

Tritium and Deuterium Labelling Studies of Alkali Metal Borohydrides and their Application to Simple Reductions

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Summary

Simple and facile syntheses of highly deuterated and tritiated LiBH_4 , NaBH_4 and KBH_4 were achieved by hydrogen isotope exchange with deuterium or tritium gas at elevated temperatures. The exchange products were characterized by boron, proton and deuterium or tritium NMR spectroscopy. The extent of isotope (^2H or ^3H) incorporation was calculated from the patterns of ^{11}B NMR spectra. Several samples of tritiated NaBH_4 were treated with $\text{BF}_3\text{-Et}_2\text{O}$ to produce tritiated borane-THF complex, which is an electrophilic reducing agent. The utility of both the borohydride reagents and borane-THF complex in labelling reactions was confirmed by exemplary reductions leading to specifically labelled products. The extent and orientation of labelling in the reduction products was assessed by a combination of radio-HPLC analysis, ^1H , ^2H or ^3H NMR and mass spectrometry.

Key Words: Lithium borohydride, sodium borohydride, potassium borohydride, tritiated borane, boron NMR, tritium NMR, deuterium NMR.

Introduction

A need for high specific activity tritium labelled materials exists in many areas of research and tritide reducing agents¹ provide an attractive approach for the incorporation of tritium into molecules of biological importance. The growing complexity of target biological molecules has led to the use of more sophisticated and selective tritiation reagents in labelling.¹⁻⁴ In the last few years a range of fully tritiated hydride reagents has been developed and their labelling chemistry explored.¹⁻⁸

Since the discovery of sodium borohydride many additional hydride reagents and their applications have been developed.⁹ However, alkali metal borohydrides remain the most widely

used reducing agents in organic chemistry. In general, they are mild and highly selective towards the reduction of aldehydes, ketones, acid chlorides and lactones.⁹ Labelled borohydrides are used as regio-selective hydrogen isotope labelling reagents because the labelling is strictly specific and the labelled atoms are located only on the carbon atom originally forming the unsaturated group.

Sodium borohydride can reduce aldehydes, ketones, primary and secondary alkyl halides, sulfonate esters, tertiary amines and disulfonamides without affecting carboxylic acids.^{10,11} It has greater resistance to hydrolysis than the more powerful LiAlH_4 and this allows reductions to be performed in aqueous or alcoholic media.¹² LiBH_4 is an analogue with slightly more reducing power and has been used for the reduction of epoxides, esters, and lactones.^{13,14} KBH_4 has milder reducing power than NaBH_4 , and although it is less soluble in most organic solvents, it is not as hygroscopic as the other borohydrides and is easier to handle.¹⁵

There are numerous reports on the syntheses of metal borohydrides from their corresponding hydrides^{16,17} and methoxyborohydrides.¹⁸⁻²⁰ LiBH_4 and KBH_4 may also be prepared from NaBH_4 by treatment with LiBr ²¹ or LiCl ²² and KOH ,¹⁵ respectively. Davis *et al.*²³ prepared 99% pure NaBD_4 from the reaction of $\text{NaB}(\text{OCH}_3)_4$ and B_2D_6 . Atkinson and co-workers²⁴ developed a method of synthesizing NaBD_4 and KBD_4 with 74-80% yields and 99%D. The most successful LiBD_4 synthesis²⁴ (57% yield and high isotope purity) was based on the reaction between LiD and BF_3 etherate, and this approach was recently used to synthesize LiBT_4 from LiT , but the product was not well characterized.² In practice, except for the latter reaction starting from LiT , none of these direct syntheses is readily adapted to routine preparation of highly tritiated borohydrides due to the difficulty in obtaining tritiated precursors with high specific activity (S.A.).

NaBH_4 does not undergo exchange with D_2O , but in 1970 Cornforth reported the rapid exchange of D_2O with LiBH_4 .²⁵ However, after the exchange is complete, 50% of the borohydride has been destroyed by hydrolysis.^{25,26} In 1952, Brown *et al.*²⁷ labelled sodium and lithium borohydride by simply heating them with a mixture of H_2 and T_2 gas. Exchange leading to approximately statistical isotopic distribution was reported to occur at a convenient rate with LiBH_4 at 200°C and with NaBH_4 at 350°C , and thermal decomposition was negligible in both cases.²⁷ The facile exchange of D_2 gas with KBH_4 near 500°C was reported by Mesmer and Jolly.²⁸ At high D_2 pressure (70-100 atm), only slight decomposition was reported to occur during three days of exchange at 538°C .

As a consequence of the important role of the alkali metal borohydrides in labelling chemistry and our ability to work with high levels of tritium gas,²⁹ we selected the simple and direct approach to labelling these reagents, *i.e.* exchanging carrier free tritium gas with solid borohydride salt at elevated temperatures. We took an empirical approach and developed appropriate equipment to work with tritium gas at high temperature. ¹¹B NMR was used extensively as a rapid and non-destructive method for the determination of %D or the tritium content in the exchanged borohydride products.

In addition to our need for labelled borohydrides in their own right, these reagents can readily reduce $\text{BF}_3\text{-Et}_2\text{O}$ ³⁰⁻³² and give borane, which is an extremely useful electrophilic reducing agent.^{33,34} We have recently demonstrated the synthesis of fully tritiated borane from the reaction of LiT and $\text{BF}_3\text{-Et}_2\text{O}$,³⁵ making the borohydride approach less essential to us. Nevertheless, formation of borane from borohydrides is a convenient and efficient method, and we felt that further characterization of appropriate conditions and full analysis of products was important.

Experimental

Exchange Methodology — A silica tube equipped with a small side arm, septum and a greaseless vacuum valve at the top was used as a standard exchange vessel [Figure 1(a)].

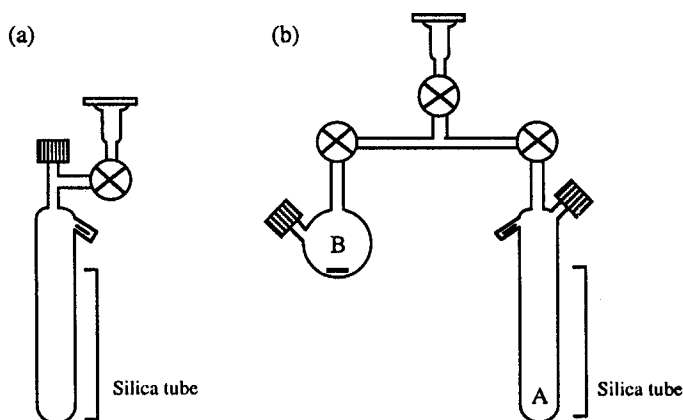


Figure 1: (a): Reaction Vessel for Borohydride Exchange and Subsequent Reduction of 2-Naphthaldehyde. (b): Reaction Vessel for Borohydride Exchange, Preparation of $\text{BT}_3\text{-THF}$, and Reduction of 2-Naphthoic Acid.

Although our experiments showed that a similar extent of borohydride labelling was attained in either pyrex or silica tubes, the silica tube was preferred because of its high thermal stability. Metal borohydride (Aldrich, typically 0.05 mmol for tritium reactions and 0.1 mmol for deuterium exchanges) was placed in the exchange vessel and a small magnetic stirrer was placed in the side arm. The vessel was evacuated to 0.13 kPa, flushed three times with dry N_2 and heated slowly with a hot air gun while under vacuum to remove any moisture which may have been present. The required pressure of D_2 or T_2 gas was introduced to the tube at room temperature.²⁹ The reactants were heated to the desired temperature for the required time, using an aluminium heating block to heat only the lower part of the vessel. After reaction, the vessel was cooled to room temperature, excess D_2 or T_2 gas was removed, and the sample was flushed twice with dry N_2 . Nitrogen gas was again admitted to a pressure of 80 kPa and 700 μ L of 1M NaOH/ CD_3OD was injected. The magnetic stirrer was then moved into the lower part of the vessel and the sample stirred. The sample solution could then be removed using a syringe with a long (6") needle for characterization by a combination ^{11}B , 1H , and 2H or 3H NMR techniques. NMR analysis was complete within 18 hours, and in many cases the sample was reinjected into the reaction vessel for a reduction experiment.

Reduction of Naphthaldehyde — A solution of 2-naphthaldehyde (typically 8.57 mg, 0.06 mmol in 300 μ L CH_3OH) was injected into the vessel [Figure 1(a)] containing a solution of NaBT₄ (e.g. 2.3 mg, 0.06 mmol in 700 μ L 1M NaOH/ CD_3OD , and 78%T, 3320 GBq/mmol = 90 Ci/mmol) and stirred at room temperature. The pressure inside the vessel was maintained at 70 kPa. After 2 hours of stirring the reaction was quenched with 2 mL of 1M methanolic HCl and the excess gas and solvent were removed by evacuation. The vessel was then filled with dry nitrogen and detached from the vacuum line. The reduction product, 2-naphthalene methanol, was then dissolved in 2.5 mL of ether and washed three times with 1 mL of H_2O . The ether was removed under a flow of N_2 gas and the sample dissolved in CD_3OD for NMR and HPLC analyses: yield 70%; specific activity 740 GBq/mmol (20 Ci/mmol, by HPLC); 750 GBq/mmol (20.3 Ci/mmol, by 3H decoupled 1H NMR in CD_3OD); 1H NMR: δ 4.76 (CH_2 , 29.0%) and δ 4.73 (CHT, 71.0%).

Preparation of BT₃-THF and Reduction of Naphthoic Acid — Typically 4.9 mg (0.13 mmol) of NaBH₄ was placed in side A of the vessel shown in Figure 1(b) and the exchange reaction was carried out with (25% T_2 and 75% H_2) gas using the same procedure described above. At the

end of the exchange reaction excess gas was removed and the NaBH_4 was flushed twice with dry N_2 . Nitrogen gas was again admitted to a pressure of 80 kPa and 700 μL of dry THF was injected. The magnetic stirrer was then moved into the lower part of the vessel and the solution was degassed twice. Nitrogen gas was introduced to 80 kPa and borontrifluoride etherate ($\text{BF}_3\text{-(C}_2\text{H}_5)_2\text{O}$, 22 μL , 0.18 mmol) was injected dropwise into the flask at room temperature, after which the solution was kept at 60°C with constant stirring. While the borane-THF reagent was being formed in flask A, a solution of naphthoic acid (10.5 mg, 0.06 mmol) in THF (300 μL) was injected into flask B and degassed twice. After 1 hour the product $\text{BT}_3\text{-THF}$ complex in flask A was vacuum transferred to flask B. This flask was filled with dry N_2 to 70 kPa and the reaction mixture stirred for 40 minutes at room temperature. The reaction was quenched with 2 mL of methanol to destroy excess borane, after which the solvent was removed by evacuation. The solid reduction product was dissolved in CD_3OD (1 mL), and analyzed by radio-HPLC followed by both ^1H and ^3H NMR spectroscopy; yield 90%; S.A. = 325 GBq/mmol (8.8 Ci/mmol, by HPLC); ^1H NMR (CD_3OD): δ 4.78 (CH_2 , s) δ 4.75 (CHT, d), ^1H decoupled ^3H NMR (CD_3OD): δ 4.75 (CHT), δ 4.72 (CT_2). By combining the information from these spectra,³⁶ it is possible to calculate mole fractions for each of these isotopomers: R- CH_2OH (73.2%), R-CHTOH (24.9%), R- CT_2OH (1.9%), and compute the S.A. = 305 GBq/mmol (8.3 Ci/mmol).

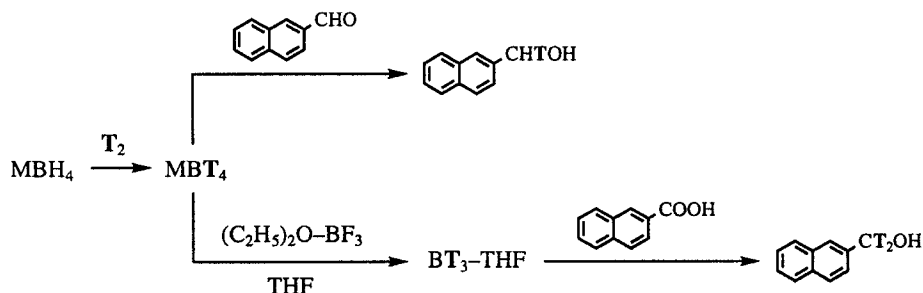
Radio HPLC—A radio HPLC equipped with a Waters 1/4" x 25 cm C-18 column and Waters model 510 pumps was used to analyze the reduction products. The mobile phase was 60% methanol/water and was pumped at 1.5 mL/min. Mass peaks were observed by UV detection at 275 nm on a Hewlett Packard 1040A diode array spectrophotometer, and radioactivity was monitored by a IN/US β -Ram LS HPLC flow detector. The specific radioactivity of the reduction products was determined by liquid scintillation counting (Packard 1500) of the isolated HPLC peak effluents. In some cases the specific activity of the tritiated reduction products was verified by using ^1H or ^3H NMR.³⁶ The %D in deuterated reduction products was determined by mass spectrometry.

NMR Spectroscopy—All NMR spectra were obtained on a Bruker AF-300 instrument. Sample sizes of 250 μL for tritiated samples (doubly encapsulated 5 mm tubes)³⁷ and 500 μL for deuterated samples were used. The borohydrides were dissolved in 1M $\text{NaOH}/\text{CD}_3\text{OD}$ to reduce the rate of hydrolysis.^{19,38} Referencing of tritium chemical shifts was achieved by the generation of a ghost ^3H TMS signal from internal TMS in the ^1H NMR spectrum.³⁹ ^{11}B

spectra were resolution enhanced by zero filling the FID to 32k data points. ^{11}B in the glass of the NMR tubes gave rise to signals with a very short T_2 , which contributed to a rolling base line in spectra. These signals were minimized by delaying data acquisition until 500 μs after RF excitation. ^{11}B spectra were referenced to $\text{BF}_3\text{--}(\text{C}_2\text{H}_5)_2\text{O}$ ($\delta = 0$ ppm) in THF-D_8 .⁴⁰

Results And Discussion

The overall approach taken in this work is illustrated in Scheme 1. A series of borohydrides were labelled by exchange with deuterium or tritium gas. These borohydrides were used in the reduction of 2-naphthaldehyde to demonstrate the specific reduction and labelling of simple substrates. In a small number of cases the borohydride was used to form labelled $\text{BH}_3\text{--THF}$, which was used in the reduction of 2-naphthoic acid.



Scheme 1: Summary of Borohydride Exchange Labelling, Borane Synthesis, and Exemplary Reduction Reactions with the Labelled Borohydride or Borane Reagents.

A summary of the exchange experiments with D_2 or T_2 gas, along with the reduction results using tritiated metal borohydrides is given in Table 1. Many preliminary reactions were carried out with deuterium gas to explore optimum exchange temperatures and other critical parameters for tritium reactions.

The initial exchange of LiBH_4 was carried out with D_2 gas at 200°C for 4.5 hours, and the labelling was found to be slow. At 270°C LiBH_4 was exchanged to 54% of the equilibrium value, but at an exchange temperature of 300°C the borohydride was decomposed and turned into yellow powder.

In early studies it became clear that the exchange of NaBH_4 with D_2 gas is facile at 450°C , whereas only slight exchange was observed at 375°C for the same reaction time. At

450°C the exchange was essentially complete (96% of the equilibrium deuterium content) within 4.5 hours without any thermal decomposition. The operational exchange temperatures we determined for NaBH_4 and LiBH_4 were higher than those previously reported.²⁷ As expected,²⁸ KBH_4 exchange with deuterium was found to be facile at 500°C and gave >80% of the statistical deuterium incorporation within 4.5 hours.

As shown in Table 1, the tritium exchange reactions with all three borohydrides gave >80% of the theoretical equilibrium tritium content. For both LiBH_4 and KBH_4 the tritium exchange gave clearly higher isotope incorporation than deuterium labelling, while NaBH_4 had >90% equilibrium isotope content with both D_2 and T_2 . NaBH_4 and KBH_4 both turned gray during the exchange reaction, while LiBH_4 gave a white powder.

Table 1: Summary of MBH_4 Exchange with D_2 or T_2 , and Naphthaldehyde Reduction Results.

Exchange Reaction	Exchange Parameters					Reduction Products		
	Temp (°C)	Time (hours)	D or T (%)	Equil. (%)	S.A. [§] GBq/mmol	Yield (%)	Theoretical S.A.	Observed S.A.
$\text{LiBH}_4 / \text{D}_2$	270	4.5	44	54	—	—	—	—
$\text{LiBH}_4 / \text{T}_2$	270	4.5	73	83	3100 (84)	81	780 (21.1)	790 (21.4)
$\text{NaBH}_4 / \text{D}_2$	450	4.5	73	96	—	—	—	—
$\text{NaBH}_4 / \text{T}_2$	450	6.0	78	93	3320 (90)	70	830 (22.5)	740 (20.0)
$\text{KBH}_4 / \text{D}_2$	500	4.5	65	84	—	—	—	—
$\text{KBH}_4 / \text{T}_2$	500	4.5	83	94	3530 (95.5)	94	880 (23.8)	870 (23.5)

[§] The maximum theoretical S.A. of tritium is 1063 GBq/milliatom = 28.76 Ci/milliatom. Numbers in parentheses are in Ci/mmol.

The ^{11}B NMR analyses of deuterated and tritiated metal borohydrides gave very similar spectra, and indicated negligible thermal decomposition in all cases under the chosen reaction conditions, as summarized in Table 1. A typical ^1H decoupled 96 MHz ^{11}B spectrum of deuterated NaBH_4 is shown in Figure 2. ^{11}B chemical shifts of LiBH_4 , NaBH_4 and KBH_4 are centered around -45.4 ppm similar to published data,⁴⁰ and the observed coupling constants and isotope effects in ^{11}B spectra are given in Table 2. A small peak at 3.4 ppm was observed in all ^{11}B spectra of metal borohydrides and it appears to arise from $\text{MB}(\text{OCH}_3)_4$,⁴¹ which is the product of the reaction between MBH_4 and CD_3OD .³⁸ The patterns of peaks due to NaBH_4

Figure 2: ^1H Decoupled ^{11}B NMR Spectrum of the $\text{NaBH}_4 / \text{D}_2$ Exchange Product in 1M $\text{NaOH} / \text{CD}_3\text{OD}$.

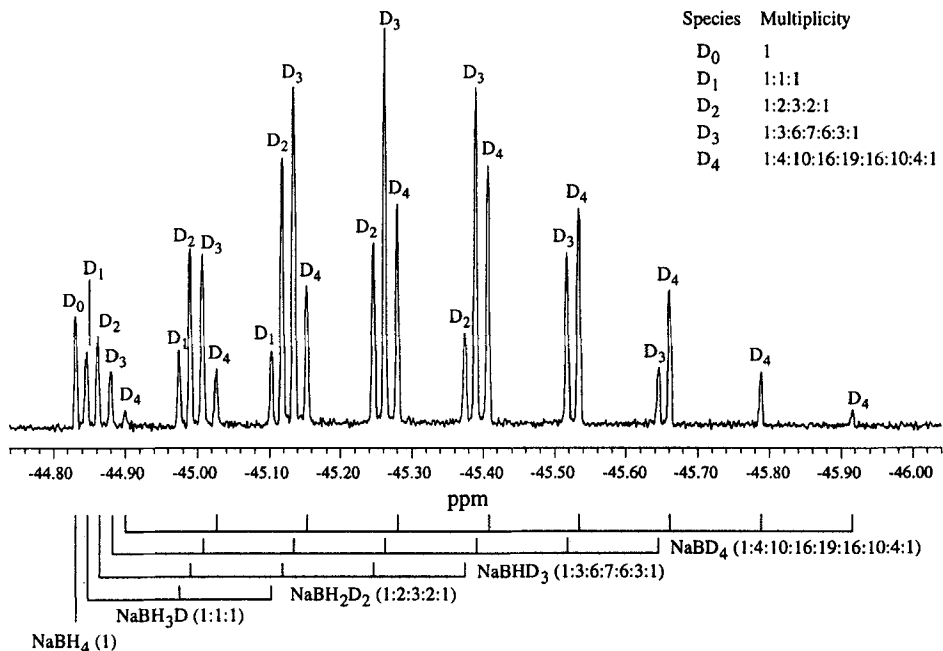


Table 2: Coupling Constants and Isotope Effects Observed in the ^{11}B NMR Spectra of Deuterated or Tritiated Exchanged Borohydrides, in Alkaline CD_3OD Solution.

Reagent	$J_{\text{B-H}}$ (Hz)	$J_{\text{B-D}}$ (Hz)	$J_{\text{B-T}}$ (Hz)	$\Delta J_{\text{B-D}}$ (Hz)	$\Delta J_{\text{B-T}}$ (Hz)	$\Delta\delta_{\text{D}}$ (ppm)	$\Delta\delta_{\text{T}}$ (ppm)
LiBH_4	80.6	12.40	86.2	0.05	0.57	0.145	0.21
NaBH_4	80.6	12.40	86.2	0.05	0.56	0.144	0.21
KBH_4	80.6	12.41	86.3	0.06	0.58	0.144	0.21

$J_{\text{B-H}}$ values were measured on solutions of commercial borohydrides. $J_{\text{B-D}}$, $J_{\text{B-T}}$, $\Delta J_{\text{B-D}}$, $\Delta J_{\text{B-T}}$, $\Delta\delta_{\text{D}}$, and $\Delta\delta_{\text{T}}$ values were all measured on labelled samples. Based on the digital resolution of the spectra (0.61 Hz/point), the error for ^1H and ^3H data was ± 0.610 Hz (0.013 ppm). ^{11}B spectra of ^2H labelled samples were collected at higher resolution, with an error of ca. 0.061 Hz (0.0013 ppm). $J_{\text{B-D}}$ was also found to be 12.23 Hz in ^2H NMR of deuterated NaBH_4 exchange product.

(singlet, 1), NaBH_3D (triplet, 1:1:1), NaBH_2D_2 (quintet, 1:2:3:2:1), NaBHD_3 (septet, 1:3:6:7:6:3:1) and NaBD_4 (nonet, 1:4:10:16:19:16:10:4:1) are apparent because of the coupling of ^{11}B to ^2H ($I = 1$, $J = 12.40$ Hz). We calculate that the J decreases by about 0.05 Hz per D replacement (*i.e.*, $J_{\text{B-D}}$ for the isotopomer NaBH_3D is 12.40 Hz, while in NaBD_4 $J_{\text{B-D}} = 12.25$ Hz). In previous work this effect was very difficult to quantify,^{38,42} but we had the advantage of working at much higher field, and the multiplicity of each D-containing species (as given above) worked to our advantage in making reliable measurements. The isotope effect ($\Delta\delta_{\text{D}} = 0.144$ ppm at 96.28 MHz) on the ^{11}B chemical shift induced by D substitution may also be extracted, and the measured value compares well with literature values for deuterium primary isotope effects on ^{11}B chemical shifts.^{38,42} Since each isotopomer gives rise to a distinct and separate multiplet it is possible to determine the mole fraction of each isotopomer by integrating the individual signals, and thereby make a determination of the total %D in the sample. For example, the calculated total %D in the exchanged product shown in Figure 2 was *ca.* 72%, with the mole percent of each isotopomer equal to: NaBH_4 (3.0%), NaBH_3D (6.0%), NaBH_2D_2 (21.4%), NaBHD_3 (40.2%) and NaBD_4 (29.4%). There are two minor assumptions in such a calculation: i). that M^{10}BH_4 and M^{11}BH_4 have very similar hydrogen isotope exchange characteristics, and ii). that the NMR intensity measurements are carefully made, and are not affected by the different relaxation properties of the various isotopomers in the product.

Similarly, tritiated borohydrides obtained by exchange were analyzed by ^1H , ^3H and ^{11}B NMR with various isotope decoupling, and a full suite of nine spectra could be finished in 6-8 hours. Typical spectra recorded from tritiated NaBH_4 are shown in Figure 3(a-i), and very similar spectra were also obtained from the exchange of LiBH_4 and KBH_4 . The observed coupling constants and isotope effects from all of these spectra are combined in Table 2 (derived from ^{11}B spectra) and Table 3 (from ^1H and ^3H spectra).

The ^3H decoupled ^{11}B spectrum [Figure 3(a)] indicates the coupling of ^{11}B to the residual ^1H atoms ($I = 1/2$, $J = 80.58$ Hz). The pattern of peaks due to NaBH_4 (T_0 , quintet, 1:4:6:4:1) is too small to be readily observed at this vertical expansion, due to the low abundance of this species (0.2%). However the patterns arising from NaBH_3T (T_1 , quartet, 1:3:3:1), NaBH_2T_2 (T_2 , triplet, 1:2:1), NaBHT_3 (T_3 , doublet, 1:1) and NaBT_4 (T_4 , singlet, 1) are apparent, centered around $\delta = -45.5$ ppm. The effect on the ^{11}B chemical shift ($\Delta\delta_{\text{T}}$) for each tritium addition to the molecule was measured as 0.21 ppm. The $J_{\text{B-T}}$ value decreases by about 0.56 Hz

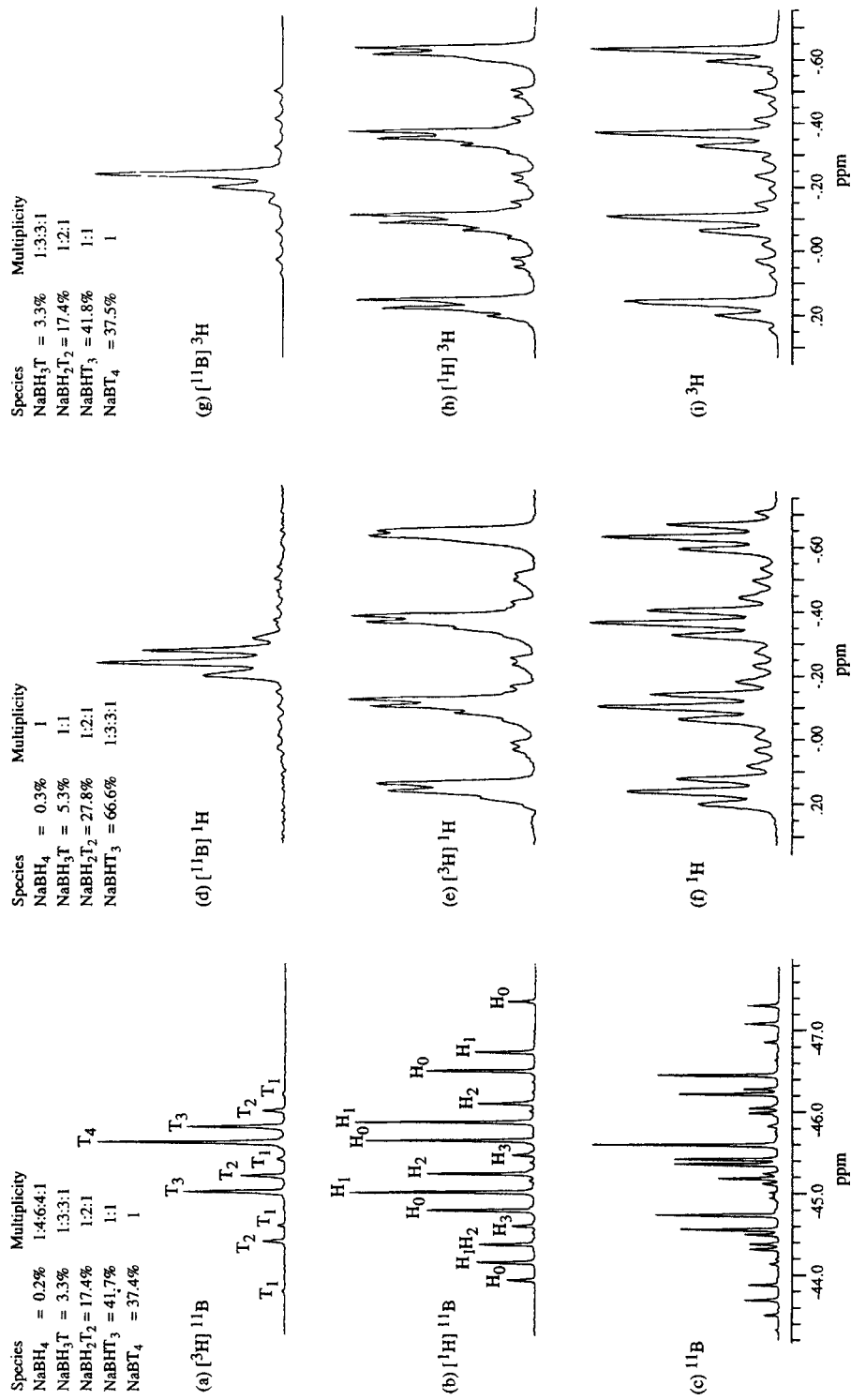


Figure 3: NMR Spectra of $\text{NaBH}_4 / \text{T}_2$ Gas Exchange Product in 1M $\text{NaOH}/\text{CD}_3\text{OD}$. Tritium Content = 78%.

per replacement of H with T. The %T in the sample was calculated from this spectrum as described above for %D, where the calculated mole percent of each isotopomer was NaBH_4 (0.2%), NaBH_3T (3.3%), NaBH_2T_2 (17.4%), NaBHT_3 (41.7%) and NaBT_4 (37.4%) and the total %T was 78% (S.A. = 3320 GBq/mmol = 90 Ci/mmol).

Table 3: Coupling Constants and Tritium Isotope Effects Observed in the ^1H and ^3H NMR Spectra of Tritium Exchanged Borohydrides, in Alkaline CD_3OD Solution.

Reagent	^1H NMR Data [¶]				^3H NMR Data [§]		
	$J_{\text{H-T}}$ (Hz)	$J_{\text{B-H}}$ (Hz)	$\Delta\delta_{\text{T}}$ (ppm)	$\Delta J_{\text{B-H}}$ (Hz)	$J_{\text{B-T}}$ (Hz)	$\Delta\delta_{\text{T}}$ (ppm)	$\Delta J_{\text{B-T}}$ (Hz)
LiBH_4	11.9	80.6	0.021	0.53	86.2	0.024	0.55
NaBH_4	11.8	80.0	0.019	0.40	86.2	0.023	0.55
KBH_4	11.8	80.7	0.021	0.55	86.2	0.024	0.57

[¶] The nominal error for ^1H measurements is one data point (± 0.610 Hz). This translates to an error of *ca.* 0.004 ppm ($2 \times 0.610 / 300.13$) for the isotope effect measurement ($\Delta\delta_{\text{T}}$). The measurement of $\Delta J_{\text{B-H}}$ is improved because the multiplets allow the averaging of J over 2, 3 or 4J ranges.

[§] The nominal error for the ^3H measurements is one data point (± 0.488 Hz). We estimate the error of the isotope effect measurement ($\Delta\delta_{\text{T}}$) as *ca.* 0.003 ppm. The measurement of $\Delta J_{\text{B-T}}$ is improved because the multiplets allow the averaging of J over 2, 3 or 4J ranges.

The ^1H decoupled ^{11}B spectrum of the same borohydride sample is shown in Figure 3(b). The pattern of peaks due to NaBH_4 (H_4 , singlet, 1), are not visible at this vertical expansion, but the NaBH_3T (H_3 , doublet, 1:1), NaBH_2T_2 (H_2 , triplet, 1:2:1), NaBHT_3 (H_1 quartet, 1:3:3:1) and NaBT_4 (H_0 , quintet, 1:4:6:4:1) patterns are apparent, due to the coupling to ^3H ($I = 1/2$, $J = 86.20$ Hz). The total %T was also calculated from this spectrum and found to be identical (78%) to that obtained from the ^3H decoupled ^{11}B spectrum ($[\text{H}]^{11}\text{B}$, Figure 3(a)). The ^{11}B spectrum coupled with both ^1H and ^3H is presented in Figure 3(c) for completeness, but we found it of little analytical value. The total %T in LiBH_4 and KBH_4 were calculated in the same fashion and found to be 73% (S.A. = 3100 GBq/mmol = 84 Ci/mmol) and 83% (S.A. = 3530 GBq/mmol = 95.5 Ci/mmol), respectively (as given in Table 1).

^1H NMR spectra of tritiated NaBH_4 are illustrated in Figure 3, insets (d–f). In general, the spectra appear complex because of the coupling of protons to both ^3H and ^{11}B nuclei. The fully coupled spectrum in Figure 3(f) is dominated by the overlaid spectra of the two most

abundant isotopomers. The patterns from NaBH_2T_2 (quartet of triplets) due to coupling to the dominant boron isotope (^{11}B , $I = 3/2$, 80.42%) and ^3H , and NaBHT_3 (quartet of quartets) were observed centered at $\delta = -0.2$ ppm. The lower intensity peaks between the quartets are a septet from coupling to the less abundant ^{10}B ($I = 3$, 19.58%). When ^3H was decoupled from ^1H [Figure 3(e)] an unresolved composite of three quartets from NaBH_3T , NaBH_2T_2 and NaBHT_3 was observed and the $\Delta\delta_{\text{T}}$ was estimated to be 0.019 ppm per addition of T. The large quartets collapsed when ^{11}B was decoupled [Figure 3(d)] and a multiplet was observed. With prior knowledge⁴³ of $J_{\text{H-T}}$, the tritium isotope effect on the ^1H chemical shift ($\Delta\delta_{\text{T}}$, above), and the isotopomer distribution, the multiplet is attributable to the overlaid spectra of the four isotopomers, viz. singlet (NaBH_4), doublet (NaBH_3T), triplet (NaBH_2T_2) and quartet (NaBHT_3) patterns. Small peaks from the septet of multiplets due to the fully coupled ^{10}B and ^3H isotopomers were also observed around the base of the intense multiplet.

320 MHz ^3H NMR spectra of tritiated NaBH_4 are presented in Figure 3(g-i) and the patterns were similar to the ^1H spectra. The ^1H and ^{11}B coupled ^3H spectrum [Figure 3(i)] showed an overlaid spectrum of three quartets of multiplets, attributable to the three most abundant isotopomers viz. NaBH_2T_2 (quartet of triplets), NaBHT_3 (quartet of doublets) and NaBT_4 (quartet). Once again, the small peaks are due to the ^{10}B isotopomers. These multiplets in the quartets collapsed when ^1H was decoupled and showed only a mixture of quartets of the above three isotopomers, Figure 3(h). The pattern from NaBH_3T was not observed because of the low concentration (3.3%) of this isotopomer, which is also split into a quartet by coupling to ^{11}B . When ^{11}B was decoupled from ^3H [Figure 3(g)] all the quartets collapsed and a multiplet was observed, due solely to the coupling of ^3H to ^1H in the mixture of species present. Once again, with prior knowledge⁴³ of $J_{\text{H-T}}$, the tritium isotope effect on the ^3H chemical shift ($\Delta\delta_{\text{T}}$), and the isotopomer distribution, the multiplet is attributable to the overlaid spectra of the four isotopomers, viz. NaBH_3T (quartet), NaBH_2T_2 (triplet), NaBHT_3 (doublet) and NaBT_4 (singlet). The coupling constants and isotope effects observed in the tritium and proton NMR spectra are given in Table 3, and are quite similar to those reported by Altman,⁴³ and calculated from analogous deuterium studies.^{38,42}

Reaction conditions for the exemplary reduction of 2-naphthaldehyde to 2-naphthalene methanol were optimized using commercial NaBH_4 or NaBD_4 prior to use of the tritium labelled borohydride. The tritiated reduction products were analyzed by radio HPLC, and the chemical

yields were found to be high (70-94%) in all reductions, with essentially all radioactivity in the desired labelled products. The observed specific activities in the products were very close to the theoretical maximum, based on the %T in the reducing agent (*i.e.* ca. 25% of the borohydride S.A., see Table 1).

The 300 MHz ^1H NMR spectra of the product from reduction of 2-naphthaldehyde by tritiated NaBH_4 are shown in Figures 4(a) and 4(b). The (^3H coupled) ^1H spectrum in Figure 4(b) shows two peaks near 4.75 ppm, which arise from coincidence of a singlet ($\text{R-CH}_2\text{-OH}$, $\text{R} = \text{Naphthyl}$) and doublet due to the ^1H - ^3H coupling in the R-CHT-OH species. The doublet collapsed into a singlet with double the intensity when ^3H was selectively irradiated [Figure 4(a)]. The separation between these two isotopomer peaks in Figure 4(a) shows the tritium isotope effect ($\Delta\delta_{\text{T}}$) on the ^1H chemical shift. Both the coupling constant ($J = 13.67 \text{ Hz}$) and tritium isotope shift ($\Delta\delta_{\text{T}} = 0.031 \pm 0.001 \text{ ppm}$) were as expected from previous studies.^{44,45} The 320 MHz (^1H coupled) ^3H NMR spectrum in Figure 4(d) shows a doublet from the R-CHT-OH species. As expected, the doublet collapsed to a singlet when ^1H was irradiated [Figure 4(c)]. The product spectra shown in Figures 4(a-d) were very clean and revealed no other labelled positions.

The reduction of 2-naphthoic acid by tritiated borane-THF complex, produced from the exchange product of NaBH_4 and 25% T_2 gas, gave a 90% yield of clean reduction product, 2-naphthalene methanol. The ^1H and ^3H NMR spectra of the product are shown in Figure 5. The ^1H spectrum in Figure 5(c) shows two peaks which arise from overlay of a large singlet ($\text{R-CH}_2\text{-OH}$) and very small doublet due to the ^1H - ^3H coupling in the singly tritiated R-CHT-OH species. The 320 MHz (^1H coupled) ^3H NMR spectrum in Figure 5(b) shows a doublet which is an overlay of a large doublet from R-CHT-OH and a small singlet ($\text{R-CT}_2\text{-OH}$). The doublet collapsed to a large singlet when ^1H was irradiated [Figure 5(a)]. The S.A. of the product was calculated as 325 GBq/mmol (8.8 Ci/mmol) from HPLC measurements and 305 GBq/mmol (8.3 Ci/mmol) from NMR spectra, whereas the theoretical S.A. is 370 GBq/mmol (10.0 Ci/mmol), assuming that the tritium exchange of NaBH_4 reached 90% equilibrium. We have observed similar discrepancies in other deuterium and tritium experiments with borane, and believe the slightly low S.A. is due to the loss of isotope by exchange between the labelled $\text{BH}_3\text{-THF}$ complex and the labile hydrogen of naphthoic acid during the reduction.³⁵

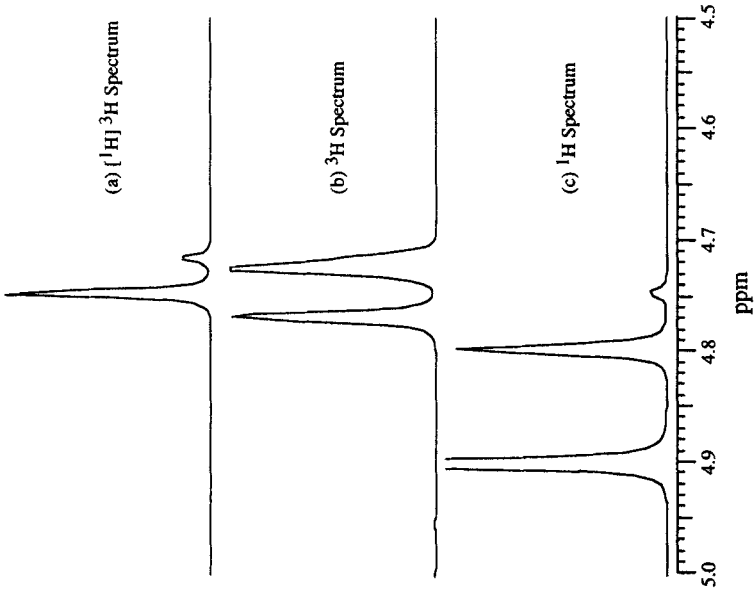


Figure 5: ^1H and ^3H NMR Spectra of 2-Naphthalene Methanol Obtained from the Reduction of 2-Naphthoic Acid with Tritiated $\text{BH}_3\text{-THF}$ Complex.

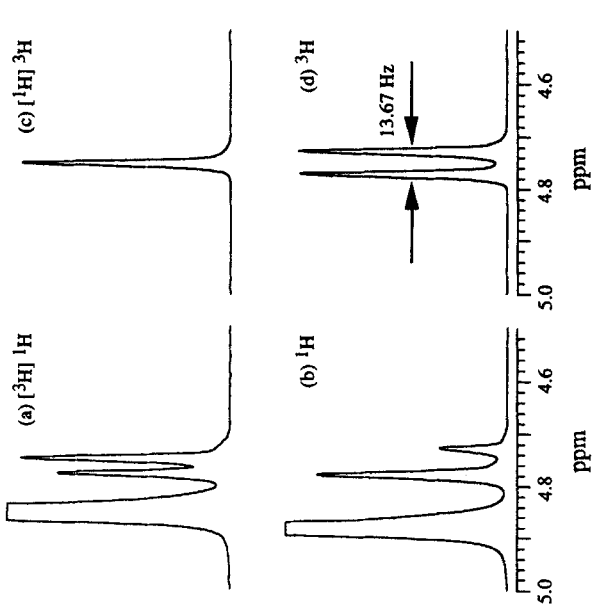


Figure 4: ^1H and ^3H NMR Spectra of 2-Naphthalene Methanol Obtained from the Reduction of 2-Naphthaldehyde by the $\text{NaBH}_4 / \text{T}_2$ Exchange Product.

Conclusions

We have demonstrated the production of LiBT_4 , NaBT_4 and KBT_4 with high tritium content ($>70\%T$), and the ability to react these reagents in a one-pot reaction to yield highly labelled reduction products, while also demonstrating retention of specific activity from reagent to reduction product. The labelling of borohydrides was achieved in one exchange cycle in a simple silica reaction tube, and no metal liner was used to protect the surface of the exchange vessel as in previous work.²⁷ Examples of the total activity of each borohydride obtained during 4-6 hours of a single exchange cycle, along with the quantity of each borohydride and tritium gas used are given in Table 4. Hence, 1.1 mg of LiBH_4 may be exchanged for 4.5 hours at 270°C in the presence of 1660 GBq (*ca.* 45 Ci) of T_2 , and yield tritiated borohydride with a tritium content of 73%, equating to 155 GBq (4.2 Ci) of product at a S.A. of 3100 GBq/mmol (84 Ci/mmol).

Table 4: Tritium Incorporated into Borohydride Reagents by a Single Exchange Cycle.

Borohydride Reagent	Amount of Borohydride (mg)	(mmol)	Tritium Gas Used [GBq (Ci)]	Tritium (%)	Equil. (%)	S.A. [#] (GBq/mmol)	Total Activity [GBq (Ci)]
LiBH_4	1.1	0.05	1660 (45)	73	83	3100 (84)	155 (4.2)
NaBH_4	2.3	0.06	1390 (38)	78	93	3320 (90)	199 (5.4)
KBH_4	2.7	0.05	1630 (44)	83	94	3530 (95.5)	177 (4.8)

[#] Theoretical maximum specific activity for $\text{MBT}_4 = 4252 \text{ GBq/mmol}$ (115 Ci/mmol). The figures given in this column reflect the mole fractions of hydrogen in the borohydride substrate vs tritium gas, and also that the samples have not exchanged to statistical equilibrium.

The specific activities obtained in all borohydrides by a single cycle of exchange were very high (*cf.* commercial tritiated NaBH_4 50-75 Ci/mmol) and the total tritium gas used was also very reasonable compared to other types of labelling techniques. This is especially true since the solid phase exchange technique allows the recovery of all the used tritium gas without fear of contamination by solvent or other reagents. Furthermore, by replacing the residual gas in the vessel with new tritium gas, it is possible to carry out multiple cycles of exchange to achieve almost 100%T in borohydrides. The relatively short exchange times used in these experiments (4-6 hours) were important to us, since we preferred to complete the exchange reaction and recovery of used tritium during a single work day. In the case of LiBH_4 we selected a higher

exchange temperature than others²⁷ to give a shorter exchange time. For NaBH₄ our results are inconsistent with the previous report,²⁷ (*i.e.* our exchange of NaBH₄ required a significantly higher temperature) and we assume that the nickel foil used in the reaction vessel for the prior work acted as a catalyst. As a consequence, we made a preliminary study of catalytic exchange of borohydrides, and decided that the potential benefits of this approach were outweighed by the wide variation in the results and the difficulty of separating the labelled product from the catalyst.

Over the past 25 years, the utility of alkali metal borohydrides has been greatly extended beyond simple reduction reactions. Techniques have been developed to effect reduction of a broad range of organofunctional groups in a wide variety of solvents. For example, solutions or suspensions of LiBH₄ or NaBH₄ in CH₃OH or dimethylformamide in the presence of transition metal salts (Ni, Co, Sn, Pd or La) are useful reducing agents which do not perturb aromatic derivatives.^{46,47} NaBH₄ on alumina was reported⁴⁸ to be a very gentle reducing agent and can be used in aprotic solvents. A number of new reducing agents have been synthesized from alkali metal borohydrides for the reduction of specific molecules, with each reagent having its own merits and characteristics, *e.g.*, NaBH(OAc)₃,^{49,50} NaBH₃CN⁵¹⁻⁵³ and KBH(*i*-PrO)₃.⁵⁴ Tritiated NaBT₄ may be used as a source of tritium gas for small scale labelling without employing any sophisticated apparatus.⁵⁵

The experiments reported here have allowed us to conveniently use all three reagents, LiBH₄, NaBH₄, and KBH₄ for high level tritiation reactions. The reagents are freshly made, have very high specific activity, and are readily prepared in convenient chemical and radioactive quantities. We have also demonstrated the preparation and use of tritiated borane from these reagents, and expect that many other derivative reagents such as NaBH(OAc)₃ and NaBH₃CN will be routinely available to us. We found ¹¹B NMR spectroscopy essential for analysis of the labelled borohydrides, and regard it as the preferred method for determination of deuterium content (%D) or specific activity (S.A.) of these reagents.

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