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# Letter to the Editor: Assignment of selectively $^{13}$C-labeled cellopentaose synthesized using an engineered glycosynthase

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## Biological context

The remarkable advances made in the fields of protein and nucleic acid NMR spectroscopy depend intimately upon our ability to selectively and uniformly $^{2}$H, $^{13}$C, and $^{15}$N enrich these macromolecules. Although chemical and biochemical methods have been reported for the labeling of saccharides and glycoproteins (e.g., Wu et al., 1998; Junicke et al., 2000; Yamaguchi et al., 2000), spectroscopic analyses of carbohydrates have suffered due to the lack of general and facile approaches for specific incorporation of isotopic labels. Recently, we have developed a chemo-enzymatic method for the synthesis of oligosaccharides using inexpensive sugar precursors and readily available enzymes (Mackenzie et al., 1998; Mayer et al., 2000). Briefly, this method exploits the well-known transglycosylation activity of a retaining glycosidase, while preventing its normal hydrolytic function by site-directed mutation of an essential nucleophilic carboxyl (Asp or Glu) residue. The engineered 'glycosynthase' retains its active site, allowing specific substrate recognition, as well as a catalytic general acid/base residue to facilitate nucleophilic attack of an acceptor sugar on an activated glycosyl donor. Previously, we utilized this approach to synthesize nitroxide spin-labeled cello-oligosaccharides for paramagnetic relaxation measurements of their interactions with cellulose-binding domains (Johnson et al., 1999). In this letter, we describe the use of glycosynthases for the site-specific $^{13}$C-labeling of cello-oligosaccharides.

## Methods and experiments

Cellopentaose, $^{13}$C-labeled in all six positions of the glucopyranosyl ring second from the reducing end (β-D-glucopyranosyl-(1,4)-β-D-glucopyranosyl-(1,4)-β-D-glucopyranosyl-(1,4)-β-D-[$^{13}C_6$]-glucopyranosyl-(1,4)-β-D-glucopyranose, henceforth GGGG*G) was prepared essentially as described (Mackenzie et al., 1998) using the Glu358Ala mutant of *Agrobacterium* sp. β-glucosidase. $^{13}C_6$-glucose (99%; Martek), converted to α-glycosyl fluoride, was used as the activated donor for the first coupling to p-methoxyphenyl-β-D-glucose. The resulting disaccharide was subjected to three subsequent couplings with unlabeled α-glycosyl fluoride, yielding GGGG*G after final de-blocking of the reducing end. At each step, the product was purified by HPLC, and a portion acetylated and characterized by NMR and mass spectrometry according to standard methods. NMR spectra of GGGG*G (3.3 mM) in $D_2O$ were recorded at 25 °C on a Varian Unity 500 MHz spectrometer and processed using FELIX v2.30 (Biosym Technologies; San Diego, CA). $^{1}$H and $^{13}$C chemical shifts were referenced to an external standard of DSS at 0.00 ppm for both nuclei.

## Extent of assignments and data deposition

As illustrated in Figure 1A, the $^{1}$H-NMR spectrum of cellopentaose is highly degenerate. With the exception of the α-anomeric H1 from the reducing end of

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the molecule (5.35 ppm), the resonances from protons in corresponding positions of each of the five glucosyl rings are essentially superimposed. Indeed, the complete $^{1}$H and $^{13}$C assignment of cellopentaose was only recently accomplished using a battery of 2D and 3D homo- and natural abundance heteronuclear experiments recorded at 750 MHz (Flugge et al., 1999). In contrast, the $^{13}$C-edited $^{1}$H-NMR spectrum of GGGG*G is greatly simplified, showing only the expected signals from the seven $^{1}$H's directly bonded to $^{13}$C nuclei in the selectively labeled glycosyl group (Figure 1B). These resonances are completely resolved in the 2D constant-time $^{1}$H-$^{13}$C HSQC spectrum of this compound (Figure 1C). The signals from the anomeric $^{13}$C1 and hydroxymethyl $^{13}$C6 were readily identified from their distinct chemical shifts and inverted signs in this spectrum (Santoro and King, 1992). Resonances from the remaining ring carbons were easily assigned with a HCCH-COSY experiment (Ikura et al., 1991), run to acquire only 2D $^{1}$H-$^{13}$C and $^{1}$H-$^{1}$H correlation spectra (not shown). Finally, one-bond scalar couplings were extracted from a high-resolution $^{1}$H-$^{13}$C HSQC spectrum recorded without $^{13}$C decoupling in the observe dimension. The assignments and coupling constants, deposited in the BMRB (accession number 4975), are: C1/H1 (105.0 ppm, 4.53 ppm, 162 Hz), C2/H2 (75.6, 3.36, 148), C3/H3 (76.8, 3.66, 142), C4/H4 (81.1, 3.67, 144), C5/H5 (77.5, 3.62, 142) and C6/H6/H6′ (62.6, 3.98, 3.82, 144). Measured $^{1}J_{CC}$ values were 48 Hz between C1 and C2, 42 Hz between C5 and C6, and an average of ∼41 Hz for the remaining carbon pairs.

In summary, we have demonstrated the feasibility of preparing selectively labeled oligosaccharides using an engineered β-glucosidase, as well as the ease of obtaining their NMR assignments via $^{1}$H-$^{13}$C correlation experiments. Efforts are underway to increase the battery of glycosynthases, as well as naturally occurring glycosyl transferases, available for the chemo-enzymatic synthesis and isotopic labeling of more complex carbohydrates.

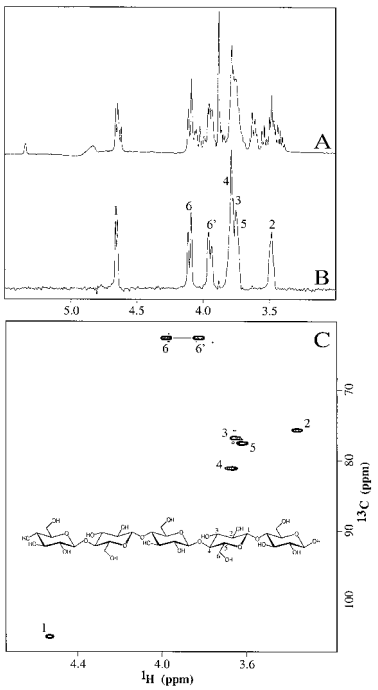


*Figure 1.* Whereas the 1D $^{1}$H-NMR spectrum of GGGG*G (recorded with $^{13}$C-decoupling) is highly degenerate (A), only the signals from the seven protons in the second glucopyranosyl ring are observed in the $^{13}$C-edited $^{1}$H spectrum of this selectively labeled compound (B). Complete resolution of these signals is obtained in a 2D constant-time $^{1}$H-$^{13}$C HSQC spectrum (C). Using a total constant-time delay of 22 ms ∼ 1/($^{1}J_{CC}$), peaks from $^{13}$C1 and $^{13}$C6 are inverted relative to those from the remaining carbons (sign not distinguished). The chemical structure of cellopentaose is shown with atom positions of the $^{13}$C-labeled ring numbered.

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