## Author

For Alpay Dermenci, interacting with professors outside the classroom is the best part of research. "The most exciting part was eliciting solutions knowing that both the professors and I faced the same challenges." He started working in the lab of Dr. A. J. Shaka in his freshman year, working in physical chemistry, organic chemistry, and structural biology. He looks forward to continuing his work in combining chemistry and biology beyond graduation, moving on to graduate school and eventually becoming a scientific researcher and professor. When he is away from his studies, Alpay enjoys reading, fishing, and spending time with his family.

## Key Terms

- Carbohydrates
- Chemical Shift
- Isotagging
- Nuclear Magnetic Resonance (NMR) Spectroscopy
- Precessing
- Protein-Like Functionality
- Structure Determination
- Trichloracetyl Isocynate

# Introducing "Protein-Like" Functional Groups into Carbohydrate Molecules: A New Way to Obtain Well-Resolved Nuclear Magnetic Resonance Spectra

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## Abstract

For protein NMR, uniform isotopic labeling with $^{13}C$, $^{15}N$, and/or $^{2}H$ has been used to aid structure determination. Proteins are usually expressed in bacteria like *E. coli* with isotopically labeled growth media, which leads to enrichment of the protein of interest. But such uniform labeling is not possible for carbohydrates because the samples are not obtained as simply as proteins. In addition, most sugars dissolve well only in polar solvents like water, which cause large residual solvent peaks, high viscosity with increased concentration, and other complicating factors. Therefore, we sought an alternative approach. We perderivatized the carbohydrate -OH groups, replacing the hydrogen with a small isotopically-enriched fragment called an isotag. The goals were to (a) improve the poor proton NMR spectral dispersion by creating electron-withdrawing inductive effects; (b) switch to organic solvents that are superior for NMR by capping the carbohydrate -OH groups with -OR groups, in which the R moiety changes the solubility; (c) apply powerful multidimensional heteronuclear protein methods to these systems by including $^{13}C$ and $^{15}N$ in the R group; and (d) see if the stereochemistry of the constituent sugar rings could be determined by analyzing the improved NMR spectra. Isotagging of carbohydrates avoids the time and expense of uniform labeling, improves chemical shift dispersion, and makes the sample soluble in organic solvents, such as $CDCl_3$ or THF-d$_8$.

## Faculty Mentor

Most biomolecular NMR spectroscopy focuses on proteins. There are a few groups working on DNA or RNA as well, but the effort in carbohydrates has been much less. The reason underlying this lopsided scientific resource allocation is not that carbohydrates are unimportant, or that we know the detailed structure of these molecules, especially as they are presented on cell surface membranes. Rather, it is that the NMR spectra of carbohydrates are much more difficult to resolve and assign than those from the other molecules. Alpay's groundbreaking work, namely to introduce small isotopically-labeled protecting groups onto each hydroxyl group present in the sugar, will tip the balance in our favor, allowing spectra with excellent resolution and sensitivity to be obtained. This high-risk, high-reward project is a perfect example of how undergraduate research can really lead to a significant advance, even in a fairly mature field.

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## Introduction

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful techniques for definitive chemical analysis of molecules in solution; it is used extensively in determining the structure and dynamic properties of molecules (Pellecchia, 2005).

The basic idea behind NMR is the detection of nuclei with non-zero spins. Typically seen are spin-1/2 nuclei such as hydrogen, carbon-13, and nitrogen-15 ($^1$H, $^{13}$C, and $^{15}$N, respectively). These nuclei behave like tiny bar magnets, partially aligning and precessing in an applied magnetic field. The frequency of precession can be observed by applying a pulse and using a pickup coil to detect the changing magnetization. This time-dependent signal is then analyzed to obtain an NMR spectrum, a plot of amplitude versus frequency. Sensitivity is often poor at natural abundance and the isotope of interest must be enriched to boost the sensitivity of the NMR experiment (Levitt, 2001). Although such enrichment may not be essential for studies involving small molecules (Figure 1a), introducing isotopes into larger molecules, such as proteins (Figure 1b), is essential for obtaining spectra that are suitable for structural determination.
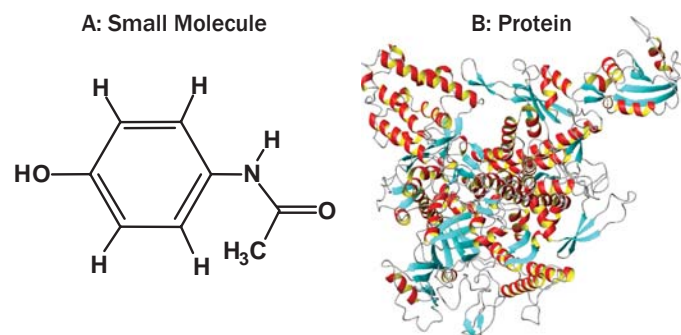


**A: Small Molecule**  **B: Protein**

Figure 1
(A) Acetaminophen is the small molecule active ingredient in aspirin. (B) Proteins—such as this subunit from RNA Polymerase II—are usually large and complex. This cartoon representation, generated as a ribbon structure using PyMol, shows the complexity involved in proteins and the need for structural analysis. This structure is from the Protein Data Bank.

In the applied magnetic field of the NMR spectrometer, magnetic nuclei precess or resonate at frequencies that are dependent on their local environment (Briuce, 2005).

Different isotopes have widely varying frequencies, but isotopes of a single type (e.g., $^1$H) also show slight changes in frequency depending on chemical structure, solvation, pH, and other subtle effects. These together define the chemical shift ($\delta$), which is measured as the relative difference in resonance frequency ($\nu_H$) from a standard like tetramethylsilane ($\nu_{TMS}$) and is quoted in parts per million (ppm) as shown in Equation 1.

$$\delta_H = \frac{\nu_H - \nu_{TMS}}{\nu_{TMS}} \times 10^6 \qquad (1)$$

The observed shift depends on how much an atom is shielded from the applied magnetic field by bonding electrons and other magnetic phenomena. The amount of the magnetic field that a nucleus "sees" determines its frequency, rather than the field that is applied. More shielded nuclei see smaller local fields for a given applied magnetic field, and usually exist in electron-rich environments of a molecule.

Magnetically equivalent nuclei resonate at the same frequency, giving rise to a single peak. Nuclei in different chemical environments, nonequivalent nuclei, have different frequencies and give rise to two or more peaks in the NMR spectrum. For example, in an $^1$H NMR spectrum, each nonequivalent $^1$H will have a characteristic chemical shift ($\delta_H$). Table 1 shows some representative chemical shifts for common functional groups (Briuce, 2005).

Table 1
Representative $^1$H Chemical Shifts

| Type of proton | $\delta$, /ppm | Type of proton | $\delta$, /ppm |
|---|---|---|---|
| —C$H_3$ | 0.9 | O=C—$H$ | 9.0 – 10.0 |
| —C$H_2$— | 1.3 | R—N$H_2$ | 1.5 – 4.0 |
| —C$H$— | 1.4 | R—O$H$ | 2.0 – 5.0 |
| O=C—C$H_3$ | 2.1 | O=C—O$H$ | 10.0 – 12.0 |
| ⬡—$H$ | 6.5 – 8.0 | O=C—N$H_2$ | 5.0 – 8.0 |

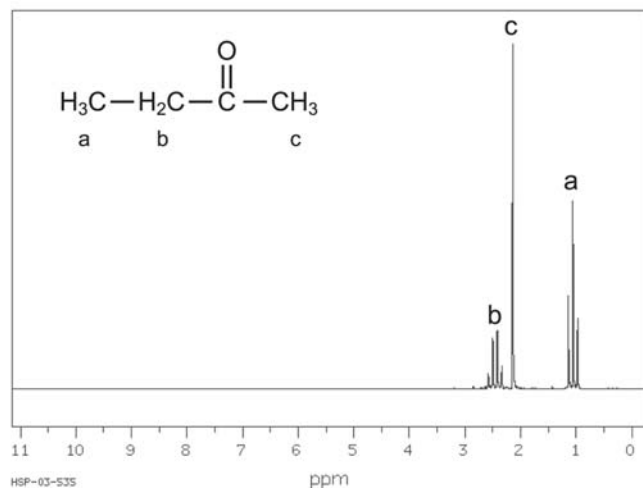NMR spectra can become quite complex even for small molecules. Part of this complexity comes from the splitting

Figure 2
The $^1$H NMR spectrum of 2-butanone. The letters above the peaks correspond to the $^1$Hs labeled on the structure of 2-butanone. The methyl group (c) does not split because the adjacent carbon does not have any $^1$H atoms. However, the other sets of $^1$H split each other, resulting in the sets of peaks labeled (a) and (b). This spectrum is from the SBDS data bank.



Figure 3
Cellulose and starch are sugars that have the same monomers (subunits), but differ in their spatial connectivity. Cellulose contains a β-linkage, where the oxygen from the starred carbon maintains an upward orientation, while starch has an α-linkage, where the oxygen from the starred carbon maintains a downward orientation. This simple difference makes the biological activity of these molecules unique.

of single resonance peaks (singlets) into multiple peaks (multiplets) by neighboring nuclei, that may orient parallel or opposite to the applied magnetic field (Figure 2).

NMR spectroscopy is fairly unique in that it is not limited to one-dimensional (1D) measurements, and can expand into a second frequency dimension. That is, we can plot a two-dimensional (2D) spectrum, an amplitude surface as a function of two independent frequency variables, such as two different kinds of NMR-active isotopes. These 2D spectra show markedly better resolution when the 1D spectra become too congested to analyze easily.

Heteronuclear Single Quantum Correlation (HSQC) is a common 2D NMR technique for which the major advantage is its ability to identify directly bonded nuclei (Keeler, 2005). An HSQC spectrum consists of two axes, each belonging to a different nucleus (e.g. $^1$H and $^{15}$N). When a peak appears at the same frequencies as a peak in the $^1$H dimension and the $^{15}$N dimension, it illustrates that the two nuclei are directly linked. HSQC also offers the further dispersion of peaks if they happen to overlap in a single dimension. When used together, $^1$H NMR and HSQC-NMR can be extremely useful in eliciting the structures of complex molecules, such as proteins.

Biologically important molecules, such as carbohydrates, can be particularly complex even though they are not as large as proteins (Alberts, 2002). The complexity of carbo-
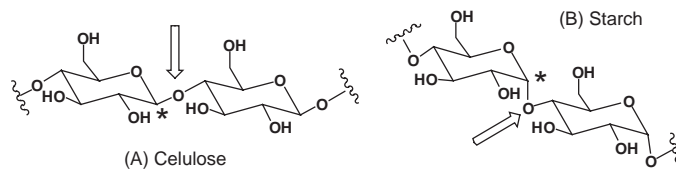
hydrates is largely due to their stereochemistry, namely the different spatial arrangement of atoms in molecules with the same connectivity. For example, cellulose and starch have the same connectivity, but differ in stereochemistry at the point at which glucose monomers are linked—the glycosidic bond—as shown in Figure 3. This small difference can lead to big consequences. Humans can digest starch but not cellulose, as the available mammalian enzymes can only break down the α-glycosidic bond (Alberts, 2002). Therefore, probing the structure of carbohydrates and learning their stereochemistry would contribute to understanding their function as biologically active compounds.

NMR would be a suitable choice for studying carbohydrates, but studying natural sugars that have not been chemically derivatized presents several problems. First, the spectrum of a considerably complex sugar displays spectral crowding, where the peaks belonging to individual $^1$H nuclei overlap with other peaks and obscure useful information. For instance, a large peak might overlap a small peak and prevent the resolution of the smaller peak. Second, sugars are typically only soluble in aqueous solvents, producing large residual solvent peaks, which can also overlap and obscure peaks of structural importance. To solve these problems we kept three goals in mind: (a) try to disperse the proton peaks to get spectra of sugars where the peaks resonate at different chemical shifts, thereby eliminating peak overlap; (b) move from aqueous to organic solvents, which are superior for NMR; and (c) use multi-dimensional NMR spectroscopy to further improve the resolution.

Although natural sugars are difficult to characterize by NMR, we found an effective method to realize our goals. Synthesizing our sugars with an electron-withdrawing, highly-reactive reagent called trichloracetyl isocynate (TAI) offers a combination of clean reaction with all of the –OH groups in the sugar, good sample stability in the absence of

moisture, and superior NMR spectra. TAI offers well-resolved and dispersed spectra both in one- and multidimensional NMR, and offers insight to the structure and linkage-isomerism for sugars. As shown in Figure 3, sugars, such as cellulose and starch, contain numerous hydroxyl groups (–OH), which are nucleophilic and capable of forming a chemical bond by donating electrons to an oxygen atom. On the other hand, TAI is an electrophilic compound, meaning that it is attracted to electrons. When a TAI molecule encounters a hydroxyl group, a chemical reaction takes place as proposed in Figure 4 (Goodlett, 1965). During the reaction, there are two possible transition states in which the TAI molecule and the hydroxyl group must orient in the correct conformation in order to push the reaction to completion (Vodicka, 2003). This transformation causes hydroxyl groups to assume a "protein-like" structure bearing similarity to a protein backbone (Figure 5). As a result, there are several advantages to NMR gained from reacting these hydroxyl groups with TAI.

TAI simplifies the NMR spectra and structure determination of these sugars. One feature of TAI is the ability to "count" the number of hydroxyl groups on a sugar. TAI converts each broad hydroxyl proton peak upfield (the right side of the NMR spectrum) to a corresponding sharp –NH peak downfield (the left side of the NMR spectrum), isolating them from the rest of the peaks (Goodlett, 1965). Moreover, the electron-withdrawing properties of TAI also shift proximal ring protons so that they can be identified on different carbons (Fritz et al., 1969). That is, the derivative lets us identify if the carbons constituting the ring are bound to one or two other carbons. Finally, TAI is reactive enough that it reacts even with hindered hydroxyl groups, making it especially useful for determining the stereochemistry of sugars (Bose et al., 1979).

Although TAI proves useful under the right conditions, there are drawbacks and minor complications with its reactivity. Beyond simple alcohols, the reaction of TAI can become more involved. A study done on a derivatized carbohydrate showed that a TAI analog has an intermediate with unique properties (Knapp et al., 1990) as shown in Figure 6.

While in its transition state, the hydroxyl proton makes a hydrogen bond to the nearby oxygen and creates a complex that slightly slows down the reaction without affecting the final product. However, a small amount of water in solution, especially when working with small amounts of sample, can consume all the TAI and result in unwanted side products. For every molecule of water, two molecules of

TAI are consumed, as shown in Figure 7. Through having the right conditions and knowing what to avoid, TAI seems to be a powerful and effective way to derivatize sugars and we sought to test its ability.
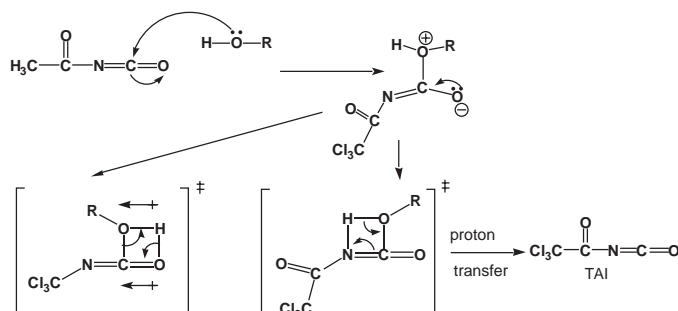


Figure 4
The reaction of trichloroacetyl isocyanate (TAI) with a generic hydroxyl group, where R is an alkyl group, shows unique properties. There are two possible transition states highlighted in brackets: one that reforms TAI and the other that gives the desired TAI derivative product. Although two transition states exist, TAI ultimately has one fate, which is the desired derivative.
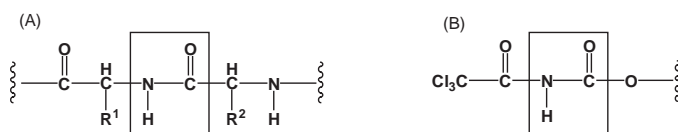


Figure 5
(A) A section from a polypeptide highlighting the amide bond, where $R^1$ and $R^2$ can be any of the 20 possible amino acids. (B) The TAI derivatized hydroxyl group consists of a carbamide group that highlights the "protein-like" similarity to (A). The similarities are identified by rectangles.
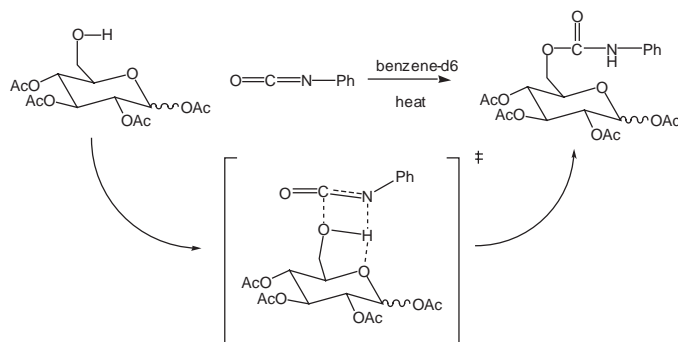


Figure 6
The phenyl isocyanate reaction can become complex when nearby groups become involved in the reaction mechanism. The oxygen from the sugar ring provides additional hydrogen bonding indicated by the dashed lines. This does not change the product, but hypothetically slows the reaction.
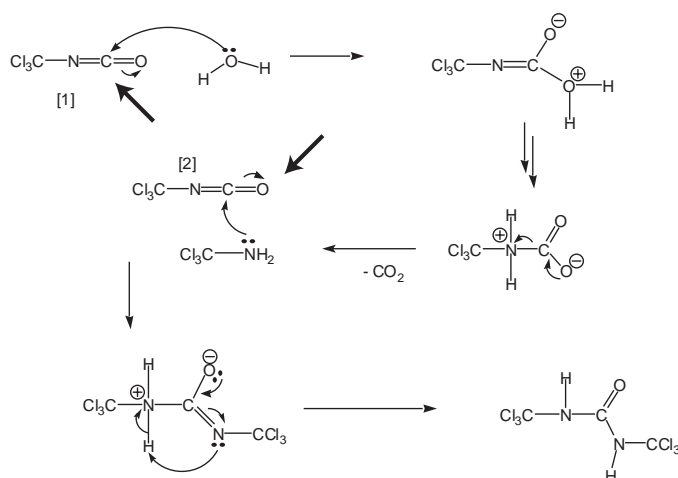
**Figure 7**
The mechanism by which water reacts with TAI and produces unwanted side products. For example, the product at the end of the reaction shows how two TAI molecules are linked due to a single water molecule. The bold arrows indicate the two separate molecules of TAI that react for every molecule of water.

## Experimental Methods

### General Experiments

All derivatizing reactions were done under nitrogen in a glovebox maintaining an inert atmosphere. The synthesis reactions were done under nitrogen connected to a Schlenk manifold containing a drying filter. All glassware was oven-dried overnight at 125 °C. J. Young 535-grade NMR tubes were used for derivatized sugar products, and Wilmad 535-grade NMR tubes were used for all other NMR experiments. 1H NMR spectra were recorded on a Varian Unity Plus (500 MHz) spectrometer with 32 coadded transients; the 13C NMR were recorded on a Bruker DRX500 (500 MHz), with enough transients to clearly identify the peaks. The 2D $^{15}N^1H$ HSQC for all derivatized sugars shown here were recorded at natural abundance. The HSQC for derivatized dodecyl-b-D-maltoside and sucralose was recorded on a Varian Unity Plus (500MHz), and the HSQC for the derivatized octyl-b-D-glucoside was recorded on a Varian Inova (800 MHz) at UCI's Biomolecular NMR Facility. The chemical shifts (d) for all NMR experiments were reported in ppm with reference to TMS. The gas chromatography purifications from the synthesis of trichloroacetyl isocyanate were performed using a GOW-MAC 350 Gas Chromatograph. The concentration of sodium hypochlorite (NaOCl) in Clorox Ultra Regular Bleach™ was determined by UV-Vis on a Jasco V-530 UV/Vis Spectrophotometer at a wavelength of 292 nm. In the synthesis of TAI, the Schlenk manifold was supplied with argon and the reaction was heated with a silicone bath.

### Preparation of Derivatized octyl-β-D-glucoside:

For the derivatization of octyl-β-D-glucoside, 0.020 mmol (5.85 mg) (Aldrich, Sheboygan, WI) was dissolved in approximately 2 mL of $CDCl_3$ (Cambridge Isotope Labs, Andover, MA) and allowed to stir in a vial for approximately 1 min to completely dissolve the sugar. With a microliter syringe, 0.170 mmol (20 μL) of TAI (Aldrich, Sheboygan, WI) was added to the solution while stirring continuously for 5 min (Figure 8). The solution maintained the same color throughout the reaction. Approximately 0.7 mL of solution was transferred to a J. Young NMR tube for analysis with NMR.

### Preparation of Derivatized dodecyl-β-D-maltoside

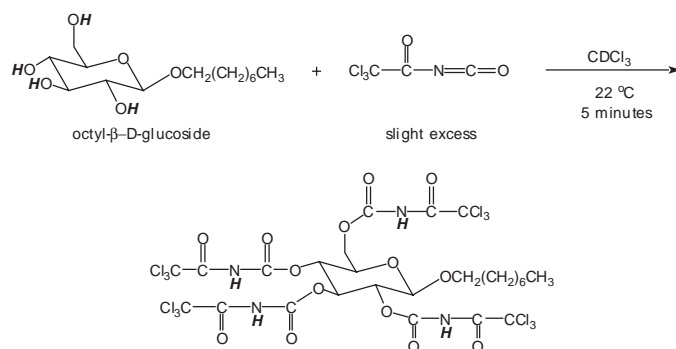For this reaction, 0.020 mmol (10.2 mg) dodecyl-β-D-maltoside (Aldrich, Sheboygan, WI) was dissolved in ca. 2 mL



**Figure 8**
Derivatization of octyl-β-D-glucoside with TAI. The hydroxyl protons are bolded and italicized to show their fate in the final product.
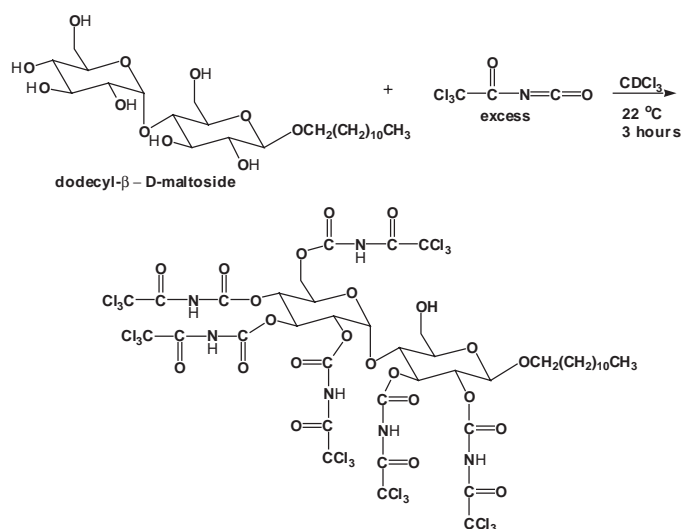


**Figure 9**
Derivatization of dodecyl-β-D-maltoside with TAI, showing the expected product. Note that the stereochemistry of the ring system is not changed by the reaction with TAI.

of CDCl₃ ("100%") (Cambridge Isotope Labs, Andover, MA) and allowed to stir in a vial for approximately 1 min. The sugar did not completely dissolve in CDCl₃ and produced a cloudy solution. Upon addition of 0.26 mmol (30 μL) of TAI (Aldrich, Sheboygan, WI) the solution gradually turned clear with 3 hr of stirring at room temperature (Figure 9). The derivatized product in CDCl₃ was then transferred to a J. Young NMR tube.

### Preparation of Derivatized Sucralose

0.010 mmol (3.976 mg) sucralose (Forbest International USA, Kendall Park, NJ) was dissolved in ca. 1 mL of THF-d₈ ("100%", Cambridge Isotope Labs, Andover, MA). The sucralose was readily soluble. With a microliter syringe, 0.170 mmol (20.0 μL) of TAI (Aldrich, Sheboygan, WI) was added to the mixture (Figure 9) and allowed to stir for an additional minute.

### Synthesis of ¹³C-labeled Trichloroacetyl isocyanate

For this series of reactions, 0.0593 mol (3.92 g) of ¹³C-potassium cyanide (K¹³CN, Cambridge Isotope Labs, Andover, MA) was dissolved in approximately 40 mL of Nanopure© and stirred for 10 min in an ice bath (0 °C). Once the K¹³CN was completely dissolved, 68 mL (.060 mol, .888 M sodium hypochlorite) of Clorox Ultra Regular Bleach (Albertson's, Irvine CA) was added to the solution over a 30 min period using an aluminum foil-covered additional funnel. After complete addition of NaOCl, the additional funnel was washed with 10 mL of Nanopure water and the washings were added to the reaction mixture. The mixture was allowed to stir for another 30 min at room temperature. The solution was frozen in a dry ice bath for 2 hr and immediately lyophilized and kept for 48 hr until dry. The resulting white product was ground into a fine powder and oven-dried for 2 hr at 120 °C. The product was 32% KO¹³CN and 68% side products. Proceeding oxidation of K¹³CN, 0.015 mol of KO¹³CN (3.8 g, 32% of white powder) was added to 4 mL of anhydrous 1,2,4-trichloroacetyl benzene (Aldrich, Sheboygan, WI) and stirred at 50 °C for 10 min under a vacuum. After a uniform mixture was obtained, 0.016 mol (1.8 mL) of trichloroacetyl chloride (Aldrich, Sheboygan, WI) was added to the reaction and the system once again put under argon. This solution was allowed to reflux at sequential sets of times and temperatures for a total of 8 hr in a silicone bath (Table 2 and Figure 10). The mixture
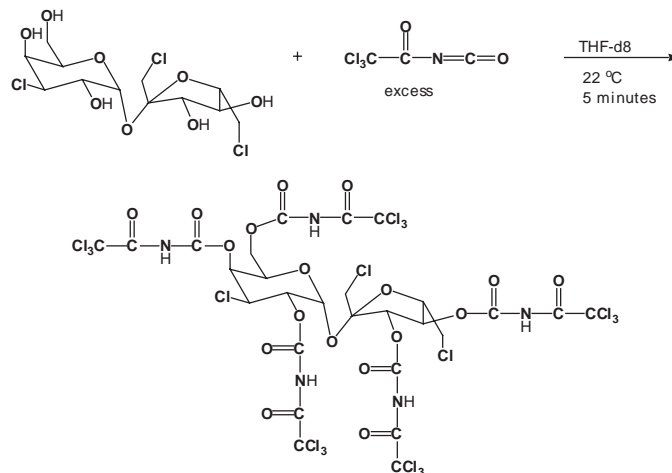


Figure 10
Reaction steps for the synthesis of ¹³C-labeled TAI. The cyanide is first oxidized in reaction A and then, through a bimolecular reaction of isocyanate and trichloroacetyl chloride, TAI is produced in reaction B.



Figure 11
Derivatization of sucralose with TAI. The hydroxyl groups react with TAI while the Cl groups do not.

was then distilled and TAI collected at boiling points ranging from 135 to 160 °C (Figure 11). The product was then further purified via preparative gas chromatography.

## Results

In this investigation, unlabeled TAI was used to test its viability for sugars by looking at the ¹H NMR spectrum before and after derivatization. In Figure 12, the ¹H NMR spectrum of octyl-β-D-glucoside shows crowding of peaks mainly between 3 and 4.5 ppm where the hydroxyl peaks are not identifiable. After synthesis, however, the ¹H NMR of derivatized octyl-β-D-glucoside shows dispersed peaks between 3 and 6 ppm and four peaks downfield, which belong to the -NH groups of the TAI derivative (Figure 13). Furthermore, the dispersed peaks between 3 and 6 ppm have excellent resolution and precise splitting patterns important for structural information.
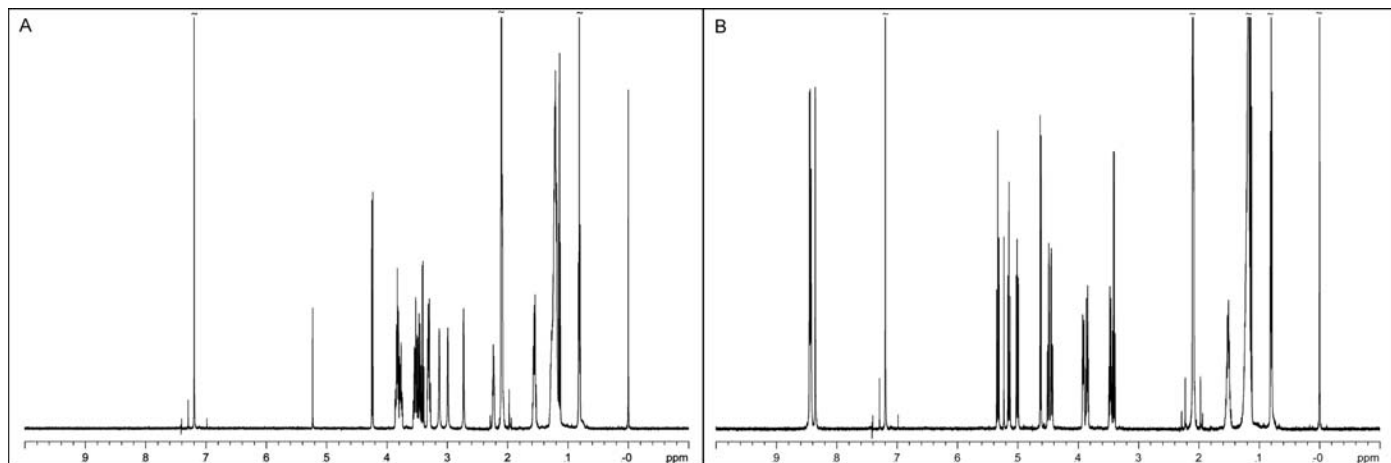
Table 2
Reaction Conditions Explored in the TAI Synthesis

| Duration | Temperature |
|----------|-------------|
| 1.0 hr | 135 °C |
| 1.5 hr | 145 °C |
| 2.5 hr | 160 °C |
| 3.0 hr | 185 °C |

Figure 12
(A) $^1$H NMR spectrum of underivatized octyl-β-D-glucoside shows poor dispersion in the 3 to 4 ppm region. The peak at approximately 7.2 ppm is from the solvent (CDCl$_3$). (B) $^1$H NMR of derivatized octyl-β-D-glucoside shows -NH peaks between 8 and 9 ppm and dispersion of the ring protons from 3 to 6 ppm. This dispersion of peaks and clarity of the spectrum makes analysis of sugars ideal for determining the structure of sugars.
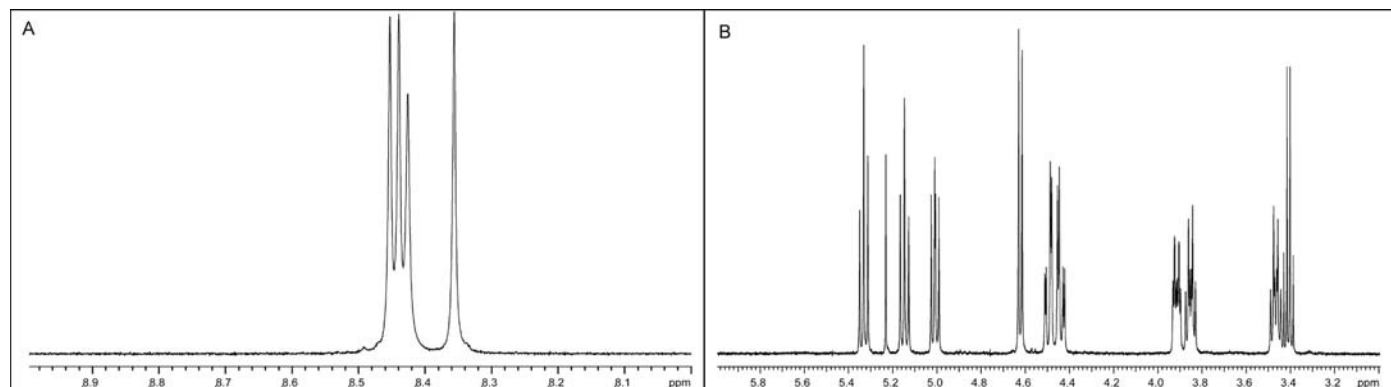


Figure 13
(A) There are four -NH peaks corresponding to four hydroxyl groups on octyl-β-D-glucoside, but with more dispersion than before derivatization. (B) The sugar ring proton peaks show excellent dispersion between 3 and 6 ppm, with noticeable splitting patterns useful for characterizing the sugar.
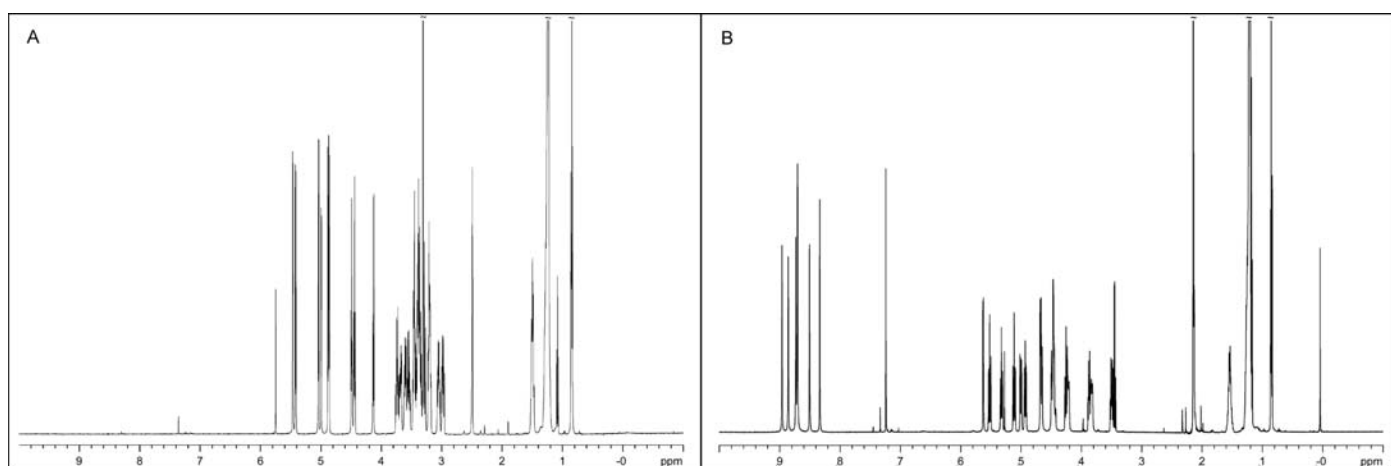


Figure 14
(A) This illustrates the complexity and crowding of dodecyl-β-D-maltoside, where not much information may be obtained—especially in the δ 3 to 4 ppm region. The $^1$H NMR of TAI derivatized dodecyl-β-D-maltoside (B) has NH peaks in the 8 to 9 ppm region and outstanding dispersion of ring protons in the 3 to 6 ppm region.
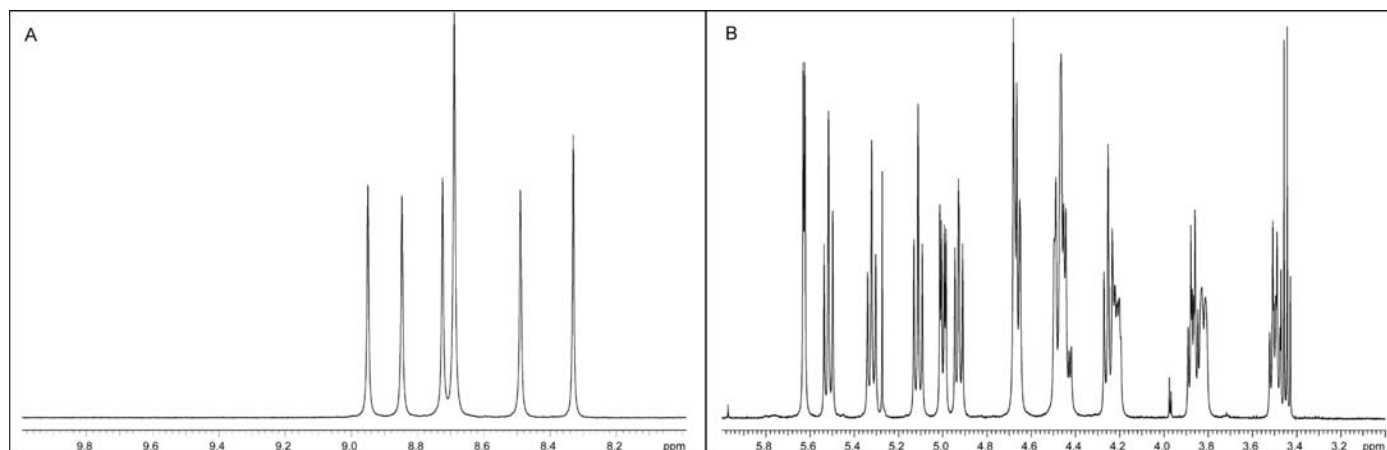
Figure 15
Magnifications of the NH region (A) and ring proton region (B) of derivatized dodecyl-β-D-maltoside showing outstanding dispersion due to TAI.
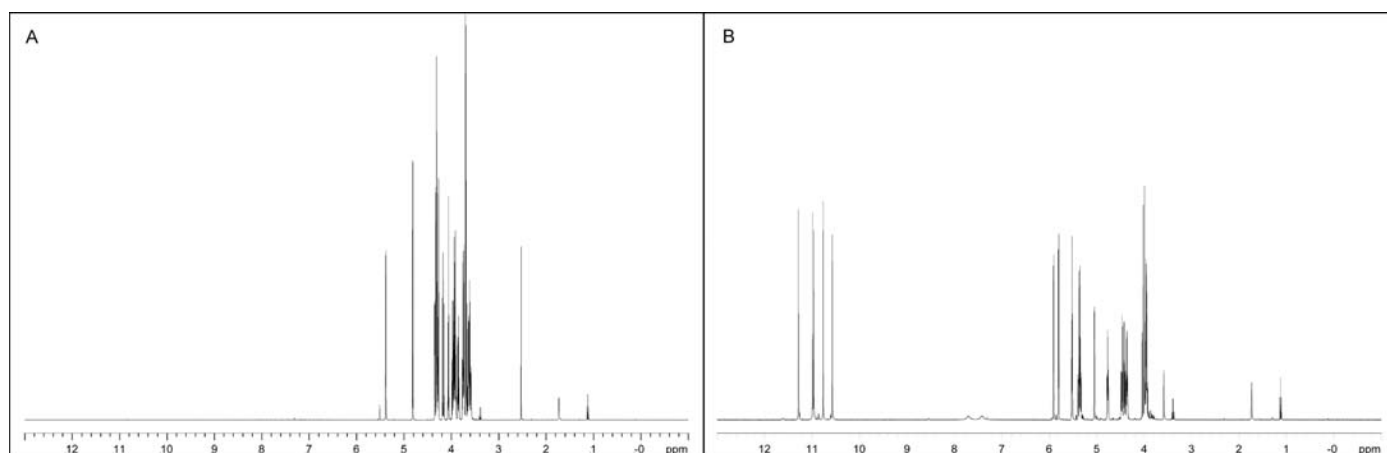


Figure 16
(A) Unaltered sucralose. (B) The TAI-derivatized form of sucralose with the peak dispersion offered by TAI. The peaks between 7 and 8 ppm are side products formed by TAI reacting with water.
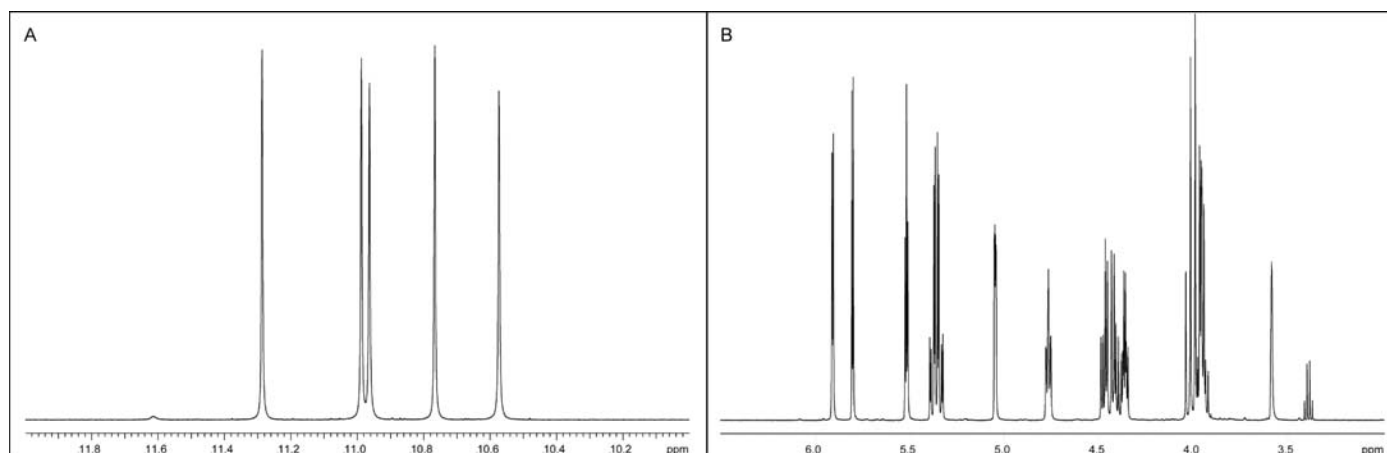


Figure 17
The expansion of the NH region from the sucralose-derivatized molecule shows five distinct peaks corresponding to the five hydroxyl groups before derivatization. Spectrum A shows the excellent dispersion of the ring protons of sucralose, while spectrum B shows the ring proton region of sucralose with clear splitting patterns of the protons.

The next sugar investigated was dodecyl-β-D-maltoside. The $^1$H NMR of dodecyl-β-D-maltoside shows the same spectral crowding as octyl-β-D-glucoside in the 3 to 5 ppm range, where peaks, such as the ring protons and hydroxyl peaks are not identifiable. Subject to derivatization, the $^1$H NMR of the derivatized dodecyl-β-D-maltoside clears up significantly (Fig. 14). Six distinct peaks, which integrate to seven protons, correspond to the seven hydroxyl groups on the sugar and appear far downfield between 8 and 9 ppm. Although there are not seven distinct peaks, due to two of the peaks overlapping, the spectrum still shows much more dispersion than before derivatization. Likewise, the ring proton peaks have tremendous dispersion compared to pre-derivatization with TAI (Figure 16).

The third sugar investigated was sucralose, a two-ring sugar slightly different in structure than octyl-β-D-glucoside and dodecyl-β-D-maltoside. The crowding experienced in the $^1$H NMR of sucralose is no different from that of octyl-β-D-glucoside and dodecyl-β-D-maltoside, where peaks found in the δ 3.5 to 4.5 ppm region prevent interpretation of any useful structural information (Figure 15). But when sucralose is derivatized with TAI, the same pattern of dispersion is witnessed. Five NH peaks are found far downfield at approximately 11.0 ppm and the ring protons show high resolution splitting. More importantly, derivatizing sucralose shows that TAI is not limited to a particular type of sugar.

In addition to $^1$H NMR, we sought to run the HSQC experiment on our derivatized sugars. An HSQC experiment was performed on each derivatized sugar, and the number of peaks that appeared in the $^1$H NMR for each sugar also appeared in its HSQC experiment. For example, octyl-β-D-glucoside gave four NH peaks in the $^1$H NMR, and these same four peaks appeared in the HSQC experiments (Figure 18). Dodecyl-β-D-maltoside gave six peaks in the $^1$H NMR, but the HSQC helped resolve the peaks in the nitrogen dimension showing seven peaks (Figure 19). The opposite is true for sucralose, where there is moderate dispersion in the $^1$H NMR spectrum, but incomplete separation of peaks in the HSQC spectrum (Figure 20). Overall, the HSQC spectra show the same pattern of dispersion and encourage further research.

To prove that TAI is a powerful "isotag" and derivatizing agent, $^{13}$C-labeled TAI was synthesized and reacted with octyl-β-D-glucoside in CDCl$_3$. The $^{13}$C NMR, like that of $^1$H NMR of unlabeled TAI with octyl-β-D-glucoside, shows four well-resolved peaks. The $^{13}$C carbonyl peaks
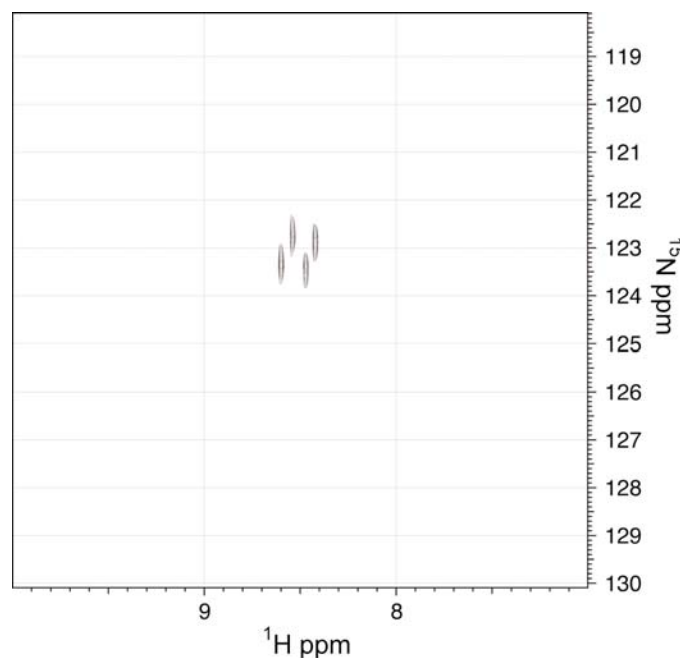


Figure 18
The HSQC of derivatized octyl-β-D-glucoside (896 x 200) at 800 MHz shows four distinct points corresponding to each derivatized hydroxyl group. This supports that the nitrogen is connected to the proton of interest.
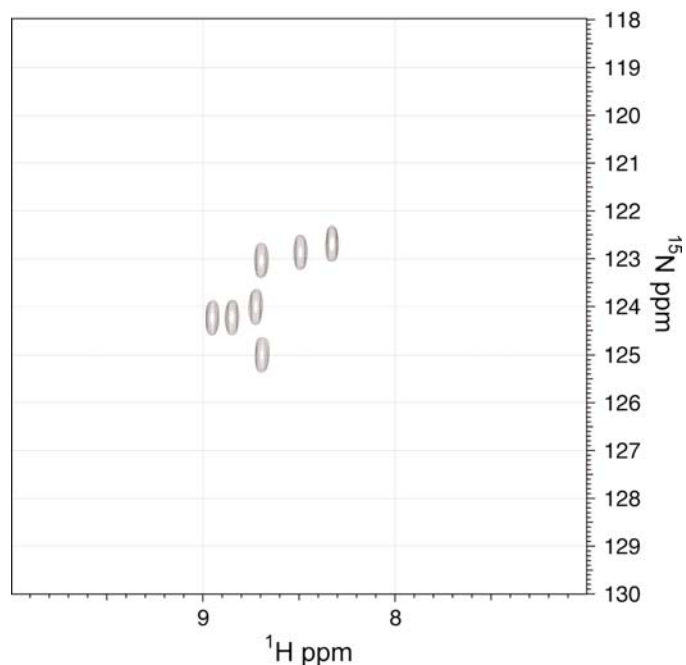


Figure 19
The HSQC for derivatized dodecyl-β-D-maltoside at 500 MHz shows seven distinct peaks characteristic of the seven derivatized hydroxyl groups. The NH peaks that originally overlapped in the $^1$H dimension (Figure 15) are now dispersed in the $^{15}$N dimension. This demonstrates the advantage and importance of expanding beyond one dimensional NMR and into multidimensional NMR.
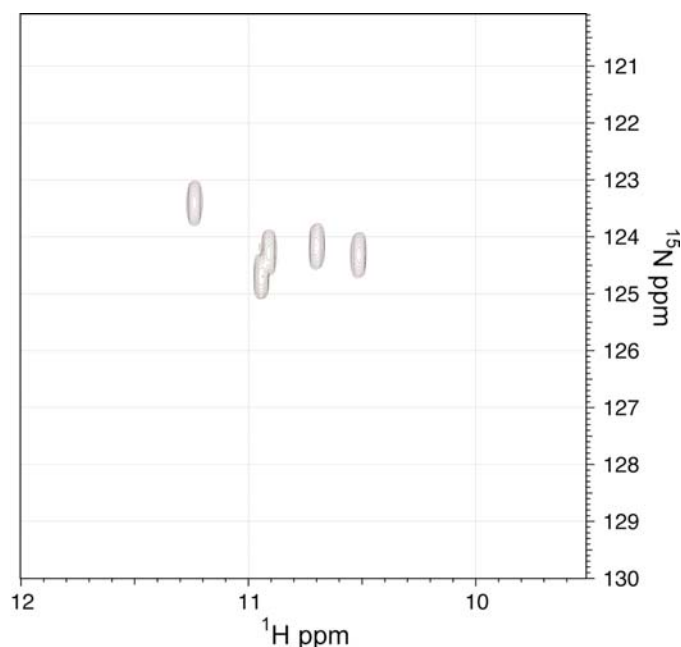
**Figure 20**
The HSQC for derivatized sucralose provides five peaks. The spectrum shows that there are five peaks, but two of the five peaks slightly overlap. These two peaks can be separated using a third dimension in which the carbonyl $^{13}C$ chemical shift supplies additional resolving power.
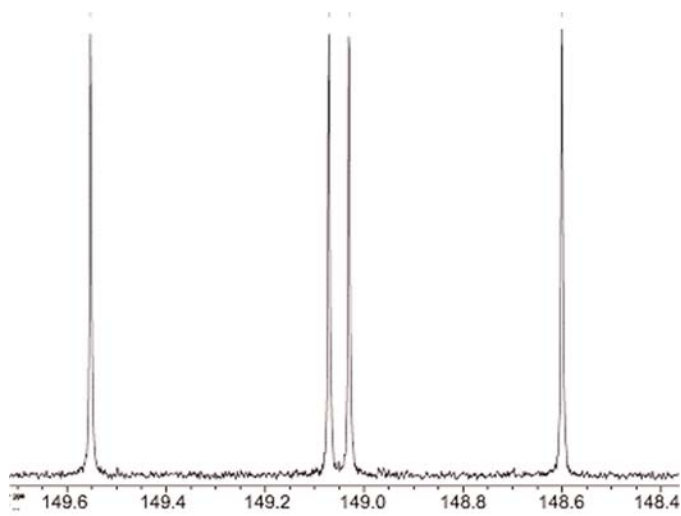


**Figure 21**
Four peaks corresponding to the four hydroxyl groups of octyl-β-D-glucoside that have been "isotagged" with $^{13}C$ TAI.

appear around 150 ppm and show very sharp lines. As typical for an acyl carbamate, the peaks of the four "isotagged" hydroxyl groups appear around 150 ppm (Figure 21). These peaks are moderately dispersed and, like the $^1H$ NMR, allow us to determine the number of hydroxyl groups originally present on the sugar. The combination of labeling with

both $^{15}N$ and $^{13}C$ will be a very powerful way to resolve all the peaks of interest in a three-dimensional NMR spectrum.

## Discussion

In this investigation, we show the possibility of derivatizing sugars with trichloroacetyl isocyanate (TAI) to simplify carbohydrate NMR and to introduce a method for gaining insight into the structure and linkage-isomerism of complex carbohydrates. The three carbohydrates used were octyl-β-D-glucoside, dodecyl-β-D-maltoside, and sucralose, in which the $^1H$ NMR for each sugar is usually extremely complicated and uninformative. However, after derivatization, the peaks in the $^1H$ NMR disperse and give valuable information. More specifically, the derivatized sugars produced the same number of NH peaks as hydroxyl groups, allowing one to count the number of hydroxyl groups; they also produced highly resolved ring protons with distinct splitting patterns that provide structural information. Likewise, the HSQC of the three sugars provided verification that the protons that appeared far downfield in the $^1H$ NMR were directly linked to the nitrogen groups of the TAI derivatives.

Additionally, we have shown that TAI can react with more than one type of sugar: it is not limited to sugars with just one ring or sugars that have a particular polarity. Both octyl-β-D-glucoside and dodecyl-β-D-maltoside lack the chlorine atoms that sucralose has, yet this does not make a difference in the ability to improve their spectra. Furthermore, while octyl-β-D-glucoside is a one ring sugar and dodecyl-β-D-maltoside and sucralose have two rings, there is still no difference in the optimal spectra that they produce. Size, however, might become an issue when sugars exceed two rings, as was illustrated by the overlap of peaks in the spectra for dodecyl-β-D-maltoside and sucralose. Consequently, future research will involve finding a method to avoid spectral crowding of peaks in the NMR spectra of carbohydrates.

Possible future work would involve synthesizing a doubly-labeled TAI with $^{13}C$ and $^{15}N$ as well as other reagents that might offer more dispersion than TAI. Having $^{13}C$, $^{15}N$-TAI would give the opportunity for reaction with any of the three sugars we have already studied, and apply powerful multidimensional heteronuclear methods to assess the ability of $^{13}C$, $^{15}N$ doubly-labeled TAI to give structural information through 3D and 4D NMR experiments. Should TAI provide incomplete resolution, other analogues can be tried. Two molecules, p-toluene sulfonyl isocyanate and pentachlorobenzoyl isocyanate, shown in Figure 22, may be test-
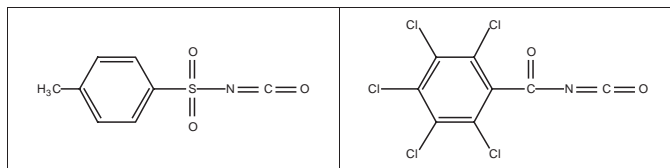
Figure 22
TAI analogues currently under consideration for the derivatization of sugars. The rings might create the possibility for producing greater dispersion than TAI has to offer.

ed in hopes of showing more dispersion than TAI, due to their electron-withdrawing capabilities.

The method of studying "isotagged" sugars by NMR proves to be superior to other methods for a number of reasons. One is that the high resolution afforded by the strong magnetic field of NMR spectrometers gives clean and informative structural information. Another is that NMR experiments are performed on solutions rather than other phases. For example, x-ray crystallographers can only work with crystals, and getting crystals with polysaccharides might not always be possible. Therefore, many researchers in the field are using NMR to help them solve challenges with complex molecules (Shriver, 2004).

## Conclusion

We have introduced a method to "isotag" carbohydrates that will help determine their structures with NMR. Sugars give a ^{1}H NMR spectrum with spectral crowding and little spectral dispersion that makes it almost impossible to obtain any information about their structure. We have shown that with a derivatizing agent called trichloroacetyl isocyanate (TAI), we can alter sugars to obtain spectra that provide valuable information. Moreover, we synthesized ^{13}C-TAI, and a ^{13}C NMR spectrum shows that ^{13}C-TAI also works. We suggest that further experiments expand into 3D and 4D NMR experiments to synthesize a doubly-labeled ^{13}C, ^{15}N-TAI that will provide even more information about the structure of sugars and give insight to their biological importance.

## Acknowledgements

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