

## Research Article

# Tritium labelling of bromhexine via amide reduction with $\text{LiB}^3\text{H}_4$

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## Summary

The mucolytic agent bromhexine is useful in the evaluation of the performance of inductively coupled plasma mass spectrometry (ICP-MS) as compared to radiolabelling. A prerequisite for this comparison is access to radiolabelled bromhexine. [ $^3\text{H}_2$ ]-bromhexine was synthesized in three steps introducing tritium in the final step by reduction of 2-amino-3,5-dibromo-*N*-methyl-*N*-cyclohexylbenzamide. Good chemical yields, high specific activity (54 Ci/mmol) and high radiochemical purity (98.5%) were achieved. Reduction with  $\text{LiB}^3\text{H}_4$  in dichloromethane proved to be very efficient and easy to handle. Copyright © 2005 John Wiley & Sons, Ltd.

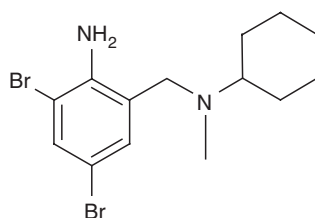
**Key Words:** tritium labelling;  $\text{LiB}^3\text{H}_4$ ; bromhexine; amide reduction

## Introduction

Information on properties such as absorption, distribution, metabolism and excretion (ADME) of new drug candidates is important in drug discovery and development. Radiolabelled tracers are currently an essential part of these *in vivo* and *in vitro* drug metabolism studies as the tracers can be detected and quantified by the nuclear decay. However, in many situations the use of radioactive isotopes is problematic or impossible. This is true in the early discovery phase where a radiolabelled version of the candidates is not available or during the first clinical trials where documentation for administration of

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## Figure 1. Bromhexine

radioisotopes to humans has not been generated. In addition it is generally appealing to avoid both the synthesis and the handling of a radiotracer if possible.

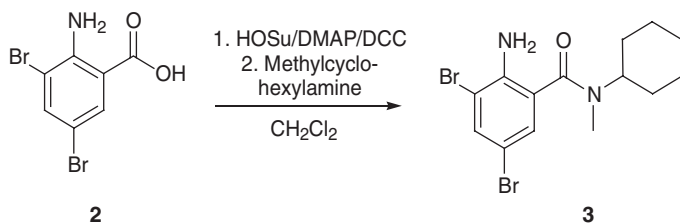
Within the last few years inductively coupled plasma mass spectrometry (ICP-MS) have been described as alternative to the use of radiotracers in drug metabolism studies. ICP-MS has been applied in the analysis of compounds containing elements such as bromine,<sup>1,2</sup> chlorine,<sup>3</sup> iodine,<sup>4</sup> phosphorus<sup>5</sup> and sulphur.<sup>6</sup>

Bromhexine (**1**) (Figure 1) is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus.<sup>7–9</sup> Bromhexine is suitable for evaluation of ICP-MS compared to radiolabelling. The comparison requires access to a radiolabelled form of bromhexine. We now report a procedure for labelling of bromhexine with tritium in excellent yield, specific activity and radiochemical purity.

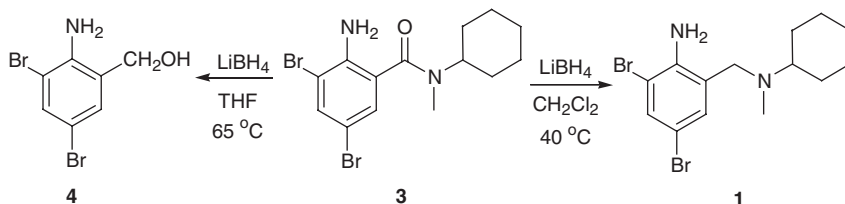
## Results and discussion

The first approach for the labelling of bromhexine (**1**) was a classical method for tritium labelling of aromatic compounds involving dehalogenation of a halogenated precursor. As bromine is present in the labelled target molecule, iodine, which is easier to reduce than bromine,<sup>10</sup> had to be inserted at one of the two vacant positions in the aromatic moiety of bromhexine (**1**). However, iodination of bromhexine (**1**) by electrophilic aromatic substitution failed. Treatment of **1** with *N*-iodosuccinimide in acetonitril/TFA,<sup>11</sup> pure TFA or trifluoromethane sulphonic acid<sup>12</sup> did not yield the desired iodinated precursor. Treatment with *N*-iodosuccinimide and Hg(TFA)<sub>2</sub><sup>13</sup> also proved unsuccessful. Apparently, iodination of bromhexine (**1**) is not feasible, possibly due to congestion around the aromatic moiety.

Therefore a three-step sequence introducing tritium through reduction of an amide functionality was investigated. Thus, commercially available 2-amino-3,5-dibromo-benzoic acid (**2**) was converted to the activated ester by treatment with *N*-hydroxysuccinimide (HOSu), dimethylaminopyridine (DMAP) and



**Scheme 1. Preparation of 2-amino-3,5-dibromo-*N*-methyl-*N*-cyclohexylbenzamide (3)**



**Scheme 2. Reduction of benzamide (3) with LiBH<sub>4</sub>**

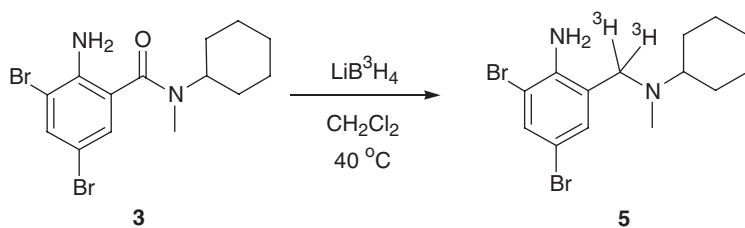
dicyclohexylcarbodiimide (DCC). Subsequent treatment with *N*-methylcyclohexylamine gave 2-amino-3,5-dibromo-*N*-methyl-*N*-cyclohexylbenzamide (3) in 78% yield (Scheme 1).

Reduction of amides with LiAlH<sub>4</sub> is sometimes problematic due to absorption of the formed amines to the precipitated aluminium salts. This can be avoided by quenching the reaction mixture very carefully with aqueous NaOH or saturated aqueous Na<sub>2</sub>SO<sub>4</sub>. These procedures are, however, difficult to handle in the small scale employed on a tritium manifold. If LiAlH<sub>4</sub> is replaced with LiBH<sub>4</sub> the reduction of amides produces a precipitate that easily dissolves when the reaction mixture is quenched with methanol.

Preliminary experiments reducing 3 with LiBH<sub>4</sub> in THF gave 2-amino-3,5-dibromobenzyl alcohol (4) as the major product and the desired amine 1 as the minor. Replacing THF with dichloromethane as the solvent improved the yield of bromhexine (1) significantly and only traces of 4 were formed (Scheme 2).

Test reactions with freshly produced LiB<sup>2</sup>H<sub>4</sub> gave almost 100% incorporation of deuterium as determined by the absence of benzylic protons in the <sup>1</sup>H-NMR spectrum. Quantitative incorporation of deuterium required quenching of the reaction mixture with 10% acetic acid in methanol, as the absence of acidic quenching gave less incorporation of deuterium due to base-catalyzed D/H-exchange of the benzylic deuterons.

Adopting the protocol to the tritium manifold system using LiB<sup>3</sup>H<sub>4</sub> prepared from Li<sup>3</sup>H and BBr<sub>3</sub> in hexane<sup>14</sup> afforded [<sup>3</sup>H<sub>2</sub>]-bromhexine (5) in 52% crude yield (Scheme 3). A small portion of this was purified by HPLC to



**Scheme 3. Tritium labelling of bromhexine**

give **5** in 43% isolated yield with a specific activity of 54 Ci/mmol and in 98.5% radiochemical purity.

### Experimental

The reaction with tritium gas was performed on a custom build tritium handling unit from RC Tritec AG, Switzerland, who also supplied the tritium source ( $U^3H_3$ ). Tritium gas was prepared *in situ* by heating  $U^3H_3$ . HPLC was performed using a Merck Hitachi Intelligent Pump L6200A equipped with a Merck Hitachi Column Thermostat T5025 with a Rheodyne injector, and a Merck Hitachi UV Detector L4000A (detection at 254 nm). Detection of tritium was performed on a Canbarra Packard flow detector A500. Analytic and preparative HPLC were performed on a RP C18 column ( $4.6 \times 250$  mm, 5  $\mu$ m, OdDMeSi 120 Å, Novo Nordisk) with a flow of 1.0 ml/min. and 0.1% TFA, 25% MeCN, 75%  $H_2O$  as the mobile phase. Radioactivity measurements were performed on a Packard Tri-Carb 1000 liquid scintillation analyzer using Ultima Flo<sup>TM</sup> M (Packard Bioscience) as the scintillation liquid.  $^1H$ - and  $^{13}C$ -NMR spectra were recorded on a Bruker DRX 300 spectrometer (carbon atoms from the cyclohexyl ring appear as very weak signals in standard  $^{13}C$ -NMR spectra due to conformational mobility, and for this reason these signals are not reported). Flash chromatography was performed with silica gel 60 Å (Merck, 40–63  $\mu$ m).

#### *2-Amino-3,5-dibromo-N-methyl-N-cyclohexylbenzamide (3)*

2-Amino-3,5-dibromo-benzoic acid (**2**) (110 mg, 0.34 mmol), HOSu (50 mg, 0.43 mmol) and DMAP (18 mg, 0.15 mmol) were dissolved in  $CH_2Cl_2$  (5 ml). Then, DCC (100 mg, 0.48 mmol) in  $CH_2Cl_2$  (2 ml) was added drop-wise over 10 min. The mixture was stirred for 1 h and a white precipitate of dicyclohexylurea appeared within a few minutes. *N*-Methylcyclohexylamine (200 mg, 1.7 mmol) was added and the mixture was stirred for another hour. Glacial acetic acid (1 ml) was added to quench excess of DCC. Filtration and concentration in vacuum followed by flash column chromatography (1% TEA, 30% EtOAc, 69% heptanes) gave **3** as white crystals (103 mg, 78%) Mp:

124–126°C;  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.95–1.90 (10H, m, cyclohexyl) 2.85 (3H, br s, NMe), 3.45 (1H, br m, NCH) 4.66 (2H, br s,  $\text{NH}_2$ ) 7.10 (1H, d,  $J=2.2$  Hz, H-4 aryl) 7.53 (1H, d,  $J=2.2$  Hz, H-6 aryl);  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  25.5, 108.5, 111.1, 123.4, 128.8, 135.2, 141.8, 168.4.

### $[\text{}^3\text{H}_2]$ -bromhexine (**5**)

*n*-BuLi (100  $\mu\text{l}$ , 1.5 M in hexanes, 0.15 mmol) and TMEDA (30  $\mu\text{l}$ , 0.20 mmol) were added to a 1 ml flask filled with a  $^3\text{H}_2$ -atmosphere (600 mbar). Under decreasing pressure a white precipitate of  $\text{Li}^3\text{H}$  separated during 1 h. The reaction flask was cooled in liquid nitrogen and excess tritium gas was captured onto an uranium waste bed. TMEDA and hexanes were then lyophilized to a cold finger cooled by liquid nitrogen and a 700 mbar  $\text{N}_2$ -atmosphere was established in the reaction flask. Dry THF (100  $\mu\text{l}$ ) and  $\text{BBr}_3$  (30  $\mu\text{l}$ , 1.0 M in hexanes, 30  $\mu\text{mol}$ ) were added and the mixture was heated to 60°C for 30 min. The reaction flask was cooled in liquid nitrogen, the  $\text{N}_2$ -atmosphere was removed under vacuum and THF was lyophilized to a cold finger cooled by liquid nitrogen. A 700 mbar  $\text{N}_2$ -atmosphere was established in the reaction flask and 2-amino-3,5-dibromo-*N*-methyl-*N*-cyclohexylbenzamide (**3**) (4.0 mg, 10  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (200  $\mu\text{l}$ ) was added. The mixture was stirred for 1 h at 40°C and quenched with 10%  $\text{CH}_3\text{COOH}$  in MeOH (300  $\mu\text{l}$ ). The solvents were lyophilized to a cold finger cooled by liquid nitrogen and MeOH (500  $\mu\text{l}$ ) was added and lyophilized three times to remove labile tritium. Finally, the residue was dissolved in ethanol to give the crude product **5** (283 mCi, 5.2  $\mu\text{mol}$ , 52%). 7.0 mCi of this was further purified and analyzed by HPLC to give  $[\text{}^3\text{H}_2]$ -bromhexine (**5**) (5.8 mCi, 43%) with a specific activity of 54 Ci/mmol and 98.5% radiochemical purity. The specific activity was established by HPLC.

### Conclusion

We have developed a synthetic route for the labelling of bromhexine with tritium in good yield (43%) with high specific activity (54 Ci/mmol) and high radiochemical purity (98.5%). The results demonstrate that  $\text{LiB}^3\text{H}_4$  in dichloromethane serves as an effective labelling agent for the labelling of amines obtained by reduction of amides. The use of  $\text{LiB}^3\text{H}_4$  renders handling and work up of the labelled product significantly easier than in  $\text{LiAl}^3\text{H}_4$ -based reduction.

### Acknowledgements

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