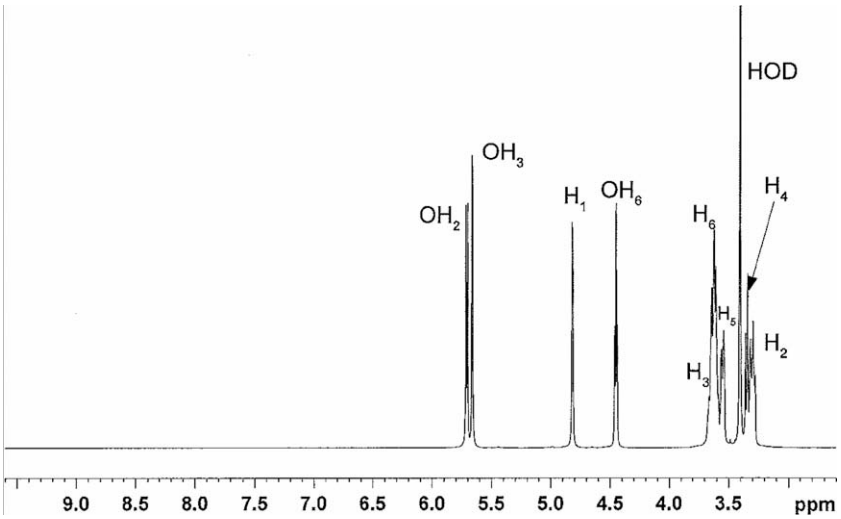

Fig. 3. ^{1}H NMR spectrum of β-cyclodextrin in d_6-DMSO.

nyl. From coupling patterns and spectral intensities, we can assign *ortho* protons to 7.8 ppm, *meta* protons to 7.5 ppm, *para* proton to 7.4 ppm, and the triazole proton at 7.5 ppm. The peaks beyond 8.0 ppm are some byproducts produced prior to the click reaction and are as yet unidentified.

For comparison, the ^{1}H chemical shift positions of the unreacted CD and phenyl-derived CD are listed in Table 1. It is clear that there is a slight shift upfield for the H-5 peaks. It is well known that such upfield shift is characteristic of the formation of an inclusion compound (e.g., Bilensoy, Vural, Bochot, Renoir, Duchene et al., 2005; Jiang et al., 2007; Zhao, Yang, Hu, & Ma, 2003). Since the phenyl group is hydrophobic, it is not surprising that it gets inserted in the CD cavity.

The ^{1}H NMR spectra for the same materials dissolved in d_6-DMSO are shown in Figs. 3 and 4. In d_6-DMSO, the CD shows more peaks because the OH groups appear (Song et al., 1997). The assignments are shown directly in Fig. 3. Note that the peak at 3.4 ppm comes from residual water (actually HOD) in d_6-DMSO. In contrast, the click reaction product gives very broad peaks. Part of the reason for line-broadening is the decreased solubility of the phenyl-derived product in DMSO. Another possible reason is the self-assembly of CD phenyl adduct, such that the phenyl ring is enclosed in CD. Since there are, on the average, five phenyl groups per CD, each CD molecule is fully occupied with phenyl groups.

For additional structural information, the ^{13}C NMR spectra in d_6-DMSO are shown in Figs. 5 and 6. The ^{13}C peaks for unreacted CD are well known (e.g., Takeo, Hirose, & Kuge, 1973), and the assignments are shown in Fig. 5. In phenyl-derived CD (Fig. 6), the CD peaks are smeared out because of the effect of possible aggregation and limited solubility in DMSO. The other peaks in the ^{13}C spectrum can be readily assigned, as shown in Fig. 6, thereby confirming the structure of the product.

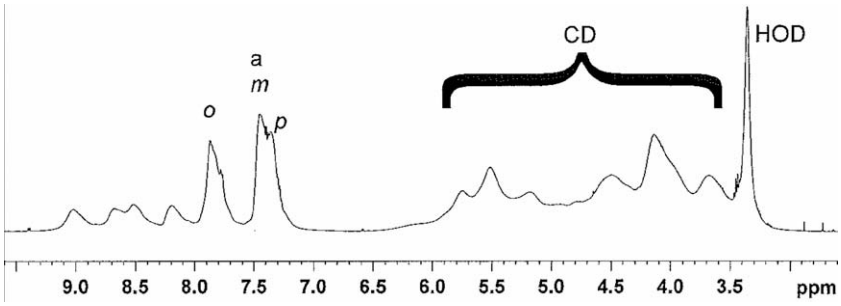

Fig. 4. ^{1}H NMR spectrum of the click reaction product (phenyl-adducted β-cyclodextrin) in d_6-DMSO.

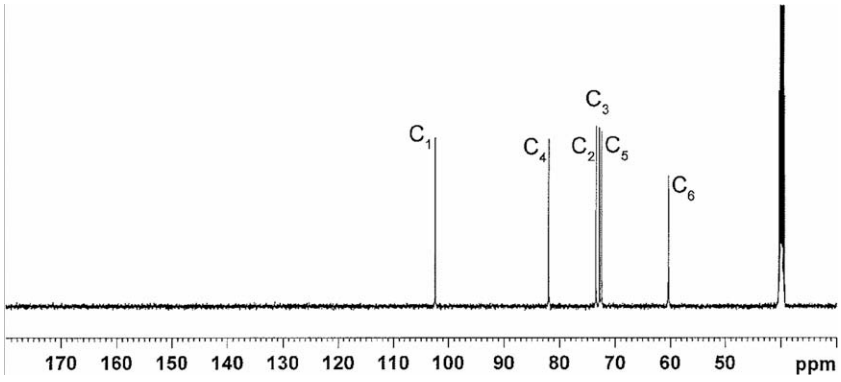

Fig. 5. ^{13}C NMR spectrum of β-cyclodextrin in d_6-DMSO.

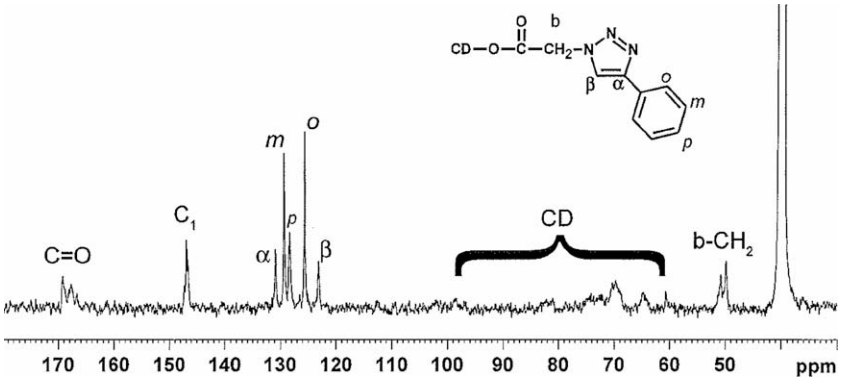

Fig. 6. ^{13}C NMR spectrum of the click reaction product (phenyl-adducted β-cyclodextrin) in d_6-DMSO.

4. Conclusions

In this work, we found the click reaction to be a facile method to incorporate a hydrophobic substitution onto an oligosaccharide. We were able to place a phenyl group onto β-cyclodextrin at a relatively high degree of substitution (0.7). The resulting material suggests self-assembly behavior in both water and DMSO.

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