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DEUTERIUM LABELLING OF A GLYCOSPHINGOLIPID USING AN ULTRASONICATED NICKEL CATALYST

Eugene A. Cioffi and James H. Prestegard

Department of Chemistry, Yale University, New Haven, CT 06511

Abstract: Raney Nickel Catalyzed hydrogen-deuterium exchange in a cerebroside and a monosaccharide is conducted under very mild conditions using a mixed solvent system and ultrasonic irradiation. The mild conditions appear to enhance the site selectivity of the exchange.

Deuterium and tritium labelling of carbohydrates and their derivatives has wide applicability in the biophysical and biochemical sciences. Deuterium labels have served as convenient NMR probes into the molecular organization and structural dynamics of micelles and membranes.[1] Both deuterium and tritium have served as probes of cellular metabolic and biosynthetic activity.[2] As a result of these applications, a great deal of effort has been directed at the incorporation of hydrogen isotopes into carbohydrates using multistep synthetic and biosynthetic procedures.[3] In cases where absolute stereospecificity is required, this is perhaps justified. In cases where isotopes incorporated at different sites need not be distinguished, or in cases where sites may be distinguished in the process of analysis, less selective catalytic means of incorporation may provide a viable alternative. This is particularly the case when the labelled product is a structurally complex natural product, such as the cell-surface glycolipids so often involved in cellular recognition and differentiation.[4]

Catalytic deuteration of non-reducing monosaccharides using Raney-Nickel in D_2O was demonstrated some years ago.[5] The high temperatures, long incubations, and limited solubility of glycolipids in D_2O would, however, raise questions as to applicability to more complex, less stable systems. Herein we present a modification of the Raney-Ni deuteration procedure which makes catalytic labelling of glycolipids a viable alternative to multistep synthesis.

We have found a solvent pair consisting of THF (83%) and D_2O (17%) to be an effective solvent pair for catalytic H $\rightarrow$ D exchange. As previously shown for other organic substrates, H $\rightarrow$ D exchange proceeds at rates comparable to D_2O alone.[6]

Further, we have found that sonication of the catalyst during the reaction allows rapid incorporation of deuterium at moderate temperatures. The successful application of ultrasonic irradiation in promoting heterogeneous and homogeneous organic reactions is well documented.[7-15] In heterogeneous reactions, the degree of enhanced reactivity has been hypothesized to arise, inter alia, from acoustic cavitation[10] and increased bulk transport.[11,12] However, preliminary results with sonication before, rather than during the reaction suggest the enhancement here to be more closely associated with modification of the catalyst surface.

In a typical experiment, a 3-necked 25 ml flask equipped with a central ground-glass

joint (~14 cm long) was charged with 20 mg. of substrate, 0.5 ml (settled volume) of deuterated Raney nickel,[5a,17] 10 ml of THF, and 2 ml of deuterium oxide. The flask was immersed in a warm water bath (*ca.* 40°C), and the flask contents gently swept with a stream of inert gas. (N_2 or Ar). Sonication was conducted for ca. one-half hour using a Bransonic Model W-200 P Sonicator equipped with a titanium tip, immersed directly through the appended central joint into the reaction vessel. The product mixture was centrifuged and filtered throuht a short bed of ion-exchange resin (Dowex-100) to remove trace paramagnetic impurities.[18] Subsequent solvent evaporation (in vacuo) and lyophilization afforded pure crystalline products in nearly quantitative yields.

Deuterium incorporation can easily be followed by the disappearance of signals in proton NMR spectra. The data for a simple glycolipid, galactosyl cerebroside, and its headgroup analog, 1-O-Methyl-β-D- galactopyranoside, are presented in Figures 1 and 2 respectively. The spectra were acquired at 490 MHZ, and assigned in the case of the fully protonated molecules by two-dimensional NMR methods. All proton chemical shifts are in parts per million (δ), relative to residual HDO at 4.70 ppm. Inspection of Figure 1 indicates significant deuterium incorporation at C_3 (71%) and at C_4 (74%) of galactosyl cerebroside. Not shown is additional incorporation into the allylic double bond, although the vicinal methine resonance at ca. 3.9 ppm is shifted upfield to ca. 3.4 ppm upon reduction, as indicated.

The near exclusive incorporation at C_3 and C_4 among the hydroxylated carbons of the headgroup was not expected, based on the more extensive incorporations and racemizations observed with monosaccharides in refluxing D_2O.[5a,18,19] Figure 2, showing deuterium incorporation into 1-O-Me-β-D-galactopyranoside, shows similiar stereospecificities, suggesting the origin to be in the alteration of catalyst, solvent, and reaction conditions, rather than the presence of the hydrophilic ceramide moiety.

Thus, the technique presented extends methodology previously confined largely to simple saccharides to more complex glycolipids. Moreover, the selectivity of deuterium incorporation seems to be improved, in that there is no incorporation at C_6, as is normally observed under refluxing conditions. Further, there appears to be some improvement in the overall product distrubution, as evidenced by the lack of any rearranged by-products in the ultrasonicated reaction.

We are currently investigating the basis for improved selectivity, and exploring the utility of this technique for selective isotopic incorporation of deuterium and possibly tritium into other complex biomolecules.

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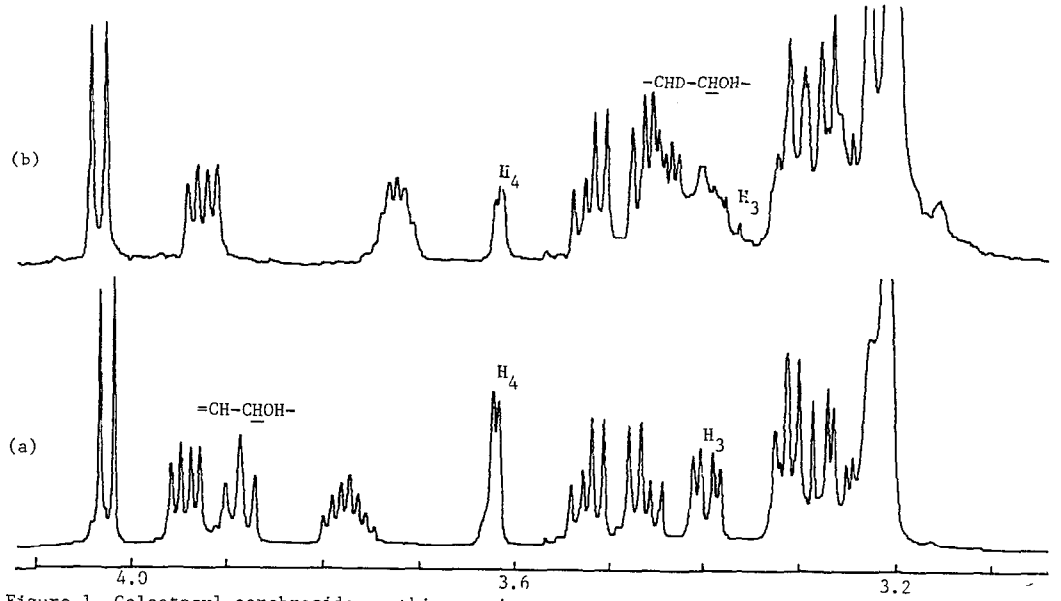

Figure 1. Galactosyl cerebroside, methine region
(a) before catalytic exchange
(b) after catalytic exchange

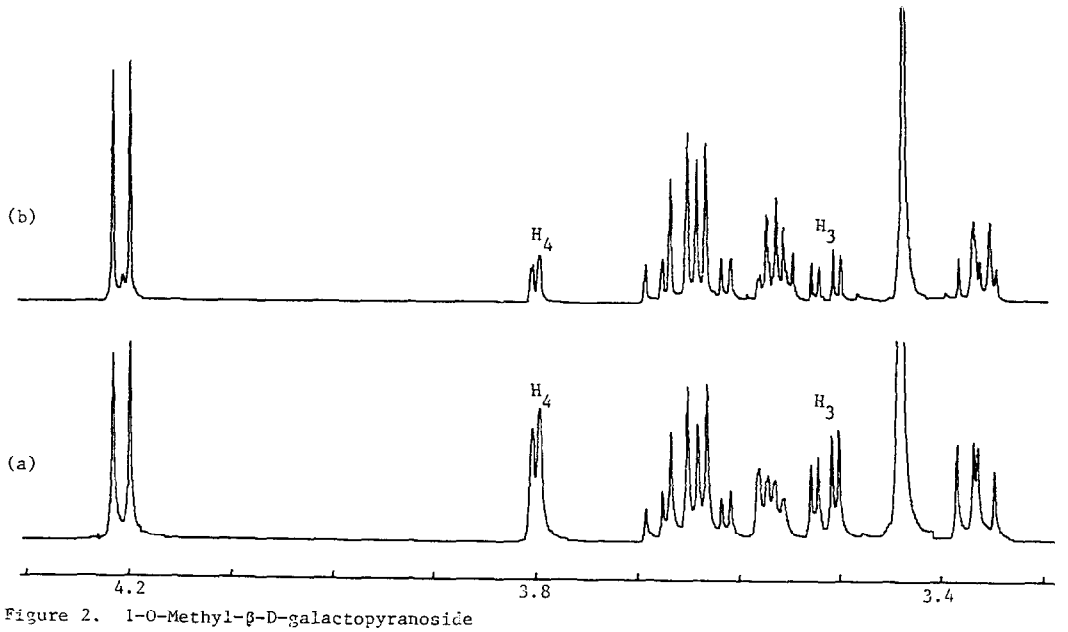

Figure 2. 1-O-Methyl-β-D-galactopyranoside
(a) before catalytic exchange
(b) after catalytic exchange

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