LA-UR-97- 3611

Approved for public release; distribution is unlimited

Title: **Stable Isotope Labeling of Oligosaccharide Cell Surface Antigens**

Author(s): C. J. Unkefer, L. A. Silks III, R. A. Martinez, R. Wu, and C. Orji, CST-4
D. Live and S. Danishefsky, Memorial Sloan-Kettering Cancer Center

Submitted to: DOE Office of Scientific and Technical Information (OSTI)

Los Alamos
NATIONAL LABORATORY

DISCLAIMER

Stable Isotope Labeling of Oligosaccharide Cell Surface Antigens

Clifford J. Unkefer,* Louis A. Silks III, Rodolfo A. Martinez, Rulian Wu and Charles Orji
Chemical Science and Technology Division, Los Alamos National Laboratory

David Live and Samual Danishefsky
Memorial Sloan-Kettering Cancer Center

Abstract

The overall goal of this Laboratory Directed Research and Development (LDRD) project was to develop new methods for synthesis of ^{13}C-labeled oligosaccharides that are required for nuclear magnetic resonance (NMR) studies of their solution conformation. Oligosaccharides are components of the cell's outer surface and are involved in important processes such as cell-cell recognition and adhesion. Recently, Danishefsky and coworkers at Slone-Kettering Cancer Center developed a method for the solid-phase chemical synthesis of oligosaccharides. The specific goal of this LDRD project was to prepare uniform ^{13}C-labeled aldohexose precursors required for the solid-phase synthesis of the Lewis blood-group antigenic determinants. We report the synthesis of ^{13}C-labeled D-glucal, D-galactal and Fucosyl precursors. We have been collaborating with the Danishefsky group on the synthesis of the Lewis oligosaccharides and the NMR analysis of their solution conformation.

Background and Research Objectives

The cellular envelopes of many types of animal cells are coated with oligosaccharides that are involved in important processes such as cell-cell recognition and adhesion. In addition, oligosaccharides are antigenic determinants that differentiate cell types, such as the the blood groups (A, B, AB, and O). Cell-surface oligosaccharides are also recognized by and serve as receptors for many infectious agents, including viruses and bacterial toxins. All of these functions of oligosaccharides involve the specific binding of the cell-surface oligosaccharide by a protein molecule. The design of pharmaceutic agents that can specifically inhibit the binding of a bacterial toxin to a oligosaccharide receptor would be greatly facilitated by knowledge of the conformation of both the free and bound forms of the oligosaccharide. Advancing our understanding of the conformation of oligosaccharides is dependent on application of solution structural methods such as NMR spectroscopy for determining their structure. As has been demonstrated in the NMR analysis of protein structures, stable-isotope labeling is required for increasing spectral

* Principal Investigator, E-mail: cju@lanl.gov

resolution and assigning the resonances. At present it is not possible to label most oligosaccharides with ^{13}C.

Because most of the interesting oligosaccharide structures derive from mammalian sources, biochemical approaches to labeling them are impossible. Recently, Danishefsky and coworkers at Slone-Kettering Cancer Center developed a method for the solid-phase chemical synthesis of oligosaccharides. The goal of this Laboratory Directed Research and Development (LDRD) project was to prepare uniform ^{13}C-labeled aldohexose precursors required for the solid-phase synthesis of the Lewis blood-group antigenic determinants. The solid phase synthesis of the Lewis oligosaccharides required three precursors : a D-glucose or N-acetyl-D-glucosamine precursor (compound **1**), a D-galactose precursor (compound **2**), and an L-fucose precursor (compound **3**). These precursors are shown in Figure 1. Compounds **1** and **2** contain three asymmetric carbons (C3–C5); compound **3** contains four asymmetric centers (C2–C5). Using uniformly ^{13}C-enriched D-glucose as a chiral precursor, we have prepared **1**, **2**, and **3** in good yield. In two steps we converted D-[U-$^{13}C_6$]glucose to **1**; the stereochemistry at C3, C4, and C5 is retained. Synthesis of **2** required the inversion of the center at C4. The conversion of D-glucose to L-fucose requires the inversion of three asymmetric centers (C2, C3, and C5). We are presently collaborating with the Danishefsky group on the synthesis of the Lewis oligosaccharides and the NMR analysis of their solution conformation.

Importance to LANL's Science and Technology Base and National R&D Needs

Los Alamos National Laboratory (LANL) was the first to separate the stable isotopes of carbon, nitrogen and oxygen and has been home to the National Stable Isotope Resource (NSIR) since the mid-seventies. The overall goal of the Los Alamos NSIR is to develop new applications of stable isotopes in biomedical research by making new labeled compounds available to the nation's biomedical researchers. The NSIR develops new methods for synthesis of labeled compounds. The current focus of the NSIR is to support the structural biology community by preparing labeled amino acids and nucleotides which are used for the structural determination of proteins and oligonucleotides. An important third class of biological macromolecules are the complex carbohydrates including oligo- and polysaccharides. It is essential to stable-isotope-label these complex carbohydrates for nuclear magnetic resonance (NMR) studies of their solution conformation.

This project allowed LANL to expand its stable isotope technology base into the labeling of carbohydrates and fulfilled a national need by providing labeled

oligosaccharides. In addition, the cell surface receptors for many of the microbial toxins including cholera, tetanus, botulinum A,B,C,E,F, perfringens and shiga toxins, and ricin are the complex oligosaccharides present as membrane-bound lipopolysaccharides. Because of their incredible potency, these toxins can be used effectively as biological weapons. LANL is developing a new generation of sensors that will detect binding of these bacterial toxins to the cell surface oligosaccharides. The technology base in carbohydrate chemistry provided by this LDRD project is applicable in biological warfare agent sensor development.

Scientific Approach and Accomplishments

As discussed above, the goal of this project was to label monomeric carbohydrates for biochemical and structural studies by NMR spectroscopy. We have been exploring both the enzymatic and chemical routes to some of the more common monomeric carbohydrates. We report the optimized syntheses of two carbohydrate monomers, of D-[1,2,3,4,5,6-$^{13}C_6$]Glucal (**1**) and D-[1,2,3,4,5,6-$^{13}C_6$]Galactal (**2**), which are useful in the solution and solid phase construction of a variety of oligosaccharides and other glycoconjugates from an economical labeled precursor source, D-[U-^{13}C]glucose. In addition, we have also developed a 12-step route for the conversion D-[U-^{13}C]glucose to a synthetic precursor (**3**) that will be used to label L-[U-^{13}C]fucosyl residues. At present we are preparing the labeled fucose.

Our high yield routes to D-[U-$^{13}C_6$]Glucal (**1**) and D-[U-$^{13}C_6$]Galactal (**2**) are diagrammed in Figure 2. Peracylation of D-[U-^{13}C]glucose (**4**) was accomplished using acetic anhydride in the presence of a catalytic amount of hydroperchloric acid. After two hours this was followed by treatment with glacial acetic acid/HBr. An hour later, the tetra-*O*-acetyl-D-glucosyl bromide was the sole product detected in the reaction mixture by thin-layer chromatography. Ultrasonic-promoted reduction of the bromide in the presence of zinc gave the tri-*O*-acetyl-D-[U-^{13}C]glucal (**1**) in a reproducible 87% yield from D-[U-^{13}C]glucose (**4**). This straightforward process is comparable to a recent report of the use of a Ti(III) species $(Cp_2TiCl)_2$ promoted formation of the glucal from 2,3,4,6-tetra-*O*-acetyl-a-D-glycopyranosyl bromide (82%).

The D-galactal (**2**) was constructed using the above labeled tri-*O*-acetyl-D-[U-$^{13}C_6$]glucal (**1**). Removal of the acetyl groups of tri-*O*-acetyl-D-glucal was effected using a methanolic solution of sodium methoxide in 97% isolated yield. It should be noted that

chromatographic purification of the triol using 5-10% methanol in diethylether as the eluent resulted in complete decomposition. Protection of the primary alcohol with *tert*-butyldiphenylsilyl chloride in pyridine proceeded in 88% yield. Selective protection of the 3-hydroxyl group with benzoyl chloride at -78°C afforded **5** in 90% isolated yield. Simply chilling the reaction to -78°C and slowly adding a methylene chloride solution of benzoyl chloride to the allylic alcohol increased the yield of the reaction from the reported 72%.

The next step required the inversion of the 4-hydroxyl group which can be considered to be fairly hindered. The use of standard Mitsunobu conditions (benzoic acid, Ph_3P, DIAD, 25°C) gave little, if any, inverted product ester. Based on a report that the acid component can have a positive effect on the course of the reaction, we investigated the use of *p*-nitrobenzoic acid. We were pleased to observe the formation of **6** using these conditions. Optimization of this step is then accomplished using toluene as the solvent, and performing the reaction at elevated temperatures gave rise to a 90% purified yield of **6**. X-ray crystallography indicates that the Mitsunobo reaction occurred with inversion of the stereochemical center at C4 (data not shown). Cleavage of the esters using methanolic methoxide afforded the diol in 90% yield. The resulting diol was treated with 1,1'-carbonylbis(2-methylimidazole) in THF to give **2** in 97% yield.

Noteworthy in this sequence of reactions is the following: 1) the optimization of the conversion of glucose to the glucal, thereby conserving the isotope; 2) the increased yield of the selective protection of the allylic alcohol from the reported 77% to 90%; 3) the successful Mitsunobu reaction on a hindered 2° alcohol; and 4) the increased yield of the carbonate from the reported 74% to our 97%. Overall, this process represents the first example of ^{13}C labeling of both the D-glucal and the D-galactal. These monomers will likely find use in both the Danishefsky oligomeric synthesis methods and in solution structural determinations by NMR spectroscopy.

Publications

Lodwig, S.N., and C.J. Unkefer, "Stereoselective Synthesis of Stable Isotope-Labeled L-a-Amino Acids: Electrophilic Amination of Oppolzer's Acyl Sultams in the Synthesis of L-[^{15}N]Alanine, L-[^{15}N]Valine, L-[^{15}N]Leucine, L-[^{15}N]phenylalanine and L-[1-^{13}C,^{15}N]Valine," *J. Labelled Compounds and Radiopharmaceuticals* **38**, 239 (1996).

Lodwig, S.N., L.A. Silks III, and C.J. Unkefer, "Synthesis of 1-Chloro-1-[^{15}N]Nitrosocyclohexane: an Electrophilic Aminating Reagent," *J. Labelled Compounds and Radiopharmaceuticals* **38,** 161 (1996).

Oppenheimer, J., and L.A. Silks III, "Synthesis of 2-Phenyl-1,2-benziso[^{77}Se]selenazol-3(2H)-one; Ebselen," *J. Labelled Compounds and Radiopharmaceuticals* **38,** 281 (1996).

Orji, C.C., J. Kelly, et al., "The First Synthesis of [9-^{15}N]-2'-Deoxyadenosine," *J. Chem. Soc., Perkin Trans. 1* **7**, 595 (1996).

Wu, R., and L.A. Silks III, "Synthesis of D-[1,2,3,4,5,6-$^{13}C_6$]Glucal and D-[1,2,3,4,5,6-$^{13}C_6$]Galactal Using D[U-$^{13}C_6$]Glucose as the sole carbon source," (submitted to *Carbohydr. Res.*).

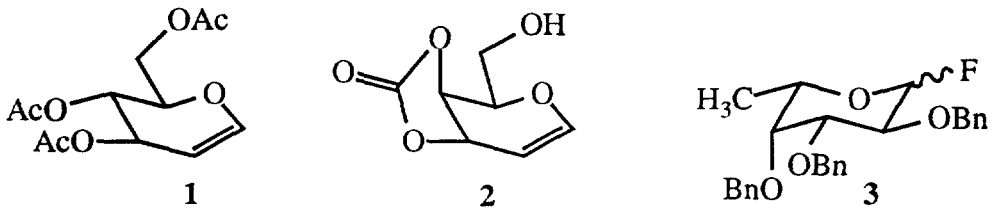

Figure 1. The precursors that will be used for solid-phase synthesis of the Lewis oligosaccharides (Ac is an acetyl group, and Bn is a benzyl group). Compound (**1**) is the precursor of D-glucose and N-acetyl-D-glucosamine, (**2**) is the precursor of D-galactose, and (**3**) is the precursor of L-fucose.

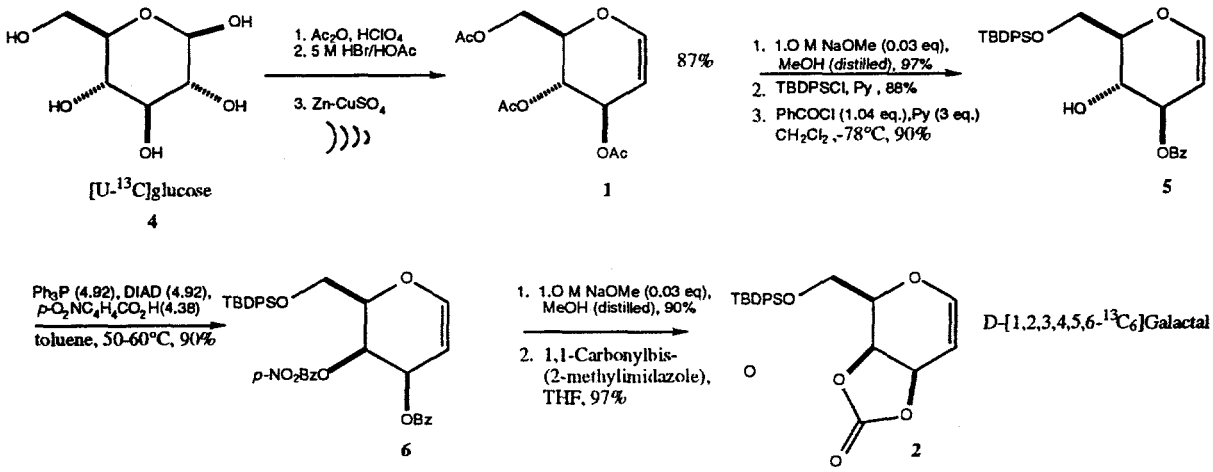

Figure 2. Chemical synthesis [U-$^{13}C_6$]Glucal and [U-$^{13}C_6$]Galactal.