A New Route to Carbohydrates Enriched with Oxygen Isotopes

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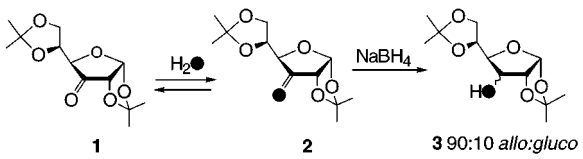
Despite the ubiquitous distribution of oxygen in organic molecules, ^{17}O NMR spectroscopy has not found extensive application in organic chemistry.[1] Although the spectral window for oxygen is large and oxygen chemical shifts are sensitive to subtle structural changes, routine use of this method has been limited because ^{17}O is a quadrupolar nucleus (spin number $I = 5/2$) of low natural abundance (0.037%).[1−3] The method is most seriously limited by rapid quadrupolar relaxation that gives rise to resonances that, at room temperature, are often so broad they cannot be distinguished from the baseline.

Nevertheless, natural-abundance ^{17}O spectroscopic investigations of a number of 2-alkoxytetrahydropyrans[4,5] and monosaccharides[6−9] have been reported by employing elevated temperatures (75−100 °C) in solvents of low viscosity to reduce line widths. These results suggested to us that ^{17}O NMR was of potential use in investigating oligosaccharide conformation. An understanding of oligosaccharide conformation is a critical prerequisite for elucidating the important roles these molecules play in biology. To date, this information has largely been obtained through the use of ^{1}H and ^{13}C NMR spectroscopy. Despite the development of powerful new techniques, these conformational studies are still especially hampered by a paucity of constraints about the glycosidic linkage that can be used to orient the individual monosaccharide rings in three-dimensional space.[10]

A serious limitation of the use of ^{17}O NMR for these conformational studies is that at the temperatures required for natural-abundance spectra to be obtained most oligosaccharides will be present as a rapidly equilibrating mixture of conformers. Obtaining meaningful information about their conformation at physiologically-relevant temperatures will be difficult. Measuring NMR spectra of specifically ^{17}O-labeled carbohydrates is possibly a way to circumvent this problem. Although the line widths in spectra obtained at room temperature will be broad, the spectral signal-to-noise ratio will be dramatically improved and the resonances will be clearly visible above the baseline.

Introduction of oxygen isotopes into carbohydrates is usually done using a hydration/reduction strategy as illustrated in Scheme 1.[11−13] A protected monosaccharide

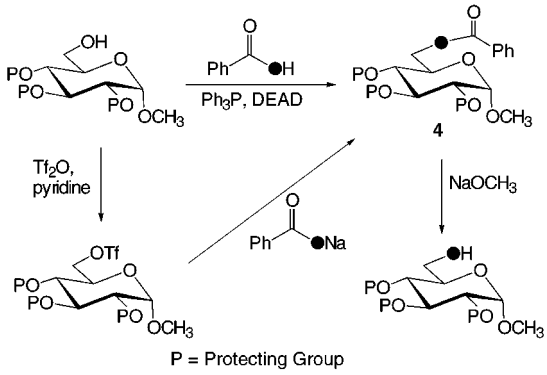
Scheme 1



bearing an aldehyde or ketone (e.g., **1**) is treated with $H_2{}^{17}O$ or $H_2{}^{18}O$ to provide the labeled ketone **2**, which is then converted to the alcohol **3** by reduction. Among the drawbacks of this approach are the following: (1) the exchange process requires a large excess of (expensive) labeled water, which must be recovered after the hydration step, and (2) for ketones, stereoselective reduction of the carbonyl is required, and when this cannot be achieved, stereoisomeric product mixtures are produced. Only two reports[11,14] describe the synthesis of sugars enriched with oxygen-17. In one, the isotope is introduced as in Scheme 1;[11] the other relies on the trapping of carbohydrate-derived radicals by $^{17}O_2$.[14] Only primary carbohydrate alcohols have been synthesized by the latter route, and it is unlikely that the reaction will proceed stereoselectively at secondary centers.

We describe here a simple, efficient method for introducing oxygen isotopes into carbohydrates. Its general applicability has been demonstrated by the synthesis of a series of ^{17}O-enriched mono- and disaccharides. We have also obtained oxygen chemical shift data for these glycans, which were recorded *at room temperature* in acquisition times of less than 10 min.

Scheme 2



In our method, isotopic enrichment is achieved through nucleophilic substitution either via a Mitsunobu reaction or by way of a triflate displacement (Scheme 2). The nucleophile used is either ^{17}O-labeled benzoic acid (prepared by adding a *stoichiometric* amount of 50% enriched $H_2{}^{17}O$ to benzoyl chloride in pyridine) or its sodium salt. The isotope is introduced via a stereoselective, irreversible reaction, which makes this method superior to those previously reported. Due to resonance, the oxygen label in the intermediate benzoate (**4**) will be equally distributed between both ester oxygens. The isotope present in the carbonyl oxygen will be lost upon methanolysis of the benzoyl group, and consequently the final compounds contain 25% ^{17}O enrichment. For the sake of clarity, only the benzoate intermediates leading to labeled products are indicated in Schemes 2−4.

Both routes were used to synthesize a series of labeled monosaccharides (Scheme 3). Starting from alcohols **5**−**9**, we have incorporated ^{17}O labels at both primary and

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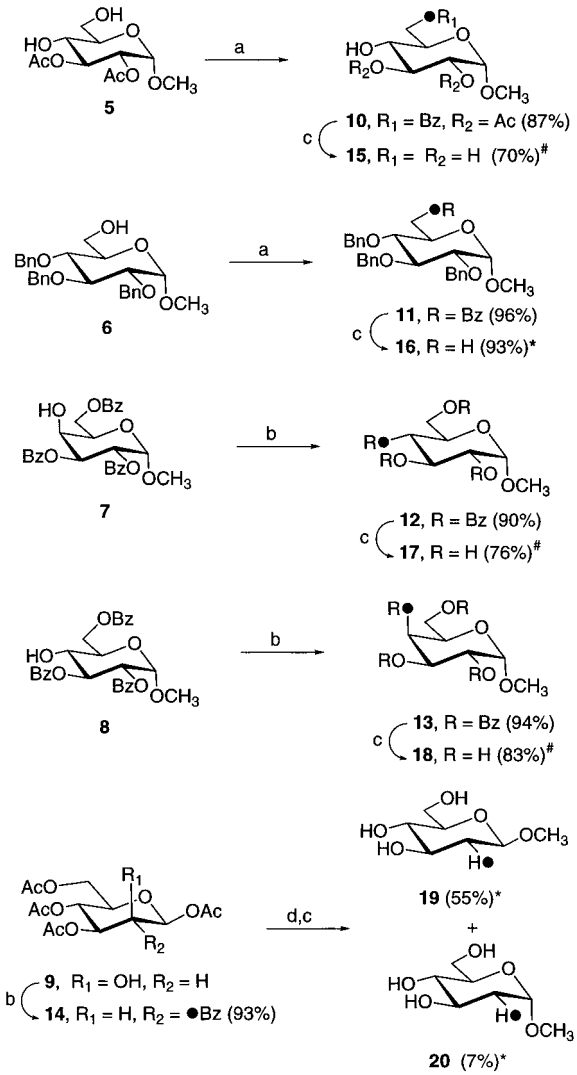
Scheme 3[a]

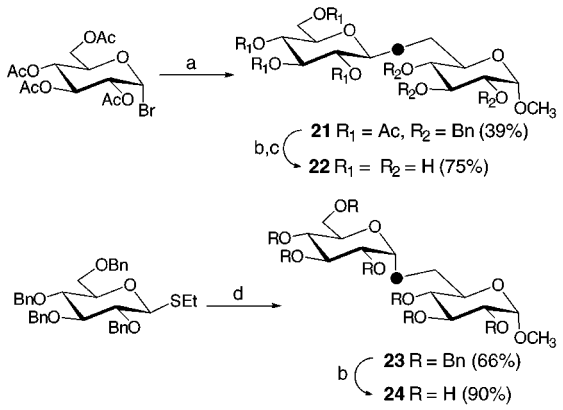


[a] Key: (a) substrate (1 equiv), PhCO●H (1 equiv), Ph_3P (2.0 equiv), DEAD (1.5 equiv), THF, rt; (b) Tf_2O (2.0 equiv), pyridine, 0 °C; then PhCO●Na (1.2 equiv), DMF, rt; (c) $NaOCH_3$, CH_3OH; (d) CH_3OH, $SnCl_4$, CH_2Cl_2, 0 °C → rt. #Yield after recrystallization. *Yield after chromatography.

secondary centers in excellent yield (**15**−**20**). These labeled products can, as expected, be used in glycosylation reactions as illustrated in Scheme 4. Disaccharides **22** and **24** represent the first reported ^{17}O-enriched oligosaccharides.

Oxygen chemical shifts of selected compounds are presented in Table 1. All spectra were recorded at room temperature in the indicated solvents at a concentration of 0.1−0.2 M. Spectra were obtained in 10 min or less with excellent signal-to-noise ratios. With such a small panel of labeled substrates, it is not possible to correlate these oxygen chemical shifts to structure, but some points are worth noting. First, inverting the 4-OH group from equatorial (**17**) to axial (**18**) results in a nearly 20 ppm upfield shift of this resonance. A similar effect has been previously reported from natural abundance spectra.[6] Second, glucosylation of **15** to provide **22** results in a 40 ppm shift of the labeled oxygen to lower field. Third, there is a 10 ppm shift difference between the two disaccharides. It should also be mentioned that, in comparison with the monosaccharides,

Scheme 4[a]



[a] Key: (a) **16**, AgOTf, collidine, CH_2Cl_2, −20 °C → rt; (b) H_2, Pd/C, CH_3OH; (c) $NaOCH_3$,CH_3OH; (d) **16**, *N*-iodosuccinimide, AgOTf, CH_2Cl_2. All yields after chromatography.

Table 1. ^{17}O NMR Chemical Shifts

compd	shift	compd	shift
10[a]	137.1;[c] 324.4[d]	**17**[b]	9.3
11[a]	141.1;[c] 335.6[d]	**18**[b]	−7.6
12[a]	154.5;[c] 335.5[d]	**19**[b]	7.9
13[a]	145.0;[c] 345.5[d]	**20**[b]	5.4
14[a]	146.9;[c] 334.7[d]	**22**[b]	32.0
15[b]	−10.8	**24**[b]	21.4
16[a]	−16.7		

[a] In $CDCl_3$, referenced to external H_2O at 0.0 ppm. [b] In H_2O, referenced to H_2O at 0.0 ppm. [c] PhCO●R shift. [d] PhC●OR shift.

the disaccharides have significantly broadened line widths. This observation is in accord with previous investigations on monosaccharides[6] in which the resonance of the ring oxygen was reported to be significantly broader than those arising from the hydroxyl oxygens.

In conclusion, we report an efficient method for introducing oxygen isotopes into carbohydrates. The method is superior to those previously reported in that it uses reactions that are irreversible and stereoselective. Furthermore, in contrast to other methods, only a slightly more than stoichiometric amount of the labeling species is required. Although we have synthesized only ^{17}O labeled substrates, the method should proceed equally well for ^{18}O enrichment. A current limitation is the 50% loss of label due to resonance in the nucleophile, and we are investigating alternate nucleophiles. We have also demonstrated that at enrichment levels of 25% in solutions of moderate concentration it is possible to obtain ^{17}O spectra at room temperature using short acquisition times. Therefore, this technique could, in principle, be used to probe oligosaccharide conformation at biologically relevant temperatures. In progress is the synthesis of a larger panel of labeled carbohydrates that will be used to further explore this possibility.

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Supporting Information Available: Representative synthetic procedures and ^1H, ^{13}C and ^{17}O NMR spectra for compounds **15**, **17**−**20**, **22**, and **24** (22 pages).

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