Synthesis of a new selective borotritide : [^{3}H]lithium9-boratabicyclo[3.3.1]nonane. Some applications to the preparation of secondary and primary [^{3}H] alcohols.

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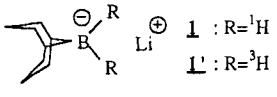
Abstract

[^{3}H]lithium 9-boratabicyclo[3.3.1]nonane, at specific activity of 52-55 Ci/mmol, was synthesized by reduction of B-OMe-9-BBN with lithium tritide. Its reducing characteristics were illustrated by the synthesis of [17-^{3}H] estradiol, [2-^{3}H] dihydrotetrabenazine and 1-(hydroxy[^{3}H]methyl)naphtalene, with good radiochemical yields and high specific activities.

Key words : Li9-BBNT, selective reduction, tritiated alcohols.

Introduction

In recent years, the emergence of new tritium labelling tools(1,2) evolved from the easy access to lithium tritide(2,3). Especially, P.G. Williams et al.(2) reported the synthesis of $LiAlT_4$ and $LiEt_3BT$ at maximum specific activity. Both are powerful reducing agents but $LiAlT_4$ lacks selectivity. In our efforts towards the development of new strategies to [^{3}H] compounds via organoboranes, we thought it interesting to carry out the synthesis of a new more selective borotritide, available at a high specific activity. [^{3}H]lithium 9-boratabicyclo[3.3.1]nonane **1'** (Li9-BBNT) appeared to be the reagent of choice for that purpose.



Indeed, H.C. Brown et al.(4) described Li9-BBNH **1** as a reagent occupying a unique position in the wide spectrum of reducing agents, exhibiting an intermediate reducing characteristic between mild $LiBH_4$ and strong $LiEt_3BH$(5b). Its reactivity with numerous classes of compounds was classified into four categories, illustrated in the following table.

Reductive characteristics of Li9-BBNH

fast reduction	slow reduction	sluggish reduction	inertness
aldehyde, ketone, ester, lactone, acyl chloride, acid anhydride, epoxide, disulfide, n-alkyl iodide, n-alkyl tosylate.	tertiary amide, alkyl bromide, aromatic nitrile.	carboxylic acid, aliphatic nitrile, primary amide, nitro compound, azoxy compound, secondary alkyl bromide, secondary alkyl tosylate.	olefin, oxime, alkyl chloride, azo compound, sulfide, sulfoxide, sulfone, sulfonic acid.

From this table, it is obvious that the tritiated agent **1'** would be a selective reductive compound, giving an easy access to numerous biologically active compounds such as alcohols and amines. For

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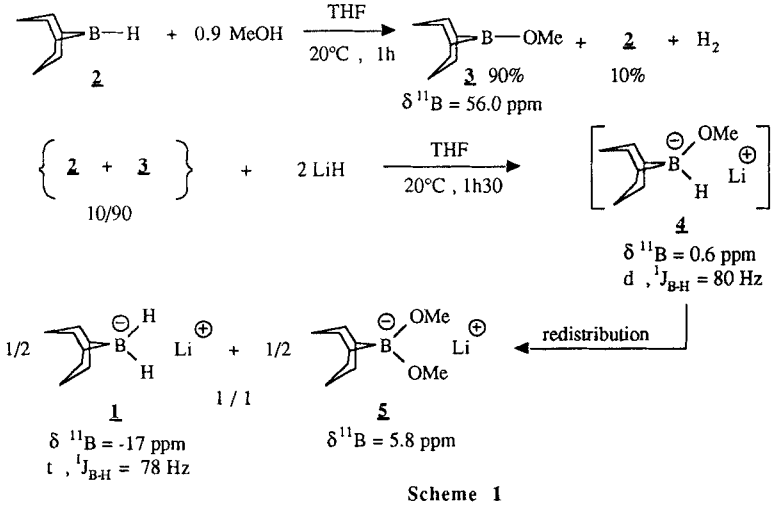
instance, 1' would be able to reduce a keto or an ester group without affecting a carboxylic group in the same molecule whereas $LiAlT_4$ would reduce both functionalities. However, unlike $LiEt_3BT$, 1' could selectively reduce a keto group in the presence of a bromine or a chlorine(5a), or in the presence of a carbon-carbon double bond. Moreover, it is worth noticing that $LiEt_3BT$ would exhibit the particular feature to reduce tertiary amides to tritiated alcohols whereas 1' would reduce them into tritiated amines. So, they are complementary to each other in that case.

In this paper, we report the synthesis of Li9-BBNT 1' with 90 % isotopic enrichment and its use for the preparation of labelled alcohols. Its reactivity is illustrated by the reduction of estrone, tetrabenazine and ethyl 1-naphtoate to alcohols which were specifically labelled at the reduced positions with a specific activity close to the theoretical maximum.

Synthesis

The synthesis of Li9-BBNH was described by H.C. Brown et al.(4). The reaction between 9-BBN and 1.5 equivalent of lithium hydride in THF under reflux conditions for 24 hours afforded compound 1. In a preliminary experiment, we noticed that "superactive" LiH(3) prepared by hydrogenation of a mixture of n-BuLi and N,N,N',N'-tetramethylethylenediamine (TMEDA), reacted with 9-BBN at room temperature whereas reflux was required with commercial LiH. Nevertheless, the application of this procedure to the synthesis of 1' from LiT could only lead to an average specific activity of 29 Ci/mmol only whereas a much higher specific activity was desired. Moreover, S. Narasimhan(6) had noted that the presence of a trace of a borinic ester in 9-BBN gave faster reactive rates with LiT. Therefore, we decided to investigate the reactivity of superactive LiH with B-OMe-9-BBN 3. The study was first carried out using non tritiated species, all the compounds in scheme 1 being characterized via their ^{11}B NMR spectra recorded at 96 MHz.

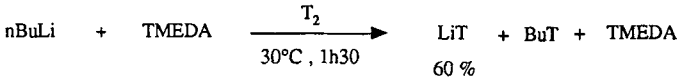


Scheme 1

The reaction of 2 with 0.9 equivalent of anhydrous methyl alcohol afforded the mixture 2/3 in the ratio 10/90. It was then reduced by a double stoichiometry of lithium hydride to lead to the intermediate 4 which redistributed to the dimethoxydialkylborane 5 and to 1 in a 40-50 % yield from 9-BBN. Compound 4 was not observed by ^{11}B NMR under these conditions. If pure B-OMe-9-BBN 3 was used, then 4 redistributed very slowly and could be observed by ^{11}B NMR. We showed

that 10 % 9-BBN were necessary for the reaction to go to completion in 1h30 instead of five days in absence of 9-BBN. **1'** was synthesized according to scheme 1, using a double stoichiometry of lithium tritide. For practical security reasons, the tritiated species were not characterized by NMR. It seems reasonable to admit that **1'** was obtained in at least 40 % yield from 9-BBN. It is worth noticing the inertness of compound **5** in the reduction process since the radiochemical yields in tritiated products are excellent, as will be described below.

The reaction between tritium gas and a mixture of n-BuLi/TMEDA provided LiT, according to scheme 2.

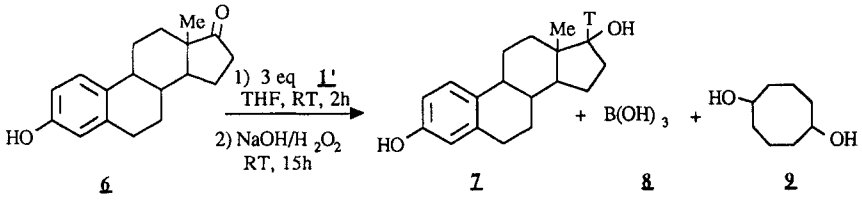


Scheme 2

The yield of LiT was determined by measurement of the amount of n-BuLi used in the reaction. The titration procedure is detailed in the experimental part.

Some examples of the use of 1' for the preparation of [^{3}H] alcohols

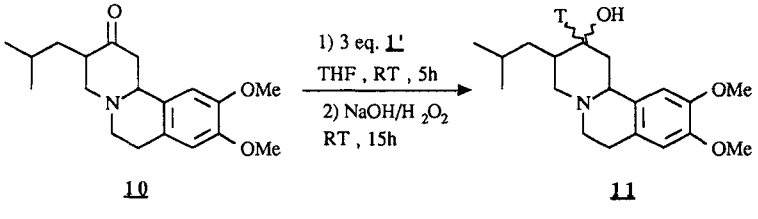
We investigated the reduction of ketones and esters to alcohols.We first carried out the reduction of estrone **6** into [17-^{3}H] estradiol **7** (scheme 3).



Scheme 3

A solution of **6** in dry THF was added to 3 equivalents of Li9-BBNT, one equivalent being consomated by the hydroxyl function. The reaction mixture was kept at room temperature for 2 hours and then oxidized by 30 % hydrogen peroxide in the presence of sodium hydroxide to give the expected [17-^{3}H] estradiol **7** (1.0 Ci, radiochemical purity>90%). An aliquot of **7** was purified by flash chromatography on silica gel. ^{3}H NMR analysis confirmed that **7** was specifically labelled at the reduced position (3.57 ppm). The value of 26 Ci/mmol for the specific activity of **7** was determined by mass spectroscopy. Consequently, that of **1'** was as high as 52 Ci/mmol, as expected. **7** was previously prepared with an average specific activity of 14 Ci/mmol using a 60 Ci/mmol KBT_4 (7). Therefore, Li9-BBNT is to be preferred when a high specific activity is sought.

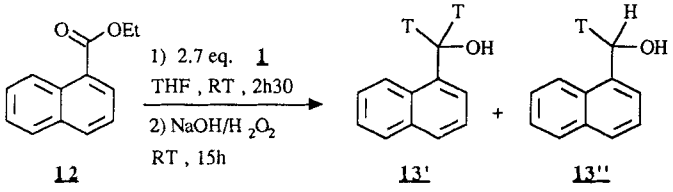
In order to check the reproducibility of this first interesting result, we carried out the reduction of tetrabenazine **10** into [2-^{3}H] dihydrotetrabenazine **11** (scheme 4).



Scheme 4

The previously described procedure was used with excess Li9-BBNT. 1.2 Ci were obtained, the radio thin layer chromatography (RTLC) essentially displaying the two isomers **11α** and **11β** in the approximative ratio 1/1, with a radiochemical purity>90%. They were purified and separated by preparative HPLC using a reversed phase column. The analysis of their ^3H NMR spectra gave chemical shifts of 3.25 ppm (**11α**) and 3.95 ppm (**11β**), thus showing again that they were specifically labelled. Their specific activity measured by mass spectroscopy was 27 Ci/mmol which was fully comparable to the previous value of 26 Ci/mmol for **7**.

We then investigated the reduction of one carboxylic ester as a way to label a primary alcohol with two tritium atoms per molecule. Ethyl 1-naphtoate **12** was chosen as substrate ; it was reduced to 1-(hydroxy[^3H]methyl)naphtalene **13** (scheme 5).



12 **13'** **13''**

Scheme 5

13 was quantitatively obtained (2.1 Ci, radiochemical purity>95%). After purification on silica gel by flash chromatography of an aliquot, **13** was analysed by ^3H NMR. The ratio between the mono and ditritiated species was 10/90, thus providing the specific activity of 55 Ci/mmol. This value was confirmed by mass spectroscopy.

Conclusion

The synthesis of 52-55 Ci/mmol Li9-BBNT was described. Three examples of its use for the labelling of alcohols were given. The use of Li9-BBNT proved to be an efficient method for the radiolabelling of alcohols starting either from ketones or esters. Interesting results were reached both in terms of radiochemical purities and specific activities. This new borotritide should allow the labelling of numerous compounds. Further investigations are currently in progress in our laboratory to explore the potential of Li9-BBNT. Especially, our attention is now directed towards the labelling of amines by reduction of tertiary amides.

Experimental section

All chemicals were purchased from Aldrich except for tetrabenazine (Fluka) and tritium gas (CEA). Tetrahydrofuran (THF), pentane, methyl alcohol and N,N,N'N'-tetramethylethylenediamine (TMEDA) were freshly dried prior to use by distillation under nitrogen from sodium benzophenone ketyl, Mg/iodine, P_2O_5 and $LiAlH_4$ respectively. The purity of 9-BBN 0.5M/THF was regularly checked by analysis of its ^{11}B NMR spectrum. All reactions were carried out under argon in glassware dried at 150°C. NMR spectra were recorded in CD_3OD for ^3H at 320.130 MHz and in THF/THF-d_8 for ^{11}B at 96.295 MHz on a Bruker 300 AC spectrometer. Chemical shifts are expressed in ppm downfield from tritiated water (external) and boron trifluoride etherate (external).

Mass spectra were recorded on a Quadripole Finnigan Mat 4600 by electronic impact (EI) or ionization by chemical desorption with ammonia (DCI NH_3). The purification of tritiated alcohols was done either on a medium pressure apparatus (10 bars) using 40g of silica gel (15-40 µm, Merck) or on a preparative HPLC using a Zorbax C18 column, these apparatuses being equipped with radioactivity and UV detections. Thin layer chromatography (TLC) was performed on glass plates coated either with a 0.25 mm layer of silica gel 60F-254 purchased from Merck or with a 0.2 mm layer of reversed phase KC18F purchased from Whatman. The radioactivity of the tritiated samples was counted in a Wallac 1409 apparatus.

Preparation of Li9-BBNT 1' : the following procedure is representative.

1.6M nBuLi/hexanes (3 mL, 4.8 mmol) and TMEDA (0.725 mL, 4.97 mmol) were mixed under argon. 0.8 mL of this mixture was hydrolyzed by ice and titrated by 0.1N HCl till pH=8.5, in the presence of phenolphtaleine. Volume V_1 amounted to 22.0 mL. 0.8 mL of the mixture was then introduced in a centrifuged vial fitted with a magnetic stirring bar. After connecting the vial to the Toepler apparatus fitted with a 50 Ci (1.72 mmol) tritium gas ampoule, the reaction mixture was frozen and the argon was removed under vacuum. It was then put under a tritium pressure and warmed to 30-35°C for 1h30 with stirring ; after that time, no more gas absorption occured. 2 mL of anhydrous pentane were added to the reaction mixture which was centrifuged : LiT precipitated as a finely divided white powder. The clear yellowish supernatant solution was transferred via syringe to another flask. The solid was washed twice with pentane (2 mL) and the washings were combined with the supernatant solution. This mixture was hydrolyzed and titrated with 0.1N HCl till pH=8.5. Volume V_2=15.8 mL was obtained. Thus, the amount n of LiT was : $n = 0.1(V_1-V_2) = 0.62$ mmol which represents a 60% yield from nBuLi. The mixture 9-BBN/B-OMe-9-BBN was then prepared by mixing a 0.5M 9-BBN/THF solution (0.62 mL, 0.31 mmol) with 0.9 equivalent of anhydrous methyl alcohol (0.011 mL, 0.28 mmol). It was left at room temperature for 1h and then added to LiT in 0.5 mL of THF. After 1h30 at room temperature, the compound to be reduced was injected to the solution of Li9-BBNT, the estimated amount being 0.124 mmol (40% from 9-BBN).

[17-^{3}H] estradiol 7

Estrone (11.15 mg, 0.041 mmol) in 0.5 mL of anhydrous THF was added to Li9-BBNT (0.124 mmol estimated). After stirring the reaction mixture for 2 hours at room temperature, it was hydrolyzed by 3N sodium hydroxide (0.1 mL, 0.3 mmol) and then oxidized with 30% hydrogen peroxide (0.11 mL, 0.9 mmol). It was kept at room temperature for 15 hours and diluted with 10 mL of diethyl ether. The organic layer was washed twice with a saturated solution of ammonium chloride (2 mL) and dried by filtration on $MgSO_4$. After removal of diethyl ether and THF under vacuum, the crude product was solubilized in methyl alcohol. The total radioactivity was measured on an aliquot (1 Ci). An aliquot of the crude product was purified by medium pressure chromatography (10 bars) on silica gel (hexane/ethyl acetate 70/30) using a UV detection at 280 nm.

Rf = 0.35 (hexane/ethyl acetate : 70/30).

^{3}H NMR, CD_3OD, δ : s, 3.57.

Specific activity : 26 Ci/mmol (mass spectroscopy, EI).

[2-^{3}H] dihydrotetrabenazine 11

Li9-BBNT was prepared as described above. A solution of tetrabenazine (16.4 mg, 0.052 mmol) in 0.5 mL of anhydrous THF was added to Li9-BBNT (0.156 mmol estimated). The reaction mixture was kept at room temperature for 5 hours and treated as described for 7 : 3N NaOH, 0.13 mL, 0.4 mmol ; 30% H_2O_2, 0.12 mL, 1 mmol ; washing with NH_4Cl. The crude product was kept in methyl alcohol. The scintillation counting of an aliquot displayed a total activity of 1.2 Ci. 11 was detected as a mixture of α and β isomers in the ratio 1/1 (RTLC). An aliquot was purified by preparative HPLC using a Zorbax C18 column (MeOH/0.030 M aqueous NH_4HCO_3 70/30), 11 being detected at 280 nm.

Rf (11α) = 0.90 ; Rf (11β) = 0.75 (SiO_2 ; CH_2Cl_2/MeOH : 9/1).

^{3}H NMR, CD_3OD, δ : 11α : s, 3.25 ; 11β : s, 3.95.

Specific activity : 27 Ci/mmol (mass spectroscopy, DCI NH_3).

1-(hydroxy[^{3}H]methyl)naphtalene 13

A solution of ethyl 1-naphtoate (8.0 mg, 0.04 mmol) in 0.05 mL of THF was added to Li9-BBNT (0.11 mmol estimated). The mixture was kept at room temperature for 2h30 and treated as previously described : 3N NaOH, 0.13 mL, 0.4 mmol ; 30% H_2O_2, 0.1 mL, 0.8 mmol ; washing with NH_4Cl. The radioactivity of the crude product was measured (2.1 Ci). An aliquot was purified by medium pressure chromatography (10 bars) on silica gel (hexane/ethyl acetate 75/25), 13 being detected at 254 nm.

Rf = 0.25 (hexane/ethyl acetate : 8/2).

^{3}H NMR, CD_3OD, δ : 13' : s, 4.96 ; 13'' : s, 4.99.

Specific activity : 55 Ci/mmol (mass spectroscopy, DCI NH_3 and ^{3}H NMR).

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7. Unpublished results.

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