JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 841–855.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.589

Research Article

Synthesis of ^{3}H-, ^{14}C-, and stable-isotope-labelled galantamine

Cor. G. M. Janssen*, Jos B. A. Thijssen and Willy L. M. Verluyten
Department of Non-clinical Pharmacokinetics, Johnson & Johnson Pharmaceutical Research & Development, B-2340 Beerse, Belgium

Summary

Reminyl® is a newly approved drug, used in the treatment of mild to moderate Alzheimer disease. The active compound, galantamine, was initially isolated from the bulbs of certain *Narcissus* species, but is at the moment also produced synthetically. In the process leading to the final approval, the synthesis of tritium-, carbon-14- and stable-isotope-labelled galantamine for pharmacokinetic studies was required.

Racemic ($\pm$)-1-bromonarwedine, a compound available as intermediate from the commercial synthesis, was transformed to racemic 1-bromo-galantamine. Catalytic bromo-tritium exchange, followed by HPLC purification and resolution afforded tritium-labelled galantamine. The [^{14}C]-label was introduced on the nitrogen as well as on the oxygen-methyl position. This was achieved by *N*- and *O*-demethylation of galantamine and reaction of the thoroughly purified intermediate with [^{14}C]-methyl iodide. Stable-isotope-labelled galantamine was obtained likewise by $^{13}CD_3OD$-methylation of *O*-demethylated galantamine under Mitsunobu conditions. Copyright © 2002 John Wiley & Sons, Ltd.

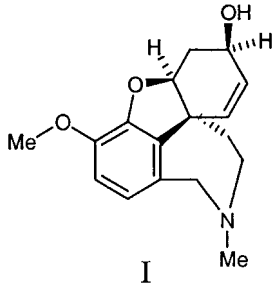
Key Words: carbon-14; tritium; stable-isotope; synthesis; galantamine

*Correspondence to: Cor. G. M. Janssen Department of Non-clinical Pharmacokinetics, Johnson & Johnson Pharmaceutical Research, B-2340 Beerse, Belgium. E-mail: cjansse1@prdbe.jnj.com

Received 19 February 2002
Accepted 7 March 2002

Introduction

Galantamine (I) is a tertiary alkaloid that has been isolated from the bulbs of the Caucasian snowdrop (*Galanthus woronowi*),[1] from the common snowdrop (*Galanthus nivalis*),[2] and also from a number of other sources e.g. from various species of *Narcissus*.[3,4]



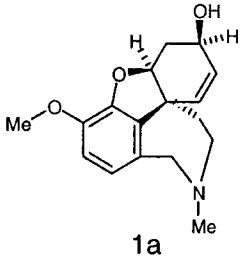
I

Galantamine shows reversible, competitive acetylcholinesterase inhibiting and nicotinic acetylcholinergic receptor modulatory properties[5–7] and readily crosses the blood–brain barrier. It is generally accepted that cognitive and functional improvement can be achieved with compounds that increase cholinergic neurotransmission in the central nervous system. Based on documented improvement of cognitive function in several animal species, galantamine has been developed for the symptomatic treatment of mild to moderate Alzheimer's disease, and was subsequently shown to have a beneficial therapeutic effect in a number of clinical trials.[8–11] In early 2001, the US Food and Drug Administration approved the compound's hydrobromic acid salt as treatment for mild to moderate Alzheimer's disease under the name Reminyl®. Pharmacokinetic research in support of the approval extensively used ^{3}H-, ^{14}C-, and stable-isotope-labelled galantamine, the synthesis of which is described here.

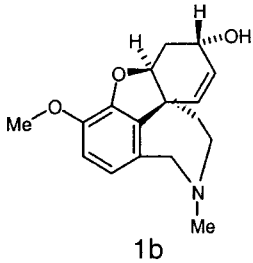
Results and discussion

The total synthesis of galantamine (1a), epigalantamine (1b) and narwedine (1c) is described in numerous papers.[12–21] Most approaches are based on a biomimetic scheme,[12] applying a phenolic oxidative cyclization process leading to the formation of the tetracyclic framework. All of the mentioned multistep sequences resulted in a very low

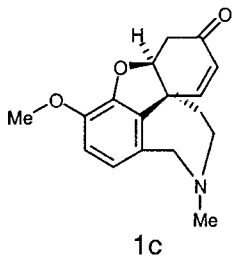
overall yield of at best 5%.[13] Only in 1988, a procedure was described, in which a preparatively interesting 11% overall yield of racemic galantamine was attained.[19] Later on, the same researchers reported a stereoselective reduction with L-selectride which eliminated the formation of epigalantamine, thus improving the overall yield of racemic galantamine to 24%.[21] Synthetic procedures for the synthesis of optically pure (−)-galantamine on kilogram scale were disclosed only recently.[22,23]



1a



1b



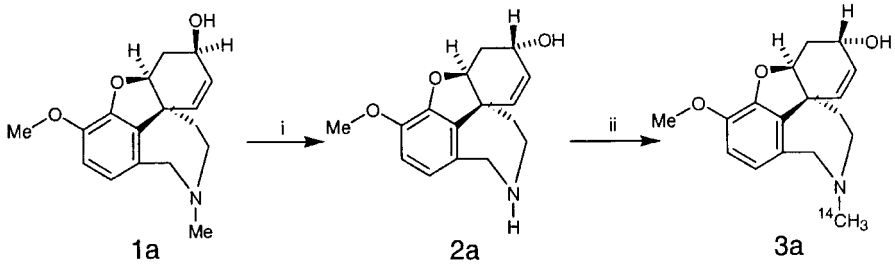
1c

A total synthesis of galantamine would offer the possibility of incorporating a carbon-14 label on a favourable skeleton-position. However, considering the tedious and time-consuming synthesis, such approach was refrained from. Moreover, since the proposed reaction sequence does not allow the introduction of a ^{14}C-unit in the elaborated pathway via an easily attainable labelled fragment, the label should be incorporated into one of its precursors. This would increase the number of reaction steps and inevitably lead to substantially diminished yields.

In order to meet the pharmacokinetic demands, instead of introducing a ^{14}C-label in the galantamine-skeleton, the introduction of a ^{14}C-label in the exterior methyl groups and also the introduction of a tritium label on the phenyl ring system was opted for. This approach would offer the opportunity to start from optically pure material which gives a direct, one-step access to optically pure galantamine. If a (partial) racemization of one of the chiral centres would occur (the most likely position would be the allylic alcohol position, leading to epigalantamine), the formed diastereomeric impurity could easily be detected and removed via HPLC.

N-[^{14}C]-methyl-labelled galantamine

The first step in the synthesis of *N*-[^{14}C]-methyl galantamine was the cleavage of the aminomethyl group (see Scheme 1). To effect these

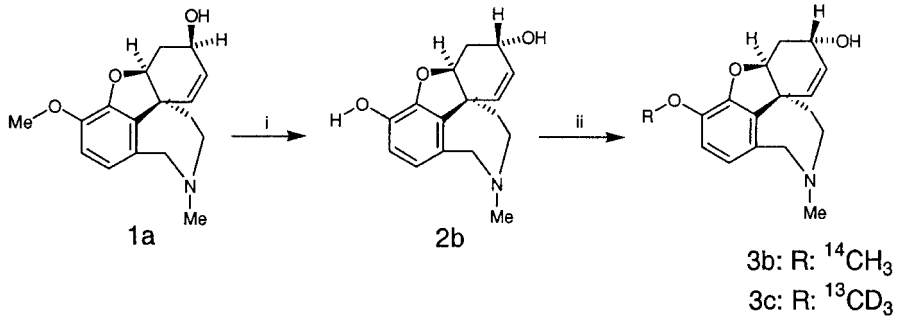


Scheme 1. Synthesis of *N*-[^{14}C]-methyl-galantamine. Reagents and conditions: (i) m-chloroperbenzoic acid, ferrous sulphate, dichloromethane and (ii) diisopropylamine, [^{14}C]methyl iodide, methanol

N-demethylations, the use of chloroformate reagents is reported to give high yields under mild reaction conditions.[24] Vinyl chloroformate was reported to demethylate 3,14-diacetyloxymorphone in essentially quantitative yield.[25] α-Chloroethyl chloroformate was used by the same authors in the demethylation of oxycodone with near quantitative yield.[26] When applied to galantamine, only a poor 3% of nor-galantamine (**2a**) could be isolated. A selective *N*-demethylation of galantamine via a non-classical Polonovski reaction[27] was reported to afford nor-galantamine in a 76% preparative yield. This method of choice gave in our hands a 51% isolated yield as the $HCl \cdot H_2O$ salt; the isolation of the desired nor-galantamine proved to be very laborious. In order to obtain *N*-[^{14}C]-methyl galantamine it seemed a matter of course to use the commercially available [^{14}C]-methyl iodide. Cold test reactions under various conditions revealed a preference for the quarternization of the secondary amine. This problem could only be addressed by using a four-fold excess of nor-galantamine, to provide after purification a 36.8% yield of labelled compound (3a), based on [^{14}C]-methyl iodide.

O-[^{14}C]-methyl-and O-stable-isotope-methyl-labelled galantamine

O-[^{14}C]-methyl galantamine was obtained in an analogous approach (see Scheme 2). Methods for *O*-demethylation are numerous,[28] but most suffer from lack of selectivity. In order to preserve stereochemistry and taking into account the labile nature of the oxygen bridge and the allylic alcohol function, a mild procedure was required. *O*-demethylation of codeine, a structure highly resembling galantamine, was reported by



Scheme 2. Synthesis of *O*-[^{14}C]- and *O*-[^{13}CD3]-methyl-galantamine. Reagents and conditions: (I) *n*-Butyllithium, butanethiol, hexamethylphosphoramide; (ii) potassium hydroxide, [^{14}C]-methyl iodide, 1,3-dimethyl-2-imidazolidinone for 3b or triphenylphosphine, $^{13}CD_3OD$, diisopropyl azodicarboxylate, tetrahydrofuran for 3c

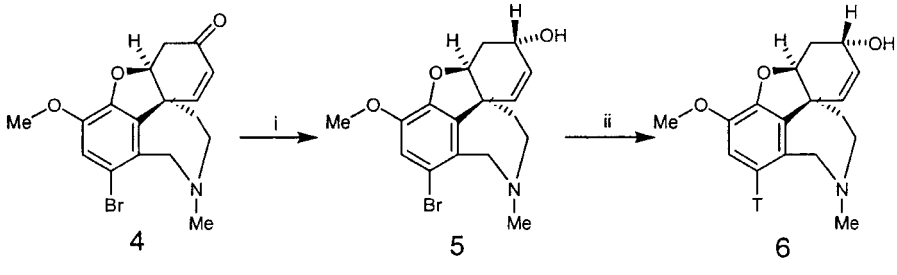
Rice[29] and Han[30] to be a high yield reaction on gram scale using boron tribromide in dichloromethane. When applied to galantamine, change of variables like stoichiometry, solvent and concentration always gave unsavoury reaction mixtures with at best a 13% isolated yield of the desired phenol (**2b**). Reaction of thiolates in *N,N*-dimethylformamide with codeine, as reported by Lawson,[31] gave better results in so far that the final reaction mixture only existed of galantamine and the demethylated product with the highest isolated yield of 21%. The low yields were ascribed to a poor solubility of the compounds in DMF. When changing to HMPT we succeeded in obtaining 69% isolated yield of *O*-demethylated galantamine (98% pure) on a multigram scale. As for the *N*-[^{14}C] methylations, [^{14}C]-methyl iodide was also used for the *O*-methylations. Unlabelled test reactions revealed even more problems than in the case of the *N*-methylations. Change of base (sodium hexamethyldisilazane, potassium carbonate, diisopropyl amine or potassium hydroxide), solvent (*N,N*-dimethylformamide, *N,N*-dimethylacetamide, toluene or 1,3-dimethyl-2-imidazolidinone) or even the use of phase transfer methods gave reaction mixtures which mainly consisted of quarternary amino compound and starting material, with small amounts of the desired product. The combination of potassium hydroxide in 1,3-dimethyl-2-imidazolidinone proved to be optimal and when applying the [^{14}C]-methyl iodide in substoichiometric amounts the *O*-[^{14}C]-methyl galantamine (**3b**) was obtained in a 18% yield, based on [^{14}C]-methyl iodide.

A certain vulnerability of the *O*- and the *N*-methyl label positions in metabolic studies might be expected. This was indeed proven for both labelled compounds (observed *N*-demethylation in humans between 4 and 7%, *O*-demethylation in humans between 5 and 33%), limiting their use to non-metabolic *in vitro* research.[32]

Modern high-throughput analysis is shifted substantially towards the use of LC–MS. In the development of a suitable high-throughout analytical method the synthesis of stable-isotope-labelled galantamine as an internal standard was desired. The compound was required to have a molecular mass raised with 4 units ($M+4$), a purity of $>98\%$ and with less than 0.1% of unlabelled galantamine ($M+0$). Galantamine was *O*-demethylated as described and the product was meticulously purified from traces of remaining galantamine. The observations made in the synthesis of the *O*-[^{14}C]-methyl label refrained us from using labelled methyl iodide. Instead, the material was reacted with $^{13}CD_3OD$ under Mitsunobu reaction conditions[33] thus circumventing the problems of *N*-quarternization. In this way, the stable-isotope-labelled galantamine (**3c**) was obtained in a 36.1% yield, keeping the amount of unlabelled galantamine below detection limit.

Tritium-labelled galantamine

In order to obtain radiolabelled galantamine which could be used in *in vivo* studies, efforts were redirected towards the introduction of a tritium label on a metabolically stable position (see Scheme 3). A literature search revealed the investigation of two alternative routes.[34] Aromatic bromine–tritium exchange with $LiAlT_4$ was shown to be theoretically



Scheme 3. Synthesis of 1-tritio-galantamine. Reagents and conditions: (i) L-Selectride, tetrahydrofuran and (ii) triethylamine, palladium 10% on carbon, butanethiol catalyst poison, tetrahydrofuran, tritium gas

feasible, but this approach was left because of the expected very lengthy reaction times (several days) and low tritium incorporation. The second alternative was the introduction of a tritium label on the allylic 6-position, effected by the reduction of (−)-narwedine with tritiated L-Selectride®. Since investigations of the metabolism showed extensive *in vivo* oxidation to (−)-narwedine, which, in turn, is reduced to both (−)-galantamine and (−)-epigalantamine,[32] this label position is by its nature unstable and synthesis of this compound to be used for *in vivo* studies was not taken into consideration. Instead, a simple catalytic bromine–tritium exchange was tried. For this reason, racemic (±)-1-bromonarwedine (**4**), a compound available as intermediate from the commercial synthesis, was reduced with L-selectride to racemic 1-bromogalantamine (**5**). The bromo-tritium exchange took place in tetrahydrofuran using a palladium catalyst poisoned with butanethiol in order to avoid a possible reduction of the allylic double bond. HPLC purification and resolution produced tritium-labelled galantamine (**6**). Recently, a procedure was described to obtain 1-bromogalantamine directly from galantamine,[35] which would avoid the final resolution step. The tritium label on the phenyl ring proved to be metabolically stable after oral dosing in rats (no formation of tritiated water was observed).[32]

Experimental

Analytical HPLC was performed on an apparatus consisting of a Gilson model 305 (master) pump and a Gilson model 306 (slave) pump, each equipped with a 10 SC pumphead. The samples were injected by a Rheodyne 7125 injector. Chemical and radiochemical purity were determined by on-line UV detection on a Gilson 118 detector and radioactivity detection on a Berthold Radioactivity monitor LB 506 C system with a flow-through cell of 0.500 ml. The eluate was mixed with Pico-Fluor TM[30] (Packard, used as a scintillation cocktail) in an FMI ultrograd mixing unit. The normalized areas of the radioactivity peaks were computed and visualized by a Compaq Deskpro computer unit. Preparative HPLC and resolution were performed with only on-line UV detection. Conditions of analytical and preparative HPLC are described in the text. Radioactivity of known aliquots was measured by liquid scintillation counting (Packard Tri-Carb 4530), using Ultima Gold (Packard) as scintillation cocktail. The specific activity was determined

by measuring the UV absorbance (on HPLC) relative to the absorbance of known amounts of injected unlabelled standards and the radioactivity contents in the HPLC-eluate by means of liquid scintillation counting. LC/MS/MS data were obtained on a Perkin-Elmer Sciex API 3000 triple quadrupole LC/MS/MS mass spectrometer.

(−)-[4aS-(4aα,6β,8aR)]-4a,5,9,10,11,12-hexahydro-3-methoxy-6H-benzofuro [3a,3,2-e,f] [2]-benzazepin-6-ol hydrochloride (N-desmethyl galantamine,* **2a***)*

The free base of galantamine (1a) was liberated from its HBr salt (4.9 g, 13.4 mmol) in a mixture of water–chloroform (55 ml, 10:1; v/v) which was made alkaline with conc. aqueous ammonium hydroxide solution (2 ml). The phases were separated and the water layer was extracted with chloroform (12 ml). The combined organic layers were washed with brine (6 ml), dried on magnesium sulphate, filtered and concentrated. m-Chloroperbenzoic acid (70% aqueous, 3.4 g, 13.7 mmol) was dissolved in dichloromethane (100 ml), the water layer was removed and this solution was added to the stirred galantamine base in dichloromethane (125 ml). Stirring was continued for 75 min, methanol (125 ml) was added and the clear reaction mixture was cooled on ice. At 2°C, ferrous sulphate · 2 aq (7.5 g, 27 mmol) was added in one portion, causing an immediate brown colouration and the temperature rise to 10°C. The mixture was stirred for 90 min on ice and then for 1 h at room temperature. It was filtered over dicalite, concentrated and dissolved in a mixture of dichloromethane–chloroform (160 ml, 4:1; v/v). Concentrated aqueous ammonium hydroxide solution (5 ml) was added, which caused the formation of a brown gelatinous precipitate. Water (200 ml) and an extra amount of dichloromethane (60 ml) were added. After 5 min, the mixture was transferred to a separation funnel and the layers were allowed to separate for approx. 1 h. The lower, organic, layer was taken and filtered over dicalite. The middle layer with incomplete separation was taken and centrifuged for 15 min at 2500 rpm. The completely separated organic layer was pipetted off and combined with the original lower layer. It was dried on magnesium sulphate, filtered and concentrated to leave a product containing residue (4.3 g). This was purified via gravity chromatography over silica (column dimensions: ID: 52 mm, height: 240 mm) using a dichloromethane–methanol eluate with increasing polarity (1200 ml 95:5, v/v; 800 ml 80:20, v/v; 400 ml 70:30, v/v; 1000 ml 50:50, v/v); TLC retention time: 0.27; eluate:

dichloromethane–ammonia saturated methanol (94:6, v/v) to yield 2.25 g of *N*-desmethyl galantamine base. This material was suspended in hot acetonitrile (20 ml) and HCl-saturated 2-propanol was added until the solution turned acidic. The material shortly solubilized and quickly thereafter a precipitate formed. The mixture was stirred for 5 min and was then, while stirring, cooled on ice. The precipitate was filtered, washed with acetonitrile and dried under vacuum at 90°C to afford *N*-desmethyl galantamine · HCl · H_2O, 2.2 g (**2a**, 51% overall yield).

(−)-[4aS-(4aα,6β,8aR)]-4a,5,9,10,11,12-hexahydro-11-methyl-6H-benzofuro [3a,3,2-e,f] [2]-benzazepin-3,6-diol hydrochloride (O-desmethyl galantamine,* **2b** · *HCl)*

A solution of butanethiol (9.7 g, 11.5 ml, 108 mmol) in hexamethylphosphoramide (50 ml) was stirred under argon at −10°C. A solution of *n*-butyllithium 2.5 M in hexanes (39 ml, 97.5 mmol) was added dropwise, maintaining the temperature below 15°C. After the addition, the temperature was allowed to rise to room temperature and galantamine · HBr (**1a**, 12.0 g, 32.5 mmol) was added as a solid in one portion. The reaction flask was immersed into an oil bath, warmed to 100°C and solvents were distilled off until the reaction mixture reached a temperature of 92°C. The remaining volume was cooled to 15°C and a mixture of dichloromethane (150 ml) and methanol (40 ml) was introduced. The material was divided into two equal portions and each portion was filtered over silica (diameter: 90 mm, height: 70 mm) using dichloromethane–methanol (80:20, v/v) as eluate. At first the elution was hampered by the formation of a gelatinous mass. The product containing fractions, as verified by means of TLC ($R_f = 0.25$; eluate: dichloromethane–methanol 80:20, v/v) were combined and the solvent was expelled at aspirator pressure. The residue (8.3 g) was chromatographed carefully over silica (column dimensions: ID: 50 mm, height: 130 mm) with dichloromethane–methanol (80:20, v/v) as elution solvent. The fractions containing both product and trace amounts of galantamine were combined and concentrated to give 3.35 g of product which contained 1.2% of starting material, according to HPLC (Kromasil RP18 100-10, 4.6 mm ID × 300 mm; flow rate: 1.0 ml/min; UV. detection at 288 nm; programmed run consisting of a linear gradient run for 25 min starting with aqueous 0.1 M ammonium acetate solution (pH 7.0) methanol–acetonitrile (93–3.5–3.5, v/v/v) to solvent composition (64–18–18, v/v/v), followed by a 5 min linear gradient run to solvent

composition (10–45–45, v/v/v) and a 10 min isocratic run at the latter solvent composition; method A). The fractions which were pure according to TLC were combined and gave after concentration 2.85 g of material. Of this residue, HPLC indicated 0.15% of starting material to be present. For use as internal standard the amount of unlabelled galantamine was required to be less than 0.1% of the amount of labelled material. For this reason, the latter residue (2.85 g) was chromatographed again over silica (column dimensions: ID: 30 mm, height: 135 mm) with dichloromethane–methanol–ammonia-saturated methanol (90:5:5, v/v/v). The collected fractions were analysed by means of LC/MS/MS (Chromatographic conditions: Column: Water Symmetry-Shield^{TM} RP18 3.5 μm, 4.6 mm ID × 50 mm; flow rate: 1.5 ml/min; isocratic run for 2 min with eluate: aqueous 0.01 M ammonium acetate solution (pH 7.0)–acetonitrile (85–15, v/v)). The fractions still containing galantamine were combined and concentrated to yield **2b**-base with 0.1% of galantamine (1.7 g). The galantamine-free fractions were combined and concentrated to give 765 mg of **2b**-base. This residue was dissolved in boiling 2-propanol (9.0 ml) and HCl-saturated 2-propanol (0.52 ml) was added. The formed crystals were filtered and washed twice with small amounts of 2-propanol to yield **2b** · HCl (777 mg).

(−)-[4aS-(4aα,6β,8aR)]-4a,5,9,10,11,12-hexahydro-3-methoxy-11-[^{14}C]-methyl-6H-benzofuro-[3a,3,2-e,f[2]-benzazepin-6-ol (N-[^{14}C]-methyl-galantamine,* **3a***)*

To a suspension of *N*-desmethyl galantamine **2a** (0.900 mmol, 319 mg) in methanol (2.0 ml) diisopropylamine (0.9 mmol, 126 μl) was added. The reaction vial was immediately stoppered, the reaction mixture was stirred to obtain a clear solution and [^{14}C]methyl iodide (0.225 mmol, 14 μl, containing 441 MBq of radioactivity and with a 92% radiochemical purity) was introduced. The reaction vessel was stoppered and the contents were stirred for 18 h at room temperature. Then the solvent was taken off at aspirator pressure, water (100 μl) was added and purification was performed by means of 50 μl injections on preparative HPLC (Hypersil ODS (5 μm), 7.1 mm ID × 300 mm; flow rate: 4.0 ml/min; UV detection at 275 nm; isocratic run with eluate: acetonitrile–water–diisopropylamine (23–77–0.2, v/v/v)) to yield after concentration and dilution with ethanol 162 MBq of compound **3a** (radiochemical yield: 40.0%) at a purity of 99.3% (HPLC conditions as described in the

synthesis of *O*-desmethyl galantamine, **2b**; method A), with an enantiomeric excess of 100% (Chiralcel OD (10 μm), 4.6 mm ID × 300 mm; flow rate: 0.9 ml/min; column temperature 0°C; isocratic run with eluate: hexane–ethanol (80–20, v/v); method B). The compound had a specific activity of 1.91 MBq/mmol.

(−)-[4aS-(4aα,6β,8aR)]-4a,5,9,10,11,12-hexahydro-3-[^{14}C]-methoxy-11-methyl-6H-benzofuro-[3a,3,2-e,f][2]-benzazepin-6-ol (O-[^{14}C]-methyl-galantamine,* **3b***)*

To a solution of galantamine-free *O*-desmethylgalantamine **2b** · HCl (0.100 mmol, 30 mg) in 1,3-dimethyl-2-imidazolidinone (1.0 ml) was added freshly ground potassium hydroxide powder (0.3 mmol, 17.7 mg) and the reaction mixture was stirred for 4 h under nitrogen atmosphere. The yellow solution was then added to the [^{14}C]-methyl iodide (0.006 mmol, containing 12 MBq of radioactivity), the reaction vessel was stoppered and the reaction mixture was stirred for 18 h at room temperature. The purification of the reaction mixture was performed by means of 50 μl injections on preparative HPLC using the same conditions as for the *N*-[^{14}C]-methyl galantamine. The product fractions were combined, evaporated, diluted with methanol–water (20:80, v/v) and again purified in two portions via the same preparative HPLC procedure. After concentration, the product which contained 2.16 MBq of radioactivity (radiochemical yield 19.6%) was diluted with ethanol. The batch had a purity of 98.7% (HPLC conditions as described in the synthesis of *O*-desmethyl galantamine, **2b**; method A), with an enantiomeric excess of 100% (HPLC conditions as described in the synthesis of *N*-[^{14}C]-methyl-galantamine, **3a**; method B). The compound had a specific activity of 1.91 MBq/mmol.

(−)-[4aS-(4aα,6β,8aR)]-4a,5,9,10,11,12-hexahydro-3-[$^{13}CD_3$]-methoxy-11-methyl-6H-benzofuro-[3a,3,2-e,f][2]-benzazepin-6-ol hydrobromide (O-[$^{13}CD_3$]-methyl-galantamine hydrobromide,* **3c***)*

To a solution of galantamine-free **2b** · HCl (410 mg, 1.5 mmol) in freshly distilled dry tetrahydrofuran (30 ml) was added polymer bound triphenylphosphine (1.1 g, containing 3.3 mmol of triphenylphosphine) and $^{13}CD_3OD$-labelled methanol (0.55 ml, 13.3 mmol) from a freshly opened ampoule. The reaction vessel was flushed with argon and sealed with a rubber septum. Diisopropyl azodicarboxylate (0.62 ml,

3.15 mmol) was slowly dropped to the reaction mixture. After stirring for 20 h at room temperature, the mixture was filtered over a fritted P4 glass filter and the remaining material was thoroughly rinsed with, respectively, tetrahydrofuran (5 ml), methanol (4 × 5 ml), and dichloromethane–methanol (1:1, v/v, 5 ml). The combined filtrates were concentrated at aspirator pressure to leave 1.14 g of residue. This was taken up in a small volume of dichloromethane–methanol (90:10, v/v) and purified over silica (column dimensions: ID: 30 mm, height: 180 mm) with dichloromethane–methanol (90:10, v/v) as eluate. The product-containing fractions were combined and the solvents were expelled at aspirator pressure. The residue (184 mg) was dissolved in warmed ethanol and to the stirred solution was dropped slowly a solution of 48% aqueous hydrobromic acid (80 µl, 0.70 mmol, 1.1 Eq) dissolved in ethanol (100 µl), causing the product to precipitate as the HBr salt. The mixture was stirred for 1.5 h, the crystals were filtered off and were washed twice with ethanol (2 × 125 µl). The material was dried in air to yield 98.5% pure [$^{13}CD_3$]-galantamine · HBr salt (**3c** · HBr, 204 mg, 36.1%) with unlabelled galantamine below the LC–MS detection limit of 0.02% (LC/MS/MS conditions as described for compound **2b**).

(±)-(4aα,6β,8aR)]-4a,5,9,10,11,12-hexahydro-1-bromo-3-methoxy-11-methyl-6H-benzofuro-[3a,3,2-e,f] [2]-benzazepin-6-ol (racemic 1-bromogalantamine,* **5***)*

To a stirred, argon-covered solution of (±)-(4aα)-4a,5,9,10,11,12-hexahydro-1-bromo-3-methoxy-11-methyl-6*H*-benzofuro-[3a,3,2-e,f] [2]-benzazepin-6-one (racemic 1-bromonarwedine, **4**, 4.18 g, 12.0 mmol) in dry tetrahydrofuran (40 ml) was added, in one portion at −10°C, *L*-selectride 1 M solution in tetrahydrofuran (13.2 ml, 13.2 mmol). An exothermic effect causing a temperature rise to −2°C was observed. The reaction mixture was stirred for 30 min at 0°C, was then allowed to come to room temperature and was stirred for 2 h at ambient temperature. Methanol (1.0 ml) was added and the solvent was evaporated at aspirator pressure. The residue was dissolved in a mixture of 2 N aqueous hydrochloric acid (40 ml) and ethyl acetate (40 ml). The layers were separated and chloroform (40 ml) was added to the water layer, which was then basified (pH > 10) with 10 N aqueous sodium hydroxide solution. The organic layer was separated and the water layer was extracted with chloroform (40 ml). The combined

organic layers were dried on magnesium sulphate and evaporated at aspirator pressure. Gravity chromatography over silica (column dimensions: ID: 40 mm, height: 160 mm) with chloroform–methanol (97:3, v/v) as eluate afforded after thorough evaporation compound **5** (2.07 g). This material was used as such in the next reaction step.

(−)-[4aS-(4aα,6β,8aR)]-1-tritio-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro-[3a,3,2-e,f] [2]-benzazepin-6-ol (1-tritio-galantamine,* **6***)*

A mixture of racemic 1-bromogalantamine (**5**, 25 mg, 0.068 mmol), triethylamine (0.040 ml), palladium 10% on carbon (40.0 mg) and a 4% *n*-butanethiol–diisopropylether solution (0.040 ml) in tetrahydrofuran (4.0 ml) was stirred and cooled on ice. It was then brought under tritium atmosphere (740 GBq of tritium gas) and bromine–tritium exchange was allowed to occur for 46 h at room temperature. Catalyst and labile tritium were removed and the residue was dissolved in methanol (100 ml). The solution contained 12.2 GBq of radioactivity with 27.5% of racemic galantamine. Part of the crude material (30 ml) was purified and resolved via HPLC (purification: Kromasil RP18 100-10, 4.6 mm ID × 300 mm; flow rate: 2.0 ml/min; UV detection at 288 nm; isocratic run with eluate: aqueous 0.1 M ammonium acetate solution (pH 7.0)–methanol–acetonitrile (80–10–10, v/v/v); resolution: HPLC conditions as described in the synthesis of *N*-[^{14}C]-methyl-galantamine, **3a**; method B. The first eluting compound is the desired enantiomer) to afford 1-tritio-galantamine **6** (238 MBq) with a radiochemical purity of 99.2% (Hypersil ODS (5 μm), 4.6 mm ID × 300 mm; flow rate: 1.0 ml/min; UV detection at 288 nm; eluate conditions: a linear gradient run for 30 min starting from water–diisopropylamine (100–0.2, v/v) to acetonitrile–diisopropylamine (100–0.2, v/v) followed by an isocratic run for 10 min at the latter solvent composition) and an enantiomeric excess of 99.6% (HPLC conditions as described in this synthesis; method B). The material had a specific activity of 94.4 GBq/mmol.

Acknowledgements

The authors wish to thank S. Van Hoegaerden for performing HPLC analyses and L. Diels for carrying out the LC/MS/MS analysis.

References

1. Proskurnina NF, Yakovleva AP. *J Gen Chem USSR* 1952; **22**: 1941.
2. Boit HG. *Chem Ber* 1954; **87**: 724.
3. Boit HG, Stender W, Beitner A. *Chem Ber* 1957; **90**: 725.
4. Boit HG, Dopke W, Beitner A. *Chem Ber* 1957; **90**: 2197.
5. Schuh FT. *Anästhesist* 1979; **25**: 444.
6. Thomsen T, Bickel U, Fischer JP, Kewitz H. *Dementia* 1990; **1**: 46.
7. Maelicke A, Samochocki M, Jostock R, Fehrenbacher A, Ludwig J, Albuquerque EX, Zerlin M. *Biol Psychiatry* 2001; **49**: 279.
8. Raskind MA, Peskind ER, Wessel T, Yuan W. *Neurology* 2000; **54**: 2261.
9. Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfield S, Ding C. *Neurology* 2000; **54**: 2269.
10. Wilcock GK, Lilienfield S, Gaens E. *Br Med J* 2000; **321**: 1445.
11. Wilkinson D, Murray J. *Int J Geriatric Psychiatry* 2001; **16**: 852.
12. Barton DHR, Kirby GW. *J Chem Soc* 1962; 806.
13. Kametani T, Yamaki K, Yagi H, Fukumoto K. *J Chem Soc (C)* 1969; **18**: 2602.
14. Kametani T, Shishido K, Hayashi E, Seino C, Shibuya S, Fukumoto K. *J Org Chem* 1971; **36**: 1295.
15. Kametani T, Seino C, Yamahi K, Shibuya S, Fukumoto K, Kigasawa K, Satoh F, Hiiragi M, Hayasaka T. *J Chem Soc (C)* 1971; **6**: 1043.
16. Kametani T, Yamahi K, Terui T, Shibuya S, Fukumoto K. *J Chem Soc Perkin I* 1972; **12**: 1513.
17. Shimizu K, Tomioko K, Yamada S, Koga K. *Heterocycles* 1977; **8**: 277.
18. Golob DJ. Studies directed toward the total synthesis of natural products and their analogs. Part I. Galanthamine. Part II. Prostaglandins and prostacyclin. Dissertation, University of California, Los Angeles, CA, USA. 1979.
19. Szewczyk J, Lewin AH, Carroll FI. *J. Heterocycl Chem*. 1988; **25**: 1809.
20. Vlahov R, Krokorian D, Spassov G, Chinova M, Vlahov I, Parushev S, Snatzke G, Ernst L, Kieslich K, Abraham WR, Sheldrick WS. *Tetrahedron* 1989; **45**: 3329.
21. Szewczyk J, Wilson JW, Lewin AH, Carroll FI. *J Heterocycl Chem* 1995; **32**: 195.
22. Küenburg B, Czollner L, Fröhlich J, Jordis U. *Org Process Res Develop* 1999; **3**: 425.
23. Czollner L, Frantsits W, Küenburg B, Hedenig U, Fröhlich J, Jordis U. *Tetrahedron Lett* 1998; **39**: 2087.
24. Cooley JH, Evain EJ. *Synthesis* 1989; 1.
25. Olofson RA, Schnur RC, Bunes L, Pepe JP. *Tetrahedron Lett* 1977; **18**: 1567.

26. Olofson RA, Martz JT, Senet J-P, Piteau M, Malfroot T. *J Org Chem* 1984; **49**: 2081.
27. Mary A, Renko D, Guillon C, Thal C. *Tetr. Lett*. 1997; **38**: 5151.
28. Greene TW, Wuts PGM. *Protective Groups in Organic Synthesis*. Wiley: New York, 1991.
29. Rice KC. *J Med Chem* 1977; **20**: 164.
30. Han SY, Sweeney JE, Bachman ES, Schweiger EJ, Forloni G, Coyle JT, Davis B, Joullié M. *Eur J Med Chem* 1992; **27**: 673.
31. Lawson JA, Degraw JI. *J Med Chem* 1977; **20**: 165.
32. Mannens GJS, Snel CAW, Hendrickx J, Verhaeghe T, Bode W, van Beijsterveldt L, Lavrijsen K, Leempoels J, Van Osselaer N, Van Peer A, Meuldermans W. *Drug Metab Dispos*, 2002; **30**: 553.
33. Mitsunobu O. *Synthesis* 1981; 1.
34. Linnemann E, Fels G. *J Label Compd Radiopharm* 2001; **44**: 661.
35. Welzig S. Neue Narwedin-und Galanthaminderivate als potentielle Alzheimer Therapeutica. *Dissertation*, TU Wien, March 1997.

