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Synthesis of deuterium-labelled (−)-galanthamine

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The synthesis of deuterium-labelled galanthamine is reported. 6-[$^2H_3$]methoxy-*N*-[$^2H_3$]methyl-(−)-galanthamine was obtained in seven steps from galanthamine. The synthesis was carried out by selective *O*- and *N*-demethylations. The [$^2H_3$]-*N*-methyl and [$^2H_3$]-*O*-methyl-groups were introduced by selective aminoreduction and *O*-methylation.

Keywords: deuterium labelling; galanthamine; sanguinine; norsanguinine

Introduction

(−)-Galanthamine **1**,[1] a tertiary alkaloid isolated from Amaryllidaceae, is a centrally acting, competitive and reversible inhibitor of acetylcholinesterase that enhances cognitive functions in Alzheimer's patients.[2] Furthermore, galanthamine is also an allosteric potentiating ligand of nicotinic receptor.[3] This drug is the most recently approved acetylcholinesterase inhibitor for use in the United States and Europe. In continuation of our work in the galanthamine series, we report the synthesis of deuterium-labelled (−)-galanthamine, which will be useful for biological studies. The introduction of six atoms of deuterium on galanthamine would permit a better detection of this compound by a method based on deuterium's analysis.

Results and discussion

During the preparation of hexadeuterated (−)-galanthamine, the formation of a quaternary ammonium must be avoided. Thus, (−)-galanthamine **1** was first selectively demethylated by reaction with L-selectride to give 6-demethylgalanthamine **2** (sanguinine) in high yield (98%).[4] The second step of the synthesis is the previously unknown *N*-demethylation of sanguinine **2**. Sanguinine **2** was first quantitatively converted into its *N*-oxide **3** by oxidation with *m*-chloroperbenzoic acid (*m*CPBA) in dichloromethane at room temperature. Subsequent treatment of **3** with a (2/1) mixture of ferrous sulphate heptahydrate and ferric chloride in methanol at 10°C provided norsanguinine **4**.[5] Protection of the amine function of **4** with di-*tert*-butyl dicarbonate furnished compound **5** (81%). Alkylation of **5** with $(CD_3O)_2SO$ in dimethylformamide (DMF) in the presence of cesium carbonate afforded *N*-BocOCD_3 norgalanthamine **6**. After removal of the *N*-Boc group of **6**, the resulting amine **7** was treated with deuterated formaldehyde, acetic acid-*d* and $NaBD_4$ to afford hexadeuterated (−)-galanthamine **8** quantitatively.

In summary, hexadeuterated (−)-galanthamine **8** was synthesized in seven steps with an overall yield of 25% from natural (−)-galanthamine **1**. This is the first synthesis of hexadeuterated (−)-galanthamine **8**, which could be a useful compound for biological studies.

Experimental

General

Proton nuclear magnetic resonance (^1H NMR) spectra were acquired at 300 MHz and ^{13}C NMR were acquired at 75 MHz on a Bruker Avance-300 spectrometer. Chemical shifts (δ) are reported in ppm relative to $CHCl_3$ (7.27 and 77.0 ppm). Mass spectra (MS) and high-resolution mass spectra (HRMS) were determined on an electrospray ionization-time-of-flight (ESI-TOF) Thermoquest AQA Navigator mass spectrometer. Infrared (IR) spectra were recorded using a Fourier Perkin Elmer Spectrum BX Fourier transform-infrared (FT-IR) spectrometer. All reactions were performed in anhydrous solvents under an inert atmosphere (argon). Dichloromethane was distilled from P_2O_5, tetrahydrofuran (THF) from sodium/benzophenone and DMF from $MgSO_4$. MeOH, EtOH and all reagents were of the highest commercially available purity. All separations were carried out under flash chromatographic conditions on Merck silica gel 60 (70-230 mesh) at medium pressure (200 mbar). Thin-layer chromatography (TLC) was performed on Merck silica gel plates ($60F_{254}$) with a fluorescent indicator.

*Synthesis of (4aS,6R,8aS)-11-methyl-5,6,9,10,11,12-hexahydro-4aH-[1]benzofuro[3a,3,2-ef][2]benzazepine-3,6-diol or sanguinine (**2**)*

A suspension of galanthamine hydrobromide **1** (654 mg) in CH_2Cl_2 was washed with saturated aqueous $NaHCO_3$ (30 ml). The aqueous layer was extracted three times with CH_2Cl_2 (30 ml). The organic layers were combined, dried over $MgSO_4$ and evaporated. The residue was dissolved in THF (20 ml) and a

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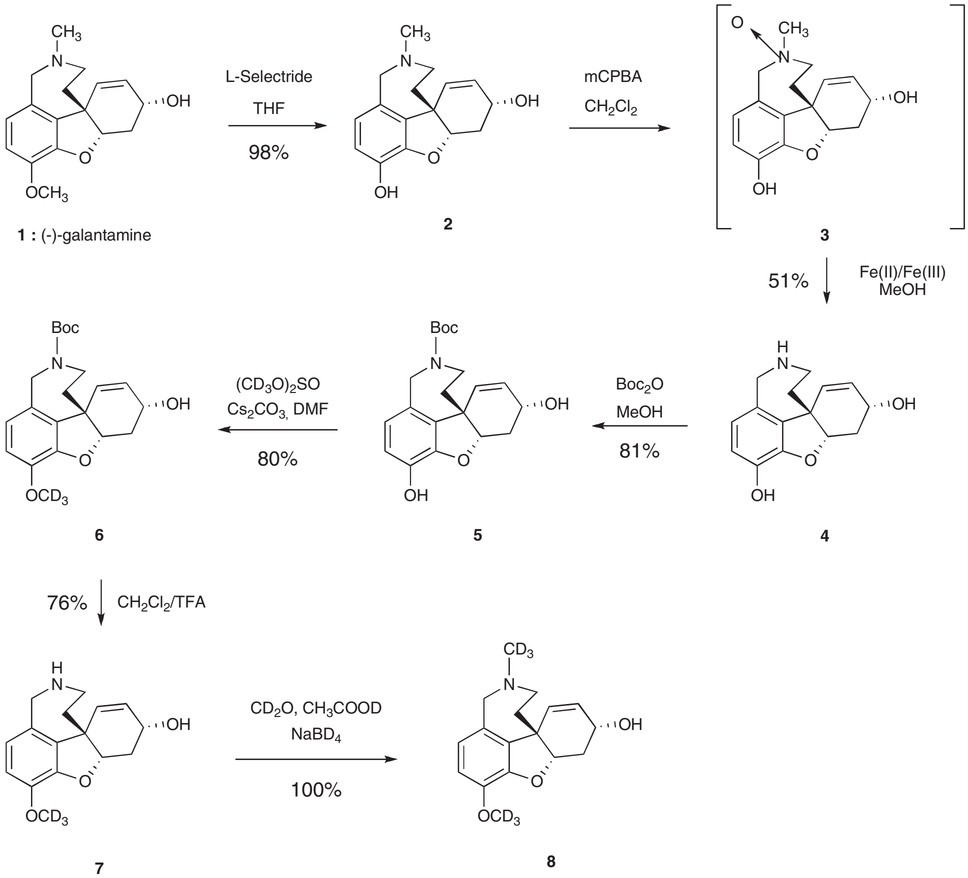

solution 1 M of L-selectride (8.8 ml, 8.8 mmol) in THF was slowly added at room temperature. The mixture was stirred at 65°C for 24 h. After cooling, the excess of L-selectride was neutralized with methanol and then with water. After a filtration on celite, the solvents were evaporated under vacuum. Silica gel flash-column chromatography (CH_2Cl_2/MeOH/28% aqueous ammonia: 94/5/1) of the residue afforded the desired compound **2** as a colourless oil (478 mg; 98%). ^1H NMR (300 MHz, $CDCl_3$) δ: 6.56 (1H, d, $J_{8-7}=8.0$ Hz, H8), 6.50 (1H, d, $J_{7-8}=8.0$ Hz, H7), 6.09 (1H, d, $J_{1-2}=10.3$ Hz, H1), 5.88 (1H, dd, $J_{2-1}=10.3$, $J_{2-3}=4.8$ Hz, H2), 4.49 (1H, bs, H4a), 4.13 (1H, bt, $J=4.8$ Hz, H3), 4.04 (1H, d, $J_{gem}=15.0$ Hz, H9α), 3.62 (1H, d, $J_{gem}=15.0$ Hz, H9β), 3.20 (1H, m, H11α), 2.99 (1H, dm, $J_{gem}=14.5$ Hz, H11β), 2.48 (1H, dm, $J=15.6$ Hz, H4α), 2.35 (3H, s, NMe), 2.02 (2H, m, H4β, H12α), 1.62 (1H, dm, $J=13.9$ Hz, H12β). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 146.7 (C6), 142.3 (C5a), 134.2 (C8b), 128.6 (C2), 128.1 (C1), 128.0 (C8a), 123.1 (C8), 116.7 (C7), 88.8 (C4a), 62.5 (C3), 61.5 (C9), 55.1 (C11), 49.3 (C4b), 42.9 (NMe), 35.3 (C12), 31.3 (C4). MS (ESI, *m/z*) 274.1 (M+H). HRMS (ESI) calcd. for $C_{16}H_{20}NO_3^+$: 274.1443, found: 274.1430. IR ν (cm^{-1}): 3030, 2920, 2888, 1621, 1235.

*Synthesis of (4aS,6R,8aS)-5,6,9,10,11,12-hexahydro-4a*H*- [1]benzofuro[3a,3,2-*ef*] [2]benzazepine-3,6-diol or* N*-desmethylsanguinine (**4**)*

*m*CPBA (71 mg, 0.29 mmol) was added to a solution of sanguinine **2** (79 mg, 0.29 mmol) in CH_2Cl_2 (8 ml) at 0°C. The mixture was stirred at 0°C for 1 h. Methanol (2 ml), $FeSO_4$ (161 mg, 0.58 mmol) and $FeCl_3$ (78 mg, 0.29 mmol) were added and the mixture was allowed to warm up to 10°C. After 3 h, saturated aqueous $NaHCO_3$ (1 ml) was added and the mixture was filtered through a pad of celite. After removal of the solvents, the residue was purified by silica gel flash-column chromatography (elution with CH_2Cl_2/MeOH/28% aqueous ammonia: 89/10/1) to give the desired compound **4** as an amorphous solid (38 mg; 51% yield). ^1H NMR (300 MHz, CD_3OD) δ: 6.54 (2H, m, H7+H8), 6.08 (1H, d, $J_{1-2}=10.1$ Hz, H1), 5.88 (1H, d, $J_{2-1}=10.1$, $J_{2-3}=4.5$ Hz, H2), 4.48 (1H, bs, H4a), 4.14 (1H, bt, $J=4.5$ Hz, H3), 4.08 (1H, d, $J_{gem}=15.4$ Hz, H9α), 3.92 (1H, d, $J_{gem}=15.4$ Hz, H9β), 3.28 (2H, m, H11α+H11β), 2.47 (1H, dm, $J=14.8$ Hz, H4α), 2.07 (1H, dm, $J_{gem}=14.8$ Hz, H4β), 1.83 (2H, m, H12α, H12β). ^{13}C NMR (75 MHz, CD_3OD) δ: 147.2 (C6), 142.8 (C5a), 134.2 (C8b), 129.5 (C8a), 128.6 (C1), 128.2 (C2), 122.2 (C8), 116.8 (C7), 88.5 (C4a), 62.4 (C3), 53.7 (C9), 49.8 (C4b), 47.4 (C11), 39.3 (C12), 31.2 (C4). MS (ESI, *m/z*) 260.1 (M+H). HRMS (ESI) calcd. for $C_{15}H_{18}NO_3^+$: 260.1287, found: 260.1284. IR ν (cm^{-1}): 3220, 3025, 2920, 2847, 1616, 1234.

Synthesis of tert*-butyl(4aS,6R,8aS)-3,6-dihydroxy-5,6,9,10-tetrahydro-4a*H*-[1]benzofuro[3a,3,2-*ef*][2]benzazepine-11 (12*H*)-carboxylate or* N*-Boc-sanguinine (**5**)*

Di-*tert*-butyl dicarbonate (289 μl, 1.3 mmol) was added to a solution of *N*-desmethylsanguinine **4** (292 mg, 1.1 mmol) in ethanol (1 ml). The mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (20 ml) and washed with saturated aqueous $NaHCO_3$ (20 ml) and then brined (20 ml). The organic layer was separated and each aqueous layer was extracted three times with CH_2Cl_2 (20 ml). The organic layers were combined, dried over $MgSO_4$ and evaporated. Silica gel flash-column chromatography (elution with CH_2Cl_2/MeOH/28% aqueous ammonia: 97/2/1) of the residue afforded the expected compound **5** as an amorphous solid (327 mg; 81%). ^{1}H NMR (333 K, 300 MHz, CD_3CN) δ: 6.58 (2H, m, H7, H8), 6.01 (1H, d, $J_{1-2}=10.3$ Hz, H1), 5.90 (1H, dd, $J_{2-1}=10.3$, $J_{2-3}=4.5$ Hz, H2), 4.66 (1H, m, H9α), 4.51 (1H, bs, H4a), 4.16 (1H, m, H9β), 4.10 (1H, m, H11α), 4.08 (1H, m, H3), 3.37 (1H, m, H11β), 2.46 (1H, dm, $J_{gem}=15.7$ Hz, H4α), 2.05 (1H, ddd, $J_{gem}=15.7$, $J=5.3$, $J=2.6$ Hz, H4β), 1.84 (1H, m, H12α), 1.71 (1H, m, H12β), 1.35 (9H, s, H3′, H4′, H5′). ^{13}C NMR (333 K, 75 MHz, CD_3CN) δ: 156.0 (C1′), 147.1 (C6), 141.8 (C5a), 133.9 (C8b), 130.9 (C8a), 128.9 (C2), 128.3 (C1), 122.2 (C8), 116.6 (C7), 89.1 (C4a), 80.2 (C2′), 62.7 (C3), 52.4 (C9), 49.9 (C4b), 46.5 (C11), 38.0 (C12), 31.4 (C4), 29.0 (C3′, C4′, C5′). MS (ESI, *m/z*) 382.1 (M+Na). HRMS (ESI) calcd. for $C_{20}H_{25}NO_5Na^+$: 382.1630, found: 382.1635. IR ν (cm^{-1}): 2976, 2925, 1688, 1682, 1237.

*Synthesis of tert-butyl(4aS,6R,8aS)-6-hydroxy-3-[$^2H_3$]methoxy-5,6,9,10-tetrahydro-4aH-[1]benzofuro[3a,3,2-ef][2]benzazepine-11(12H)-carboxylate or 6-[$^2H_3$]methoxy-N-Boc-norsanguinine (**6**)*

Dimethylsulphate-d_6 (41 μl, 0.45 mmol) and Cs_2CO_3 (405 mg, 1.2 mmol) were added to a solution of *N*-Boc-sanguinine **5** (149 mg, 0.41 mmol) in DMF (3 ml). The mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (20 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (20 ml), brined (20 ml), dried ($MgSO_4$) and evaporated. Silica gel flash-column chromatography (elution with CH_2Cl_2/MeOH/28% aqueous ammonia: 94/5/1) of the residue afforded the expected compound **6** as an amorphous solid (125 mg; 80%). ^{1}H NMR (333 K, 300 MHz, CD_3CN) δ: 6.70 (2H, m, H7, H8), 6.03 (1H, d, $J_{1-2}=10.4$ Hz, H1), 5.91 (1H, dd, $J_{2-1}=10.4$, $J_{2-3}=4.5$ Hz, H2), 4.69 (1H, d, $J_{gem}=15.5$ Hz, H9α), 4.52 (1H, bs, H4a), 4.20 (1H, d, $J_{gem}=15.5$ Hz, H9β), 4.12 (1H, m, H11α), 4.09 (1H, m, H3), 3.39 (1H, m, H11β), 2.46 (1H, m, H4α), 2.07 (1H, m, H4β), 1.85 (1H, m, H12α), 1.73 (1H, m, H12β), 1.35 (9H, s, H3′, H4′, H5′). ^{13}C NMR (333 K, 75 MHz, CD_3CN) δ: 156.6 (C1′), 148.9 (C6), 146.2 (C5a), 134.7 (C8b), 132.4 (C8a), 129.9 (C2), 128.8 (C1), 122.7 (C8), 114.1 (C7), 89.7 (C4a), 81.1 (C2′), 63.2 (C3), 53.1 (C9), 50.3 (C4b), 47.2 (C11), 38.9 (C12), 32.2 (C4), 29.6 (C3′, C4′, C5′). MS (ESI, *m/z*) 399.2 (M+Na). HRMS (ESI) calcd. for $C_{21}^{1}H_{24}^{2}H_3NO_5Na^+$: 399.1975, found: 399.1962. IR ν (cm^{-1}): 2973, 2925, 1686, 1682, 1237.

*Synthesis of (4aS,6R,8aS)-3-[$^2H_3$]methoxy-5,6,9,10,11,12-hexahydro-4aH-[1]benzofuro[3a,3,2-ef][2]benzazepin-6-ol or 6-[$^2H_3$]methoxynorsanguinine (**7**)*

Trifluoroacetic acid (TFA) (3 ml) was added to a solution of 6-[$^2H_3$]methoxy-*N*-Boc-norsanguinine **6** (84 mg, 0.22 mmol) in CH_2Cl_2 (3 ml). The mixture was stirred at room temperature for 2 h. After evaporation of the solvent, the residue was extracted with CH_2Cl_2. The combined organic layers were washed with saturated aqueous $NaHCO_3$, brined and dried ($MgSO_4$). Removal of solvent under reduced pressure afforded the desired compound **7** as a yellow oil (47 mg; 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.63 (2H, m, H7+H8), 6.02 (2H, m, H1+H2), 4.60 (1H, bs, H4a), 4.14 (1H, bt, $J=3.5$ Hz, H3), 4.04 (1H, d, $J_{gem}=15.6$ Hz, H9α), 3.96 (1H, d, $J_{gem}=15.6$ Hz, H9β), 3.38 (1H, dt, $J_{gem}=14.7$, $J=3.6$ Hz, H11α), 3.23 (1H, m, H11β), 2.68 (1H, dm, $J=15.8$ Hz, H4α), 2.01 (1H, ddd, $J_{gem}=15.8$, $J=5.1$, $J=2.4$ Hz, H4β), 1.84 (1H, ddd, $J_{gem}=13.8$, $J=3.8$, $J=2.4$ Hz, H12α), 1.75 (1H, ddd, $J_{gem}=13.8$, $J=11.7$, $J=3.6$ Hz, H12β). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 146.2 (C6), 144.1 (C5a), 133.1 (C8b), 132.1 (C2), 127.7 (C1), 126.8 (C8a), 120.8 (C8), 111.1 (C7), 88.5 (C4a), 61.9 (C3), 53.5 (C9), 48.6 (C4b), 46.8 (C11), 39.8 (C12), 29.9 (C4). MS (ESI, *m/z*) 277.1 (M+H). HRMS (ESI) calcd. for $C_{16}H_{17}^{2}H_3NO_3^+$: 277.1631, found: 277.1629. IR ν (cm^{-1}): 2920, 1682, 1274.

*(4aS,6R,8aS)-3-[$^2H_3$]methoxy-11-[$^2H_3$]methyl-5,6,9,10,11,12-hexahydro-4aH-[1] benzofuro[3a,3,2-ef][2]benzazepin-6-ol or (−)-6-[$^2H_3$]methoxy-N-[$^2H_3$]methyl-galanthamine (**8**)*

Formaldehyde-d_2 (30 μl, 0.45 mmol, 30% in water) and acetic acid-*d* (26 μl, 0.45 mmol) were added to a solution of 6-[$^2H_3$]methoxynorsanguinine **7** (25 mg; 0.09 mmol) in MeOH (0.5 ml). The mixture was heated at 65°C during 2 h and then cooled to 0°C. $NaBD_4$ (11 mg, 0.27 mmol) was added and the mixture was stirred for an additional 1 h at 0°C. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2. The combined organic layers were washed with water, brined, dried ($MgSO_4$) and evaporated to yield the desired compound **8** as a colourless oil (26.5 mg; 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.70 (1H, d, $J_{8-7}=8.2$ Hz, H8), 6.68 (1H, d, $J_{7-8}=8.2$ Hz, H7), 6.06 (1H, dd, $J_{2-1}=10.2$, $J_{2-3}=4.9$ Hz, H2), 6.01 (1H, d, $J_{1-2}=10.2$ Hz, H1), 4.64 (1H, bs, H4a), 4.27 (1H, d, $J_{gem}=15.3$ Hz, H9α), 4.16 (1H, bt, $J=4.6$ Hz, H3), 3.86 (1H, d, $J_{gem}=15.3$ Hz, H9β), 3.45 (1H, dd, $J_{gem}=13.4$, $J=13.1$ Hz, H11α), 3.20 (1H, dm, $J_{gem}=13.4$ Hz, H11β), 2.71 (1H, dm, $J=15.9$ Hz, H4α), 2.12 (1H, ddd, $J_{gem}=13.4$, $J=13.1$, $J=2.7$ Hz, H12α), 2.02 (1H, ddd, $J_{gem}=15.9$, $J=5.2$, $J=2.4$ Hz, H4β), 1.73 (1H, dm, $J_{gem}=13.4$ Hz, H12β). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 146.0 (C6), 144.9 (C5a), 132.8 (C8b), 128.5 (C2), 125.8 (C1, C8a), 122.9 (C8), 111.6 (C7), 88.6 (C4a), 61.8 (C3), 59.4 (C9), 53.1 (C11), 47.8 (C4b), 32.5 (C12), 29.8 (C4). MS (ESI, *m/z*) 316.2 (M+Na), 294.2 (M+H). HRMS (ESI) calcd. for $C_{17}^{1}H_{15}^{2}H_6NO_3Na^+$: 316.1796, found: 316.1793. IR ν (cm^{-1}) 3026, 2913, 2856, 1621, 1288, 1265.

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