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Synthesis of ^{3}H-(–)-galanthamine

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Summary

^{3}H-(–)-galanthamine (0.881 TBq/mmol) was synthesized via stereoselective reduction of (–)-narwedine with tritiated L-Selectride®. This reaction sequence was favored over an exchange of aromatic bromine with lithium aluminium tritide. Copyright © 2001 John Wiley & Sons Ltd.

Key Words: (–)-galanthamine; tritiation; radiosynthesis; Alzheimer's disease; acetylcholinesterase inhibitor

Introduction

Galanthamine, an alkaloid of the amaryllidaceae family[1] is known to act as a competitive and reversible inhibitor of the enzyme acetylcholinesterase[2] (AChE). Furthermore, galanthamine is a non-competitive nicotinic receptor agonist, and an allosterically potentiating ligand for the nicotinic response induced by acetylcholine[3] (ACh). This compound therefore has the potential to exhibit a dual function at the nicotinic synapse.[4] Based on the cholinergic hypothesis, galanthamine is a promising drug for the treatment of Alzheimer's disease (AD).[5] The hydrobromide salt of galanthamine (Nivalin®) has been approved for the treatment of AD in several European countries. In addition, this drug has been used for 30 years in the clinical treatment of different neurological illnesses and in anaesthesia.[6]

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For further investigation of the AChE inhibition by galanthamine as well as for an analysis of the interaction between galanthamine and the nicotinic acetylcholine-receptor,[7] tritiated (–)-galanthamine of high specific activity was needed.

Results and discussion

Two alternative routes for specific labelling of (–)-galanthamine **2a** have been investigated as outlined in Figure 1.

Investigation of route A

Hydrogenolysis of aromatic compounds by exchange of halogen atoms with $LiAlT_4$ is a widely known and convenient method for tritiation. Our first approach towards the synthesis of ^{3}H-(–)-galanthamine

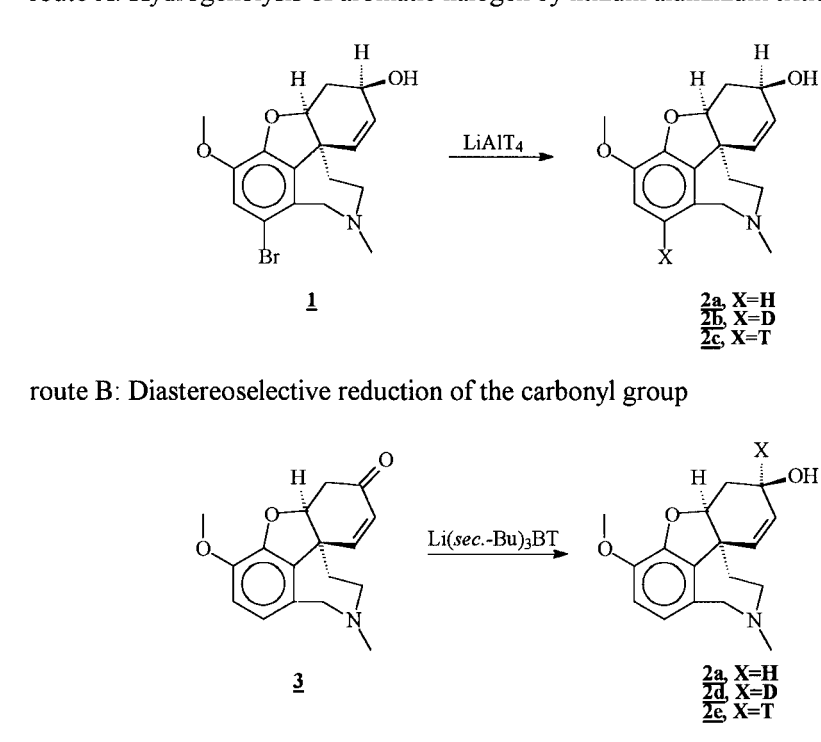


Figure 1. Potential routes to ^{3}H-(–)-galanthamine

therefore was based on the tritiation of readily available 8-bromo-(–)-galanthamine **1** with $LiAlT_4$. (–)-Galanthamine hydrobromide **4** is commercially available as Nivalin® and can be monobrominated using hydrogen-peroxide in formic acid[8] (Figure 2). Reduction of 8-bromo-(–)-galanthamine **1** with $LiAlH_4$ resulted in a complete reaction within 20 h using four equivalents of hydride reagent with a yield of 79%.[9] In order to investigate isotope effects in this reaction we substituted $LiAlH_4$ with $LiAlD_4$ and obtained 8-^{2}H-(–)-galanthamine **2b** in a similar yield of 74%; however, a reaction time of 52 h and quenching of the reaction product with D_2O (Figure 2) is required.

An isotope effect presumably is the cause for more than a doubling of the reaction time and probably a further increase of the reaction time can be expected if $LiAlT_4$ is used instead of $LiAlD_4$. In addition, in order to obtain high incorporation of deuterium (e.g. $>70\%$) it was necessary to employ deuterium oxide as quenching reagent. If H_2O is used rather than D_2O, a deuteration rate of only 20% was observed. This finding parallels the results of a deuteration of anthracene derivatives described in the literature.[10] In addition, that study reports an even higher incorporation rate when the system $LiAlH_4/D_2O$ is used rather than $LiAlD_4/H_2O$.

Investigation of route B

In the large-scale synthesis of (–)-galanthamine as described by Jordis *et al.* [11], the final step consists of a diastereoselective reduction of (–)-narwedine **3** with L-Selectride®. This reaction gives high yields with virtually no by-product formation. In order to make use of this procedure for the tritiation of **3**, tritiated L-Selectride® **7** is needed. The synthesis of this compound has been described in the literature[12] (Figure 3).

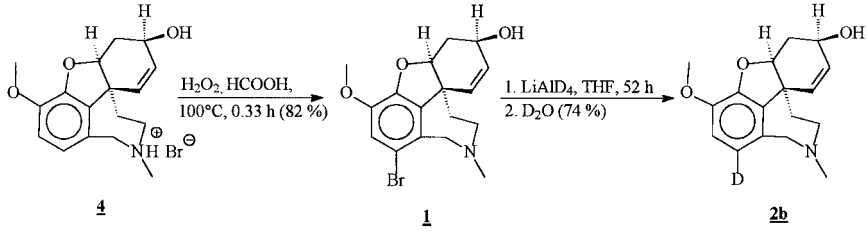


Figure 2. Synthesis of 8-^{2}H-(–)-galanthamine via route A

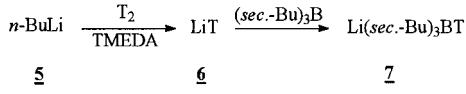


Figure 3. Synthesis of tritiated L-Selectride® 7

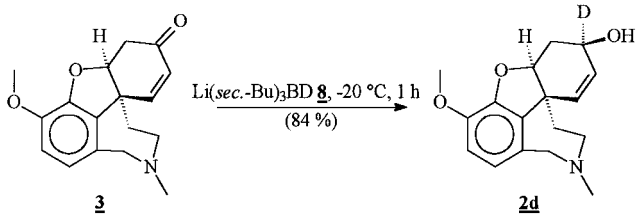
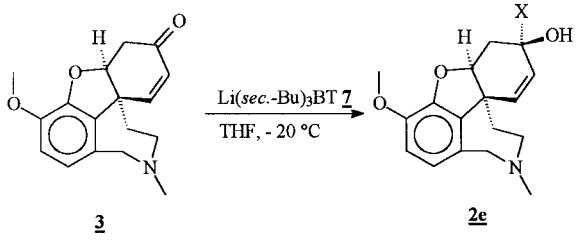


Figure 4. Reduction of (–)-narwedine 3 with ^{2}H-L-Selectride® 8

In order to investigate the applicability of this reaction to the synthesis of tritiated (–)-galanthamine we have synthesized deuterated L-Selectride® (Li(*sec*-Bu)$_3$BD) **8** based on the procedure described for the tritiated derivative. Compound **8** was used without purification in the reduction of (–)-narwedine **3**. The reaction proceeds with 84% yield at -20°C within 1 h and gives the expected deuterated (–)-galanthamine **2d** (Figure 4).

Virtually, no difference in the reaction time was observed with respect to the corresponding hydrogen reaction and the yield, purity and stereochemistry are as in the corresponding synthesis of (–)-galanthamine **2a**. NMR-analysis of the reaction product proved that the incorporation of deuterium took place exclusively in the required position. The incorporation of deuterium in compound **2d** was determined to be $>95\%$, and the coupling constant for the ^{13}C–^{2}H coupling was found to be 21 Hz. Due to these results, route B was selected for the synthesis of specifically tritiated (–)-galanthamine **2e**.



*Synthesis of 3-^{3}H-(–)-galanthamine **2e** via route B*

(–)-Narwedine **3** was reduced with tritiated L-Selectride® **7** using the conditions and work up procedure described for the corresponding deuteration reaction which gave a 61% yield and a total radioactivity of 6.4 Ci. (0.237 TBq) of 3-^{3}H-(–)-galanthamine **2e**. The specific activity was determined via MS to 23.8 Ci/mmol (0.881 TBq/mmol).

Experimental

*[4aS,6R,8aS)-1-bromo-3-methoxy-4a,5,9,10,11,12-hexahydro-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepine-6-ol (8-bromo-(–)-galanthamine) **1***

To a solution of galanthamine hydrobromide **4** (4 g, 10.8 mmol) in formic acid (40 ml) was added hydrogen peroxide solution (40 ml, 35%, v/v). The reaction mixture was heated for 20 min at 100°C. The reaction flask was cooled quickly to room temperature and the pH value was adjusted with concentrated ammonia solution (25%, v/v) to pH 10. The mixture was extracted 3 times with ethyl acetate (50 ml). The organic layer was washed once with brine (50 ml) and dried over $MgSO_4$. The solvent was evaporated and the remaining solid was purified via column chromatography (silica gel 150 g, $CHCl_3$ 92%: MeOH 8%) to yield 8-bromo-(–)-galanthamine **1** (2.22 g, 6.08 mmol, 56.3% of theory, Lit[8] 64%) as a white foam.

^{1}H-NMR ($CDCl_3$, 200 MHz): δ [ppm] = 1.62 (d, 1H, H-9, J 13.87 Hz), 1.97–2.15 (m, 2H, H-9, H-5), 2.45 (s, 3H, NCH_3), 2.67 (d, 1H, H-5′), 2.98 (d, 1H, H-10), 3.23 (d, 1H, H-10′), 3.84 (s, 3H, OCH_3), 3.96 (d, 1H, H-12, J 15.7 Hz), 4.16 (s, 1H, H-6), 4.32 (d, 1H, H-12′, J 15.7 Hz), 4.63 (s, 1H, H-4a), 5.99–6.13 (m, 2H, H-8, H-7), 6.93 (s, 1H, H-1).–

^{13}C-NMR ($CDCl_3$, 50 MHz) δ [ppm] = 30.21 (t, C-5), 34.03 (t, C-9), 42.52 (q, NCH_3), 49.17 (s, C-8a), 53.88 (t, C-10), 56.55 (q, OCH_3), 62.28 (t, C-12), 89.23 (d, C-4a), 114.94 (d, C-1), 116.19 (d, C-8), 126.68 (t, C-2), 127.89 (s, C-12a), 128.54 (t, C-7), 134.55 (s, C-12b), 144.66 (s, C-3a), 145.88 (s, C-3).–

*[4aS,6R,8aS)-3-methoxy-4a,5,9,10,11,12-hexahydro-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepine-6-ol (^{1}H-(–)-galanthamine) **2a***

Method A

$LiAlH_4$ (0.030 g, 0.76 mmol) was dispersed in dry THF (15 ml). The solution was cooled to 0°C and 8-bromo-galanthamine **3** (0.07 g,

0.19 mmol) dissolved in dry THF (5 ml) was added slowly. After 20 h complete conversion of **3** was achieved (determined by GC). The mixture was quenched with H_2O, the solvent evaporated and the residual solid purified via column chromatography (silica gel 15 g, $CHCl_3$ 96%, MeOH 4%) to yield ^{1}H-(–)-galanthamine **2a** (0.522 g, 0.15 mmol, 79% of theory, Lit.[7]: 49%) as a white foam.

Method B

A solution of *n*-buthyllithium **5** (1.4 M[13] in hexane, 0.63 ml, 0.884 mmol) and N,N,N′N′-tetramethyl-ethylendiamine (0.16 ml, 1.061 mmol) was stirred for 2 h at room temperature in the presence of a hydrogen atmosphere. After addition of the amine a white precipitate was rapidly formed. The hydrogen gas was displaced by argon and tri-*sec*-butylborane solution (1.0 M (THF), 0.89 ml, 0.884 mmol) was added to the reaction mixture thereby re-dissolving the precipitate. The solution was cooled to −30°C and a suspension of (–)-narwedine **5** (0.126 g, 0.442 mmol) in THF (0.5 ml) was slowly added. The reaction mixture was stirred for 0.5 h at −20°C and 0.5 h at room temperature. Excess of reducing reagent was quenched with methanol at 0°C. The solvent was evaporated off and the remaining solid dissolved in EtOH (4 ml). HBr solution (48%, w/v) was added to adjust the pH to 1 and the solution stored overnight at 0°C. The precipitate was filtered, washed with EtOH (1 ml) and dried under vacuum. The resulting solid was dissolved in water (4 ml), brought to pH 10 by ammonia solution (25%, v/v) and extracted 4 times with EtOAc (2.5 ml). The combined organic layers were washed with brine (2 ml) and dried over $MgSO_4$. The solvent was evaporated off and the remaining solid dried under vacuum to yield ^{1}H-(–)-galanthamine **2a** (0.103 g, 0.358 mmol, 81.1% of theory) as a white foam. The spectroscopic data were identical with literature date.[7]

^{1}H-NMR ($CDCl_3$, 200 MHz): δ [ppm] = 1.62 (dd, 1H, 9-H), 1.97–2.18 (m, 2H, 5-H, 9-H′), 2.42 (s, 3H, NCH_3), 2.71 (dd, 1H, 5-H′), 3.07 (t, 1H, 10-H), 3.30 (t, 1H, 10-H′), 3.70 (d, 1H, 12-H), 3.85 (s, 3H, OCH_3), 4.08–4.18 (m, 2H, 6-H, 12-H′), 4.63 (s, 1H, 4a-H), 5.97–6.11 (m, 2H, 7-H, 8-H), 6.62–6.71 (d, 2H, 1-H, 2-H).–

^{13}C-NMR ($CDCl_3$, 50 MHz): δ [ppm] = 30.37 (t, C-5), 34.21 (t, C-9), 42.52(q, NCH_3), 48.63 (s, C-8a), 54.25 (t, C-10), 56.30 (q, OCH_3), 61.03 (t, C-12), 62.48 (d, C-6), 89.13 (d, C-4a), 111.55 (d, C-2), 122.48 (d, C-1), 127.27 (d, C-8), 128.04 (d, C-7), 129.69 (s, C-12a), 133.43 (s, C-12b), 144.51 (s, C-3a), 146.21 (s, C-3).–

[4aS,6R,8aS)-1-[^{2}H]-3-methoxy-4a,5,9,10,11,12-hexahydro-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepine-6-ol (8-^{2}H-(–)-galanthamine) ***2b***

$LiAlD_4$ (0.032 g, 0.76 mmol) was dispersed in dry THF (15 ml). The solution was cooled to 0°C and a solution of 8-bromo-galanthamine **3** (0.07 g, 0.19 mmol) in dry THF (5 ml) was added slowly. After 52 h complete conversion of **3** was achieved (determined by GC). The mixture was quenched with D_2O. The solvent was evaporated off and the residual solid purified via column chromatography (silica gel 15 g, $CHCl_3$ 96%, MeOH 4%) to yield 8-[^{2}H]-(–)-galanthamine **2b** (0.407 g, 0.141 mmol, 74% of theory) as a white foam.

^{1}H-NMR ($CDCl_3$, 200 MHz): δ [ppm] = 1.60 (dd, 1H, 9-H), 1.96–2.17 (m, 2H, 5-H, 9-H′), 2.34 (s, 3H, NCH_3), 2.69 (dd, 1H, 5-H′), 3.06 (d, 1H, 10-H), 3.28 (t, 1H, 10-H′), 3.70 (d, 1H, 12-H), 3.84 (s, 3H, OCH_3), 4.06–4.14 (m, 2H, 6-H, 12-H′), 4.62 (s, 1H, 4a-H), 5.96–6.10 (m, 2H, 7-H, 8-H), 6.61–6.73 (d, 1.48H, 1-H, 2-H).–

^{13}C-NMR ($CDCL_3$, 50 MHz): δ [ppm] = 30.37 (t, C-5), 34.16 (t, C-9), 42.46 (q, NCH_3), 48.59 (s, C-8a), 54.22 (t, C-10), 56.30 (q, OCH_3), 60.92 (t, C-12), 62.45 (d, C-6), 89.10 (d, C-4a), 111.57 (d, C-2), 122.51 (d, C-1), 127.23 (d, C-8), 128.03 (d, C-7), 129.44 (s, C-12a), 133.42 (s, C-12b), 144.51 (s, C-3a), 146.20 (s, C-3).–

[4aS,6R,8aS)-3-methoxy-4a,5,9,10,11,12-hexahydro-11-methyl-6-[^{2}H]-benzofuro[3a,3,2-ef][2]benzazepine-6-ol (3-^{2}H-(–)-galanthamine) ***2d***

Quantities, reaction conditions and workup were identical to the procedure described for the preparation of ^{1}H-(–)-galanthamine **2a** via method B. Hydrogen gas was replaced by deuterium gas. The reaction yielded 3-^{2}H-(–)-galanthamine **2d** (0.108 g, 0.375 mmol, 85% of theory) as a white foam.

M.p.: 128°C (Lit.[8] ^{1}H-(–)-galanthamine: 128–129°C)
$[\alpha]^{25} = -96.3$ (c = 1.5; $CHCl_3$) (Lit.[14] ^{1}H-(–)-galanthamine): $[\alpha]^{25} = -93.4$ (c = 1.0; $CHCl_3$)
TLC: R_f = 0.46 (THF 93%:EtOH 4%:Et_3N 3%)

^{1}H-NMR ($CDCl_3$, 200 MHz): δ [ppm] = 1.55 (dd, 1H, 9-H), 1.94–2.07 (m, 2H, 5-H, 9-H′), 2.39 (s, 3H, NCH_3), 2.68 (dd, 1H, 5-H′), 3.03 (d, 1H, 10-H), 3.25 (t, 1H, 10-H′), 3.66 (d, 1H, 12-H), 3.81 (s, 3H, OCH_3), 4.08 (d, 1H, 12-H′), 4.59 (s, 1H, 4a-H), 5.89–6.12 (m, 2H, 7-H, 8-H), 6.58–6.68 (d, 2H, 1-H, 2-H).–

^{13}C-NMR ($CDCl_3$, 50 MHz): δ [ppm] = 30.28 (t, C-5), 34.20 (t, C-9), 42.48 (q, NCH_3), 48.59 (s, C-8a), 54.43 (t, C-10), 56.26 (q, OCH_3), 60.99 (t, C-12), 61.99 (D-triplett, C-6), 89.06 (d, C-4a), 111.50 (d, C-2), 122.43 (d, C-1), 127.31 (d, C-8), 127.94 (d, C-7), 129.71 (s, C-12a), 133.41 (s, C-12b), 144.52 (s, C-3a), 146.20 (s, C-3).–

Analytical calculation for $C_{17}H_{20}DNO_3$ [288.36]

	% C	% H + D	% N
Calculated	70.81	7.06	4.86
Found	70.30	7.46	4.86

[4aS,6R,8aS)-3-methoxy-4a,5,9,10,11,12-hexahydro-11-methyl-6-[^{3}H]-benzofuro[3a,3,2-ef][2]benzazepine-6-ol (3-^{3}H-(–)-galanthamine) ***2e***

The reaction flask was evaporated and tritium gas introduced. A solution of *n*-buthyllithium (1.6 M (hexane), 0.63 ml, 1.0 mmol) and N,N,N′,N′-tetramethyl-ethylendiamin (0.16 ml, 1.061 mmol) was added and stirred until the pressure remained constant for 30 min. The reaction mixture was degassed and the solvent removed by lyophilization. Nitrogen gas was admitted to 784 mbar and tri-*sec*-butylborane solution (1.0 M (THF), 0.89 ml, 0.884 mmol) was added to the reaction mixture thereby re-dissolving the precipitate. The solution was cooled to −30°C and a suspension of (–)-narwedine **3** (0.126 g, 0.442 mmol) in THF (1.0 ml) was added slowly. The reaction was stirred for 0.5 h at −20°C and 1 h at room temperature. Excess of reducing reagent was quenched with methanol at 0°C. The reaction mixture was degassed and the solvent removed by lypohilization. Labile tritium was removed by dissolving the solid in methanol and removing the solvent by lyophilization. The remaining solid was dissolved in EtOH (4 ml). HBr solution (48%, w/v) was added until pH 1 was reached and the solution was stored overnight at 0°C. The precipitate was filtered, washed with EtOH (1 ml) and dried under vacuum. The derived solid was dissolved in water (4 ml), brought to pH 10 with ammonia solution (25%, v/v) and extracted 4 times with EtOAc (2.5 ml). The combined organic layers were washed with brine (2 ml) and dried over $MgSO_4$. The solvent was evaporated off and the remaining solid dried under vacuum to yield 3-^{3}H-(–)-galanthamine **2e** (77.8 mg, 0.269 mmol, 60.8% of theory) as a white foam.

Specific activity (MS): 23.8 Ci/mmol (0.881 TBq/mmol)
Total radioactivity (LSC): 6.4 Ci (0.237 TBq).
TLC: $R_f = 0.46$ (THF 93%: EtOH 4%: Et_3N 3%)

Acknowledgements

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