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Microwave-assisted C–H bond activation using a commercial microwave oven for rapid deuterium exchange labeling (C–H → C–D) in carbohydrates

Eugene A. Cioffi,* Rebekah H. Bell and Baouyen Le

Department of Chemistry, University of South Alabama, Mobile, AL 36688-0002, USA

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Abstract—Studies have shown that facile hydrogen → deuterium exchange in two model carbohydrates via stereospecific C–H bond activation could be achieved using a pre-sonicated Raney Nickel® catalyst and microwave irradiation. Using a simple commercial microwave oven and a silica-gel bath, monosaccharide and disaccharide samples underwent isotopic exchange using microwave irradiation for sequential 15 s intervals. The influence of chilling between irradiation intervals was examined. The results revealed increasing levels of ^{2}H incorporation without either epimerization or concomitant decomposition seen earlier in non-optimized experiments.

1. Introduction

Deuterium labeling of carbohydrates and glycoconjugates have wide applicability in biochemistry and biophysical fields.[1] Carbohydrates play pivotal roles in cell recognition and differentiation, and function as receptors for a large suite of hormones.[2] Deuterium labeled compounds are frequently utilized in the conformational analysis of oligo- and polysaccharides, as well as glycoproteins and glycolipids, to simplify resonance overlap (and splitting) and ^{1}H NMR spectroscopy.[3] Among the many chemical and enzymatic synthetic pathways known for deuterium labeling,[4] an efficient and expeditious method for simple non-reducing carbohydrates is based upon the exchange labeling of deuterium for hydrogen using a Raney Nickel® catalyst in refluxing D_2O.[5] However, this method lacks the desired regio- and stereoselectivity, and cannot be applied to complex, thermally sensitive molecules. Harsh, extended refluxing conditions readily promote hydrolysis, racemization, and epimerization.[6]

Microwave-assisted reactions are increasingly cited in organic synthesis, since they often achieve rate enhancements, higher yields, and better selectivity in comparison to conventional heating.[7] An efficient, clean, and rapid incorporation of deuterium into carbohydrates using microwaves would be desirable. Herein we report a facile and effective microwave-assisted procedure for stereospecific C–H bond activation and concomitant deuterium labeling using a commercial microwave oven and Raney Nickel® catalyst.

A digested Raney Nickel® alloy catalyst may be used for deuterium exchange in a stereospecific C–H bond activation manner, for carbohydrates and glycoconjugates with vicinal –OH and/or N–H pairs.[8–12] Previous research has proven this by study of ultrasonic C–H bond activation on a heterogeneous metallic template,[10] stereospecific $^{1}H \rightarrow {}^{2}H$ isotopic exchange in carbohydrates,[6] morphological and surface studies of ultrasonically treated Raney Nickel® hydrogen–deuterium exchange catalysts,[7] and ultrasonically induced enhancement of isotope exchange catalysts.[8]

Microwave irradiation reactions are becoming increasingly popular, and several types of microwave apparati have been marketed that facilitate cleaner reactions, higher yields and more uniform results in a shorter reaction time.[13,14] In a preliminary study, we found that the use of a simple, multi-mode domestic microwave oven for the irradiation of a pre-activated Raney Nickel® catalyst and a deuterium isotope source readily promoted

* Corresponding author. Tel.: +1 251 460 7430; fax: +1 251 461 1765; e-mail: eacioffi@jaguar1.usouthal.edu

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stereospecific C–H → C–D exchange in carbohydrates, although the study suggested that the reaction conditions were not optimal.[15]

Polar solvents are typically used to perform organic transformations with microwave irradiation since polar solvents couple very well with microwave energy.[16] This study used the polar solvents D_2O and tetrahydrofuran (THF) to perform the reaction, since our previous studies[6–10] have shown positive results using this solvent pair in both ultrasound-promoted experiments as well as our preliminary microwave experiments.[15] These preliminary results showed that isotopic labeling can be conducted with a metallic catalyst in a microwave oven using a model carbohydrate, 1-*O*-methyl-β-D-galactopyranoside, and the goals of this study were to optimize these results using both a simple monosaccharide and a disaccharide. The model monosaccharide, 1-*O*-methyl-β-D-galactopyranoside, and the disaccharide sucrose, were used for modification and optimization of the previous procedure. By examination of known exchangeable C–H sites in the compounds using ^1H and ^{13}C NMR spectroscopy, the progression of the reactions was monitored. The NMR results revealed very favorable results, indicating an 86% ^2H incorporation in one C–H site in 1-*O*-methyl-β-D-galactopyranoside, and a 91% ^2H incorporation at one site in sucrose, in less than 10 min of total irradiation time.

2. Results and discussion

For all the experiments performed, satisfactory results were obtained and exchange occurred only where expected (Figs. 1 and 2). The results of the microwave irradiation procedure and percentages of ^2H incorporation are summarized in Tables 1–3.

For each time period irradiated, stereospecific deuterium incorporation is readily observed (Tables 1–3). Substantial ^2H incorporation occurs at the expected individual vicinal sites, and the optimization method with ice bath cooling between irradiation cycles seems to generally prevent decomposition and/or epimerization. Variability in the rates of exchange between the individual reaction vials may be attributed to the lack of mixing of the heterogeneous mixture during the irradiation period. The highest ^2H incorporation was seen at the H_3 position in 1-*O*-methyl-β-D-galactopyranoside, and the $C_{3'}$ and C_2 positions of sucrose. Due to peak overlapping at the NMR field employed (e.g., C_3 and C_4 in sucrose), integrations and percent incorporations were calculated assuming that only vicinal –OH sites are available for exchange; comparison of the ^{13}C NMR spectra (inverse-gated NOE suppression) of authentic and irradiated samples confirmed this assumption.

The experiments performed show positive results for the microwave-assisted isotopic exchange of C–H → C–D in two non-reducing model carbohydrates. The experimental results showing this incorporation can be easily followed by ^1H NMR spectroscopy and compared with the ^1H NMR spectra of unreacted, authentic compounds. This is easily done by monitoring the decrease in signal intensity in ^1H NMR spectra in the appropriate exchange region (chemical shift). The percentage of deuterium incorporation varied at each individual vicinal position, without any additional ^2H incorporation at non-vicinal sites. Between sequential irradiation times, the samples were removed from their sleeves within the oven, and allowed to cool with manual swirling. In the initial experiments, the vials were irradiated two at a time in the silica-gel bath/oven, and sample aliquots taken at 30, 60, 75, 90, and 120 s total irradiation-time intervals. Subsequent experiments were conducted for 3, 4, 5, and 6 min total irradiation times resulting in higher C–H → C–D stereospecific exchange levels (Tables 1 and 2).

To optimize the experiment, 1-*O*-methyl-β-D-galactopyranoside was used in the exchange procedure by which samples were cooled between irradiation times with an ice bath (Table 3). A past problem that had been experienced by this lab, using extended microwave irradiation and other experiments if using D_2O at reflux, was decomposition of the compounds.[1–5] In the case of

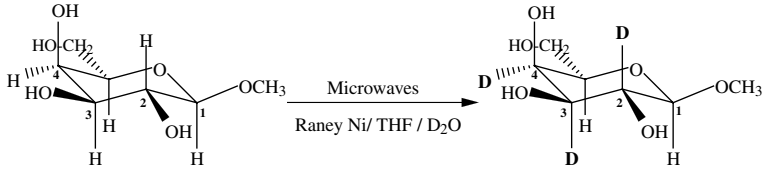

Figure 1. Deuterium exchange at H_2, H_3, and H_4 in 1-*O*-methyl-β-D-galactopyranoside.

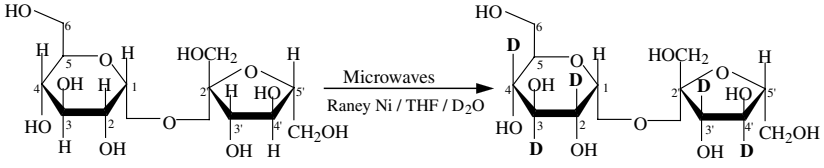

Figure 2. Deuterium exchange at C_2, C_3, C_4, $C_{3'}$, and $C_{4'}$ in sucrose.

Table 1. Commercial microwave-oven irradiation and percentages of $^{1}H \rightarrow {}^{2}H$ isotope exchange in 1-*O*-methyl-β-D-galactopyranoside

Vial	Irradiation time intervals (time/s)	Total irradiation time (time/s)	% Deuterium incorporation (±2%)		
			C_4	C_3	C_2
Reaction set 1					
1	15 s × 2	30 s	5	26	7
	15 s × 4	60 s	6	30	10
2	15 s × 5	75 s	13	39	11
	15 s × 8	120 s	20	66	15
Reaction set 2					
1	15 s × 12	180 s/3 min	10	19	<1
	15 s × 16	240 s/4 min	11	31	8
2	15 s × 20	300 s/5 min	37	37	10
	15 s × 24	360 s/6 min	47	58	11

Table 2. Commercial microwave-oven irradiation and percentages of $^{1}H \rightarrow {}^{2}H$ isotope exchange in sucrose

Vial	Irradiation time intervals (time/s)	Total irradiation time (time/s/min)	% Deuterium incorporation (±2%)			
			$C_{3'}$	$C_{4'}$	$C_3 + C_4$[a]	C_2
Reaction set 1						
1	15 s × 2	30 s	10	3	20	25
	15 s × 4	60 s	16	4	29	32
2	15 s × 6	90 s	19	6	39	35
	15 s × 8	120 s	25	11	46	42
Reaction set 2						
1	15 s × 12	180 s/3 min	8	6	17	21
	15 s × 16	240 s/4 min	57	6	28	69
2	15 s × 20	300 s/5 min	67	8	33	81
	15 s × 24	360 s/6 min	78	17	56	91

[a] In order to obtain the best incorporation values, the overlapping peaks of positions C_3 and C_4 were integrated together.

Table 3. Commercial microwave-oven irradiation and percentages of $^{1}H \rightarrow {}^{2}H$ isotope exchange in 1-*O*-methyl-β-D-galactopyranoside with ice bath cooling between irradiation intervals

Vial	Irradiation time intervals (time/s)	Total irradiation time (time/s/min)	% Deuterium incorporation (±2%)		
			C_4	C_3	C_2
Reaction set 1					
1	15 s × 24	360 s/6 min	55	53	7
	15 s × 28	420 s/7 min	62	62	15
2	15 s × 32	480 s/8 min	37	66	18
	15 s × 36	540 s/9 min	40	86	21

microwave reactions, the decomposition was hypothesized to occur because of the high temperature the silica-gel bath had reached. Between irradiation times, the vials were taken out of the bath to air cool with gentle swirling and allow the reaction vapor pressure to decrease. However, air cooling alone was not sufficient to allow the hot metallic catalyst to cool, although the temperature of the liquid solvent mixture and the attendant vapor pressure of the solvents appeared to have decreased. The hot metallic surface caused decomposition and/or epimerization of the carbohydrate, especially under extended irradiation times. Similarly, this behavior is also generally seen when reactions are conducted under refluxing conditions. Efficient cooling of the vials between microwave irradiation times would not only allow the vials to cool and depressurize, but it also would allow the metal surface of the catalyst to cool. Samples removed from the microwave oven and briefly cooled in an ice bath between irradiation intervals were irradiated for 15 s intervals for total irradiation times of 6, 7, 8, and 9 min (Table 3). In this manner, we were able to probe the extent of ^{2}H incorporation and to note if any decomposition or epimerization occurred. Sample analysis indicated that *no* decomposition had occurred,

and further, higher ^2H incorporation was achieved. The patterns of C–H → C–D exchange were readily apparent in the subsequent ^1H and ^{13}C NMR analyses.

The homogeneous nature of microwave heating inhibits localized overheating of the reaction walls, although the catalyst itself acts as a good thermal sink and could provide an undesirably hot surface, which would promote product degradation. However, the experiments involving sequential 15 s irradiations of samples of 1-*O*-methyl-β-D-galactopyranoside with intermediate ice-bath cooling and swirling for extended irradiation times demonstrated that product epimerization/degradation seen in preliminary experiments may be easily obviated by a simple optimization technique.

3. Conclusion

The Raney Nickel® catalyzed C–H → C–D technique using a simple commercial microwave oven may be used to promote significant ^2H incorporation into non-reducing carbohydrates within a short reaction time. Furthermore, the irradiation times may be extended to provide very high levels of incorporation without decomposition or epimerization, provided that ice bath cooling is used between irradiation intervals. As with the model carbohydrates 1-*O*-methyl-β-D-galactopyranoside and sucrose, it is likely that many other compounds bearing vicinal –OH pairs will react in the same manner. However, as seen with these compounds, the overall ^2H incorporation percentages did not progress at equal rates. The percentages of ^2H incorporation may depend upon the structure, flexibility, and steric hindrances indigenous to each substrate. The variability of exchange between experiments utilizing the same substrate for identical irradiation times or different reaction vials (e.g., compare 6 min irradiation in Tables 1 and 3) is likely due to the inability to stir properly or otherwise agitate the heterogeneous reaction within the microwave oven.

Expensive laboratory-grade single-mode microwave instruments allow for precise temperature, pressure, and stirring control, and thus may afford more reproducible results. Nonetheless, these results illustrate that a low cost, domestic microwave oven may be used to promote significant ^2H incorporation in the Raney Nickel® catalyzed isotopic exchange reaction, *provided that simple safety measures are employed*. Further research on the utility of this reaction methodology and simple modification of the apparatus to provide safe sample stirring is in progress.

4. Experimental

Raney Nickel® (50% Ni/50% Al) was purchased from Alfa Aesar (Ward Hill, MA) and used as received. THF, NaOH, and silica gel (−70 + 230 mesh) were purchased from Sigma-Aldrich, Incorporated (Milwaukee, WI). Deuterium oxide (D_2O; 99.9%; 99.96% + DSS) was purchased from Cambridge Isotope Laboratories, Incorporated (Andover, MA). Type I water (⩾18 MΩ) was prepared using a Barnstead Nanopure® apparatus. A 1200W Panasonic domestic microwave oven (Model #NN-L931-WF) equipped with a tunable power inverter and turntable was used in all the microwave experiments. 40 mL EPA vials (Aldrich #Z27,702-9 or equivalent) with Teflon-lined silicon rubber septa were used as reaction vessels. Argon gas was used to maintain an inert atmosphere. NMR spectra were measured using a JOEL YH 300 NMR operating at 300.53 MHz for ^1H spectral measurements and at 75.56 MHz for ^{13}C measurements. NMR samples were dissolved in 99.96% D_2O and the chemical shifts referenced to 0.01 mg/mL internal DSS. Typically, a 45° pulse angle, 10 s relaxation delays, and 32 transients were used in the ^1H NMR experiments; a 30° pulse angle, 5 s relaxation delays, and 1024 transients were used in the ^{13}C experiments. When applicable, inverse-gated ^{13}C decoupling experiments were performed (NOE suppression and quantitative analysis) subsequent to T_1 relaxation experiments.

4.1. Catalyst digestion and preparation

The preparation of the exchange catalyst is a modification of the published T-4 digestion procedure.[17] To 6.0 g of Raney Nickel® and 10.0 mL of Type I water contained in a 50 mL Erlenmeyer flask suspended in a water bath (at 50 °C), 1.2 mL of 20% NaOH was carefully added and stirred mechanically for 1.5 h. After this initial digestion period, 18.0 mL of 40% NaOH was added and the solution was stirred for an additional hour, resulting in an opaque white upper layer. The milky-white upper layer was decanted-off, and the catalyst rinsed with stirring 8 to 10 times with 45 mL Type I water (50 °C) until the rinse pH's ⩽ 8.0. After the final water washing, 3 × 45 mL EtOH rinses followed with careful decantation after each rinse. The digested catalyst was transferred to a 15 mL centrifuge tube using a minimal amount of EtOH, centrifuged down to allow for removal of the bulk residual EtOH, and covered with ≈5 mL of fresh D_2O. The catalyst in the centrifuge tube was placed under an inert atmosphere, closed, and stored in a refrigerator (≈4 °C). This amount of digested catalyst will support up to 12 reactions.

4.2. Substrate $^1H \rightarrow {}^2H$ pre-exchange

Before any compounds can be reacted with the catalyst, they must be pre-exchanged with D_2O to effect –O–H → –O–D (or –N–H → –N–D) conversion and thus insure a high ^2H pool in the subsequent reaction. In this simple procedure, 2.0 mL of D_2O was added to the weighed substrate (typically 50–75 mg) and lyophilized overnight. Both the non-irradiated and reacted substrates were pre-exchanged/lyophilized overnight prior to subsequent ^1H and ^{13}C NMR analyses.

4.3. Catalyst pre-sonication

The digested catalyst should be pre-sonicated prior to the microwave reactions. Pre-sonication activates the

catalyst and removes impurities (such as entrained bases). All reactions were performed in 40 mL EPA vials closed with a Teflon-lined Si septum cap. 10 mL THF was added to a reaction vial containing ≈0.5 mL (wet slurry) of the catalyst suspended in a 40 °C water bath. The catalyst/THF mixture was sonicated for ≈30 min using a probe sonicator (or a thermostated ultrasonic bath) under an inert atmosphere. The catalyst is was rinsed twice with 2 × 5 mL washings of THF kept under an inert atmosphere using a syringe; the pre-activated catalyst was now ready for subsequent microwave irradiation experiments.

4.4. Representative microwave exchange procedure

All microwave reactions were run at a constant 50% power level for the specified timed intervals. The experiments were carried out on two model substrates, 1-*O*-methyl-β-D-galactopyranoside, and sucrose. The basic microwave exchange procedure consisted of adding 1.0 mL of the lyophilized, pre-exchanged substrate (as a 0.05 g/mL D_2O solution), 10 mL of THF, and ≈0.5 mL pre-activated Raney Nickel® slurry to a clean 40 mL EPA vial (it was found that adding the substrate as a D_2O solution to be the most effective for uniform mixing and later microwave irradiation). An additional 1.0 mL of D_2O was added to the vials, the vials swept with an inert gas, and capped with new and unpunctured septa tops (to prevent accidental THF vapor leakage into the microwave vessel and afford a visual 'safety-check', that is, if the vials should overheat, the septa will bulge substantially, and the reaction may be safely terminated). The setup in the microwave oven was either a plastic dish or a 500 mL beaker filled with ≈10 cm depth silica gel for uniform irradiation; cardboard sleeves were constructed and placed in the vessel prior to silica-gel addition for easy addition/removal of the vials and to keep the vials upright. After placing the vials in the cardboard sleeves contained within the silica-gel bath, and placement of the silica-gel bath into the oven, the samples were irradiated for 15 s intervals at 50% power. CAUTION: *At all times, the septa tops of the vials must be kept under close observation while inside the microwave oven to insure that overheating (evident as septa bulging) does not occur. If bulging is observed, the microwave power should be terminated immediately!*

4.5. Product isolation and NMR analysis

The sample mixtures were cooled after irradiation, filtered with a simple double filtration system using a glass filter paper plug topped with a glass wool plug pre-filter placed in a disposable glass pipette. The samples were pressure filtered into small round bottom flasks, and the THF removed (water vacuum at 40 °C) in a rotary evaporation apparatus. After rotary evaporation, the samples were frozen to the sides of the flasks using a dry ice/acetone bath and lyophilized (usually overnight). To the dry, lyophilized samples was added 1.0 mL D_2O and filtered again (if necessary) using a glass wool plugged disposable pipette into NMR tubes.

The NMR spectra of the irradiated samples were compared to the NMR spectra of the authentic, pre-exchanged substrates. The percentage of ^2H incorporation was determined by the disappearance of signals in the ^1H NMR spectra in comparison to the pre-exchanged samples. For 1-*O*-methyl-β-D-galactopyranoside, exchange was observed as expected at the C_2, C_3, and C_4 sites (as shown in Fig. 1). For sucrose, the exchange was seen as expected at the C_2, C_3, C_4, $C_{3'}$, and $C_{4'}$ sites (as shown in Fig. 2).

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