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Short Research Article

Facile C–H bond activation for deuterium and tritium labeling of glycoconjugates conducted in ultrasonic and microwave fields: A review†

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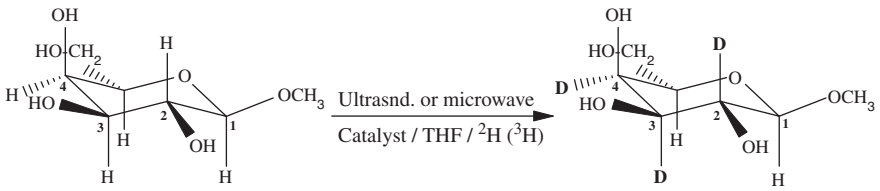
Deuterium and tritium labeling of carbohydrates and glycoconjugates has very wide applicability in the biochemical and biophysical fields. Carbohydrates function as receptors for a variety of hormones and play critical roles in cell recognition and differentiation. Deuterium labeled compounds are frequently utilized in the conformational analysis of oligosaccharides, as well as other glycoconjugates, to alleviate resonance overcrowding in ^{1}H NMR spectroscopy.

Raney® nickel catalyzed $^{1}H \rightarrow {}^{2}H$ (^{3}H) isotopic exchange is conducted under mild conditions using either ultrasonic or microwave irradiation. The initial and overall rates of isotopic exchange are modulated by the presence of transition metals. Only the Raney-nickel® type alloys afforded stereospecific C–$^{1}H \rightarrow$ C–^{2}H isotopic exchange in an ultrasonic or microwave field. Typical supported precious metal catalysts, supported Ni catalysts, singular metal catalysts, or multi-phasic catalysts were ineffective or caused substrate decomposition.

We have explored new digestion conditions for the preparation of highly active Raney nickel catalysts and have developed new protocols for the stereospecific incorporation of ^{2}H (^{3}H) in a wide variety of glycoconjugates using our facile C–H → C–D (C–T) exchange reactions. The regioselectivity of isotope incorporation is controlled by the judicious use of modified catalysts, co-solvents, and the isotopic source.



Reactions are easily performed using either a dedicated ultrasound probe, a slightly modified commercial microwave oven, or a dedicated commercial microwave synthesis unit. Research over the past several years is the subject of this attenuated *review*.

Introduction

Deuterium and tritium labeling of carbohydrates and glycoconjugates has wide applicability in the biochemical and biophysical fields.[1–5] There are a number of multi-step synthetic methods for regioselectively introducing ^{2}H and ^{3}H into simple carbohydrates,[6] which include the reduction of appropriate oxo-derivatives with metal hydrides and catalyzed exchange in solution with ^{2}H or ^{3}H gas.[7–11]

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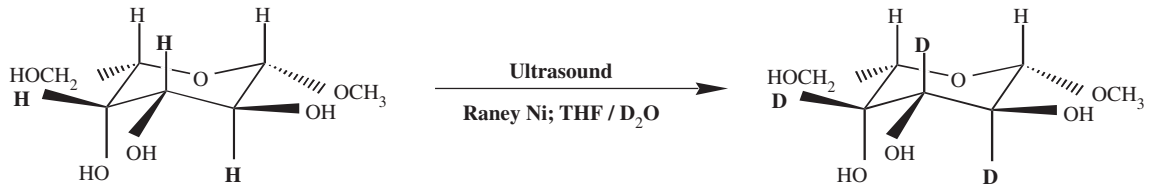

Scheme 1

We have developed methodologies for reversible and selective ^{2}H (and ^{3}H) isotopic incorporation into non-reducing carbohydrates and glycoconjugates.[6a–g] In a series of on-going investigations, we have developed new methodologies[6a–g] to address the problems of selective isotopic incorporation (Scheme 1).

Kinetic comparison of this H/D exchange reaction demonstrated that ultrasonication of the catalyst, either prior to or during the reaction, substantially ameliorated the overall selectivity and rates of ^{2}H incorporation at ambient or low temperatures.[6b,c] A subsequent combinatorial study utilized a small number of complex molecules, possessing a wide variety of 'typical' functionality, to evaluate the applications and limitations of this exchange reaction in multi-step synthetic protocols.[6e] This study found that most organic functionalities (ketones, esters, acids, etc.) where generally tolerant of Raney® nickel under ultrasonic irradiation, remarkably *atypical* of documented reactivity.[12] Isolated and primary hydroxyls, and non-vicinal secondary hydroxyls (e.g. glycerin and 1,3-dihydroxypropane) are untouched by the C–H → C–D exchange event.

Results and discussion

For successful deuterium or tritium isotopic incorporation into glycoconjugates, a number of variables were examined in order to attempt to optimize the overall C–H → C–D (C–T) exchange protocol. The variables that have been optimized are subdivided into: (1) control of regioselectivity; (2) isotopic source; (3) catalyst preparation.

Control of regioselectivity

The reversible ultrasonic exchange labeling technique for C–H bond activation in glycoconjugates has been found to be totally stereospecific in all experiments conducted to date, and the microwave reactions parallel the ultrasonic reactions, albeit in a shortened reaction time. Regioselective control has proved to be the most problematic, and we have approached this from three different, yet complimentary pathways. These pathways are catalyst-induced control (*kinetic*), solvent-induced control (*thermodynamic*), and substrate-induced control (*steric*).

Catalyst-induced control. We found that *only* the Raney® nickel type catalysts afford facile $^{1}H \rightarrow {}^{2}H$ exchange.[6e,f] Typical supported precious metal catalysts (e.g. Pd on carbon), supported Ni catalysts (e.g. Ni on kieselguhr), or singular metal catalysts (e.g. Ni) were either totally ineffective or caused substrate decomposition. Biphasic and triphasic mixed metal catalysts (e.g. Ni coated Al) similarly were ineffective. Of the Raney® type alloys, only the co-melted and digested Raney® nickel alloys afforded stereospecific isotopic exchange. Marked differences in the initial or overall exchange rates in doped or plated catalysts at individual sites (i.e. equatorial C–H's *vs* axial C–H's *vs* syn-axial C–H's) effect kinetic control of regioselectivity, if only low levels of isotopic enrichment are desired. The observed overall rates of C–H → C–D exchange for Raney® nickel catalysts are equatorial-H>axial-H ≫ *syn*-axial-H.

Solvent-induced control. A study of ultrasonically driven C–H → C–D isotopic exchange conducted in eight different solvent pairs demonstrated that simple co-solvent modification has a pronounced effect on the overall exchange regioselectivity.[6d] A co-solvent pair consisting of *p*-dioxane/D_2O promoted axial and equatorial exchange ('switching-off' *syn*-axial exchange); a 1,2-dimethoxyethane/D_2O mixture promoted *syn*-axial and axial exchange ('switching-off' equatorial C–H exchange). The solvent pairs need not be miscible for successful $^{1}H \rightarrow {}^{2}H$ exchange; a solid catalyst suspension in a mixed-pair emulsion afforded levels of ^{2}H incorporation similar to that of totally miscible solvent pairs. The reversibility of this exchange technique for site-specific ^{2}H (or ^{3}H) incorporation may be exploited using various solvent systems. Thus, deuterium incorporation at a single ring carbon has a well-defined high level of ^{2}H isotopic enrichment. The stereochemical integrity of all exchangeable carbon centers are maintained, under mild reaction conditions without need of substrate isolation or additional reagents.

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Substrate-induced control. Judicious incorporation of –OH (or –NH) protecting groups may assist in regioselective control of exchange labeling.[6e]

Isotopic source

In most investigations we used D_2O for the available ^2H pool, which unquestionably performed without complications. For ^3H incorporation, the use of T_2O appears not desirable due to handling, storage, and disposal regulations.[6a–f] Ideally, ^3H gas or a '^3H surrogate' such as R-OT (i.e. $R = C_2H_5$) appeared to be feasible. However, we have had little success using C_2H_5-OD (or CH_3-OD) under a ^2H atmosphere for C–H → C–D exchange. An effective compromise has been achieved using *in situ* generated ^3H_2O from the reaction of PtO with ^3H gas,[13] where the ^3H_2O is condensed directly into the reaction vessel containing the substrate, pre-sonicated catalyst, and co-solvent (if desired). If the labeled substrate is to be used directly in NMR investigations, D_2O (or CH_3OD) is substituted in the isolation steps.[14]

Catalyst preparation

Initial Raney® nickel catalyzed C–H → C–D exchange reactions were found to be irreproducible, dependent upon the catalyst vendor, age of catalyst prior to use, methods of storage, etc. We found that a modified 'T-4 digestion' affords an equally reactive exchange catalyst within 3 h,[12,13] affording a remarkably active catalyst and batch-to-batch reproducibility of $\pm$ 3% individual site incorporation. The digested catalyst is pre-sonicated prior to the microwave reactions, which fully activates the catalyst and removes impurities.

Microwave assisted C–H → C–D exchange reactions

We are also exploring the feasibility of microwave irradiation for isotope exchange reactions. Microwave irradiation reactions are becoming increasingly popular, and several types of dedicated microwave synthesis units are marketed that facilitate cleaner reactions, higher yields and more uniform results in a shorter reaction time.[15–17] A commercial, multi-mode domestic microwave oven outfitted with a silica-gel heat sink and simple glassware was used to stereospecifically incorporate high levels ($\geqslant$70%) of ^2H into two model carbohydrates within 6 min, although the study revealed that the reaction conditions were not optimal.[6f,g] Extended irradiation of substrates (>10 min) resulted in slight epimerization and decomposition. To minimize adventitious thermal effects, the procedure was modified to allow for brief cooling in an ice bath between microwave irradiation intervals and the irradiation power was decreased to 50% (~600 W).[18] The 'model' monosaccharide, 1-0-methyl-β-D-galactopyranoside, and the disaccharide sucrose were used for modification and optimization of the microwave procedure. NMR results revealed 86% ^2H incorporation in one C–H site in 1-0-methyl-β-D-galactopyranoside, and 91% ^2H incorporation at one site in sucrose, in less than 10 min of total irradiation time.[18,19] Substantial ^2H incorporation occurs at all expected individual vicinal sites, and the optimization method with ice bath cooling between irradiation cycles seems to generally prevent decomposition and/or epimerization. In the multi-mode domestic microwave oven, the highest ^2H incorporation was seen at the C_3 position in 1-O-methyl-β-D-galactopyranoside (as expected at the C_2, C_3, and C_4 sites; Figure 1), although contrasting with higher ^2H incorporation at the C_4 position typical to ultrasonically promoted reactions. Similarly, the highest ^2H incorporation was seen at the $C_{3'}$ and C_2 positions of sucrose (as expected at the C_2, C_3, C_4, $C_{3'}$, and $C_{4'}$ sites. Raney® nickel catalyzed C–H → C–D exchange reactions are being explored using a single-mode, dedicated microwave synthesis apparatus.[14] This apparatus allows for precise temperature and pressure monitoring and control, as well as variable sample stirring. Preliminary ^1H and ^{13}C NMR results of reactions conducted for 10 min of total irradiation time revealed an 83% and an 85% ^2H incorporation at C_4 and C_3, respectively, in 1-0-methyl-β-D-galactopyranoside. In sucrose, there is a 73 and 70% ^2H incorporation at $C_{3'}$ and C_2, respectively, and the individual exchangeable site variability is $< \pm$ 2%.[19,20] In addition, successful application of this technique for ^3H incorporation is currently underway, and the study is being expanded to encompass a large number of glycoconjugates and mixed solvent pairs.[14]

Conclusion

We are continuing to develop our Raney nickel® catalyzed C–H → C–D (or C–H → C–T) exchange technique in a heterogeneous reaction scheme. We have found that either ultrasonic irradiation or microwave irradiation effectively promotes significant stereospecific ^2H (or ^3H) incorporation into non-reducing carbohydrates and glycoconjugates within a short reaction time. Further research on the utility of this reaction methodology and direct application for facile ^3H labeling is in progress.

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