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Research Article

Application of the Bischler–Napieralski–Pschorr radiosynthesis of (*R*)-(-)-[6a-^{14}C]apomorphine, a non-selective D_1/D_2 dopamine receptor agonist

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Summary

A method has been developed for the carbon-14 radiosynthesis of non-narcotic morphine derivative (*R*)-(-)-[6a-^{14}C]apomorphine (**1**) from the starting material 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl[carboxyl-^{14}C]acetamide (**5**). The key to this synthesis was the application of the Bischler–Napieralski cyclodehydration to 1-(3,4-dimethoxy-2-nitrobenzyl)dihydro[1-^{14}C]isoquinoline (**4**), followed by *N*-methylation and reduction to 1-(3,4-dimethoxy-2-nitrobenzyl)-2-methyl-1,2,3,4-tetrahydro[1-^{14}C] isoquinoline (**3**). A final Pschorr reductive ring closure followed by chiral separation to give (*R*)-(-)-[6a-^{14}C]apomorphine dimethyl ether (**2**) and *O*-demethylation led to (*R*)-(-)-[6a-^{14}C]apomorphine (**1**) with a specific activity of 55 mCi/mmol, radiochemical purity of > 98% and chiral purity of > 99%.

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Key Words: apomorphine; cyclodehydration; Bischler–Napieralski and Pschorr

Introduction

(*R*)-(-)-[6a-^{14}C]Apomorphine (**1**), (*R*)-(-)-(5,6,[6a-^{14}C],7-tetrahydro-6-methyl-4*H*-dibenzo-[de,g]-quinoline-10,11-diol)[1] shown in Scheme 1 contains the *ortho*-aryl phenol motif and belongs to a class of natural alkaloids called the aporphines (e.g. apocodeine and bulbocapnine) which exhibit interesting biological properties.[2] The structural elucidation of apomorphine was solved

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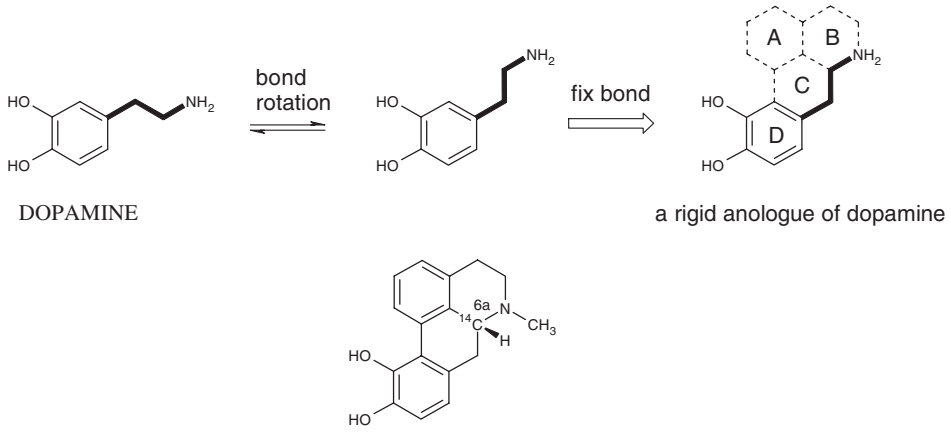

DOPAMINE

a rigid anologue of dopamine

(*R*)-(-)-[6a-^{14}C]APOMORPHINE (**1**)

Scheme 1. Chemical structures of (*R*)-(-)-[6a-^{14}C]apomorphine (1) and dopamine

by Pschorr[3] in 1907 and its absolute configuration was determined by Corrodi *et al*. in 1955 and shown to have a *R* stereogenic centre.[4] The first reported total synthesis of ($\pm$)apomorphine was carried out by Neumeyer *et al*.[5] in 1973 involving the alkylation of a *Reissert* isoquinoline with 3,4-dimethoxy-2-nitrobenzylchloride (**8**).

This drug has been found to be a non-selective D_1/D_2 dopamine receptor agonist; showing more potent D_2-like effects in the treatment of Parkinson's disease[6] resulting from dopamine imbalances in the brain. Dopamine can adopt many conformations due to single bond rotation in the alkylamine side chain. Apomorphine contains a rigid dopamine pharmacophore within its framework to prevent rotation resulting in biological activity.[7,8] Another application of this drug is the treatment of erectile dysfunction.[9] Other co-workers have demonstrated that the main metabolic *in vivo* pathways in rat and human hepatocytes are sulphonation, glucuronidation and *N*-demethylation to give norapomorphine. Elimination of these conjugates is predominantly via the kidneys.[10]

Results and discussion

The retro-synthetic analysis for the incorporation of a single carbon-14 label at the (*R*) stereogenic centre to give (*R*)-(-)-[6a-^{14}C]apomorphine (**1**) is shown in Scheme 2. The disconnection of the *ortho* aryl carbon–carbon bond in (*R*)-(-)-[6a-^{14}C]apomorphine dimethyl ether (**2**) gives the 1-(3,4-dimethoxy-2-nitrobenzyl)-2-methyl-1,2,3,4-tetrahydro[1-^{14}C]isoquinoline (**3**). A further disconnection leads to 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl[carboxyl-^{14}C]acetamide (**5**). This can be synthesized from 3,4-dimethoxy-2-nitrophe-

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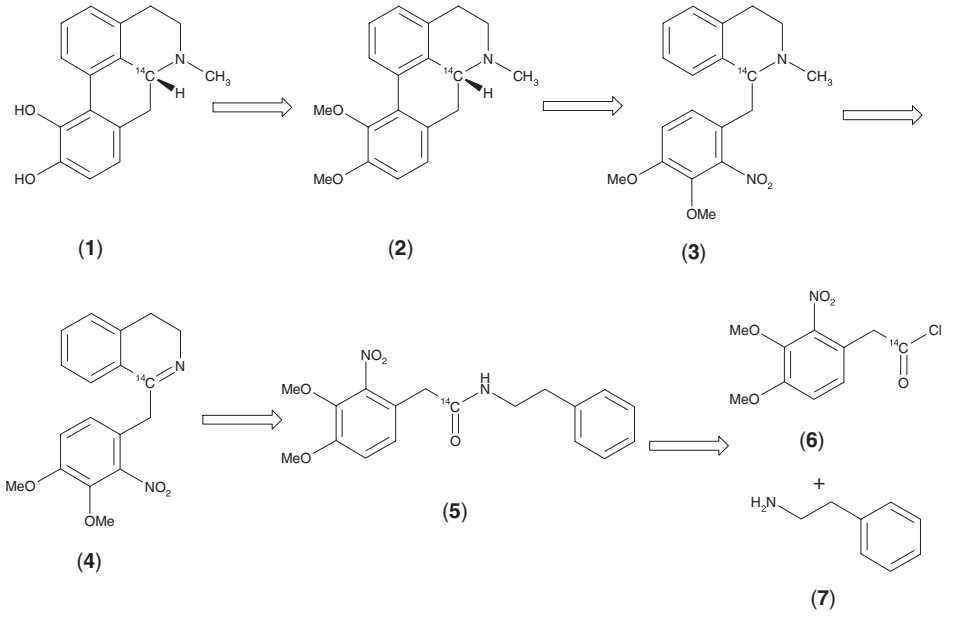

Scheme 2. Retrosynthetic analysis of (*R*)-(-)-[6a-^{14}C]apomorphine (1)

nyl[carboxyl-^{14}C]acetylchloride (**6**) and the commercially available starting material 2-phenethylamine (**7**). The application of the Bischler–Napieralski cyclodehydration on (**5**) will give 1-(3,4-dimethoxy-2-nitrobenzyl)dihydro [1-^{14}C]isoquinoline (**4**), followed by *N*-methylation and reduction to produce (**3**). A final Pschorr reductive ring closure followed by chiral separation will give (**2**) and *O*-demethylation to (*R*)-(-)-[6a-^{14}C]apomorphine (**1**).

Synthesis of ^{14}C-labelled compounds

The synthetic route to 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl[carboxyl-^{14}C]acetamide (**5**) is shown in Scheme 3. The inactive starting material 3,4-dimethoxy-2-nitrobenzylchloride (**8**) was prepared in five steps starting from the commercially available vanillin acetate.[11] Compound (**8**) gave the following ^{1}H NMR (d_6-DMSO, 200 MHz) $\delta = 7.41$ (d, 1H, $J = 8.7$ Hz), 7.32 (d, 1H, $J = 8.7$ Hz), 4.72 (s, 2H), 3.91 (s, 3H), 3.84 (s, 3H).

The incorporation of the carbon-14 radiolabel was facilitated via S_N2 displacement of the chloro in 3,4-dimethoxy-2-nitrobenzylchloride (**8**) using potassium[^{14}C]cyanide (55 mCi/mmol) in aqueous DMF at 80°C to afford 3,4-dimethoxy-2-nitrobenzyl[^{14}C]cyanide (**9**) in 90% yield from (**8**). Acid hydrolysis of (**9**) with 50% hydrochloric acid produced 86% yield of 3,4-dimethoxy-2-nitrophenyl[carboxyl-^{14}C]acetic acid (**10**).

Subsequent conversion to 3,4-dimethoxy-2-nitrophenyl[carboxyl-^{14}C] acetylchloride (**6**) with oxalyl chloride followed by the reaction with

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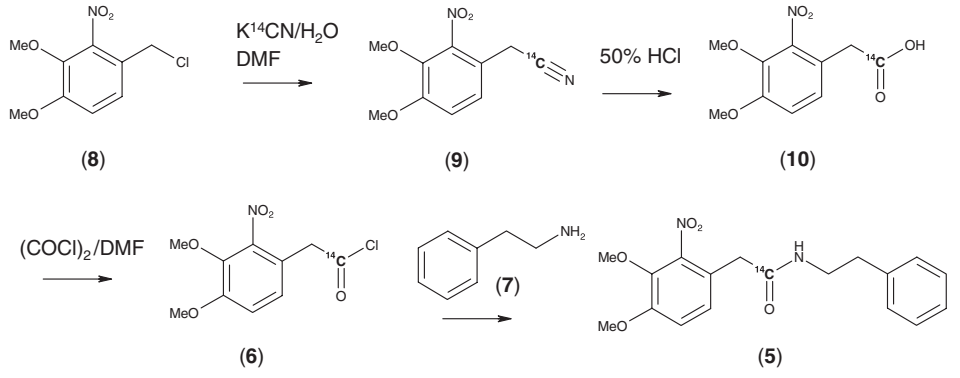

Scheme 3. Synthetic route to 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl [carboxyl-^{14}C]acetamide (5)

2-phenethylamine (**7**) produced the retro fragment 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl[carboxyl-^{14}C]acetamide (**5**) in 86% yield.

Bischler–Napieralski cyclodehydration

A successful Bischler–Napieralski cyclodehydration was accomplished by activating the amide function in 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl[-carboxyl-^{14}C]acetamide (**5**) with $P_2O_5/POCl_3$ in toluene (Scheme 4) to give *O*-phosphorylation. There is evidence that this combination of $P_2O_5/POCl_3$ provides a strong dehydrating medium which leads to the imino phosphates ($X_1 = OPO_2$, $X_2 = OPOCl_2$) and nitrilium phosphates ($Y_1 = PO_3^-$, $Y_2 = ^- OPOCl_2$) depending on which dehydration route is taken.[12,13] Fodor and Nagubandi[14] have reported that a mixture of $P_2O_5/POCl_3$ may give rise to the imino phosphate ($X_1 = OPO_2$) which facilitates a better leaving group than the dichlorophosphates ($X_2 = OPOCl_2$). The nitrilium salt ($Y_1 = PO_3^-$) can be captured via an intramolecular cyclization by the phenethylamino moiety to give the Bischler–Napieralski *endocyclic* product 1-(3,4-dimethoxy-2-nitrobenzyl)dihydro[1-^{14}C]isoquinoline (**4**).

This was confirmed by ^{1}H NMR (d_6-DMSO, 200 MHz) $\delta = 7.55$ (d, 1H, $J = 6.8$ Hz), 7.39–7.10 (m, 5H), 4.05 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.48 (t, 2H, $J = 7.3$ Hz), 2.59 (t, 2H, $J = 7.8$ Hz) and positive ion electrospray [LC–MS–ES] with a pseudo molecular ion $[MH]^+$ bundle at $m/z = 327/329$ confirming the structure as a single carbon-14 label. No triplet was observed for the isoquinoline nitrogen in (**4**) therefore indicating that the structure is the imine tautomer.

Bischler–Napieralski products

During the reaction another product was isolated (Scheme 5) and characterized by MS and ^{1}H NMR. Positive ion [LC–MS–ES] gave a pseudo molecular

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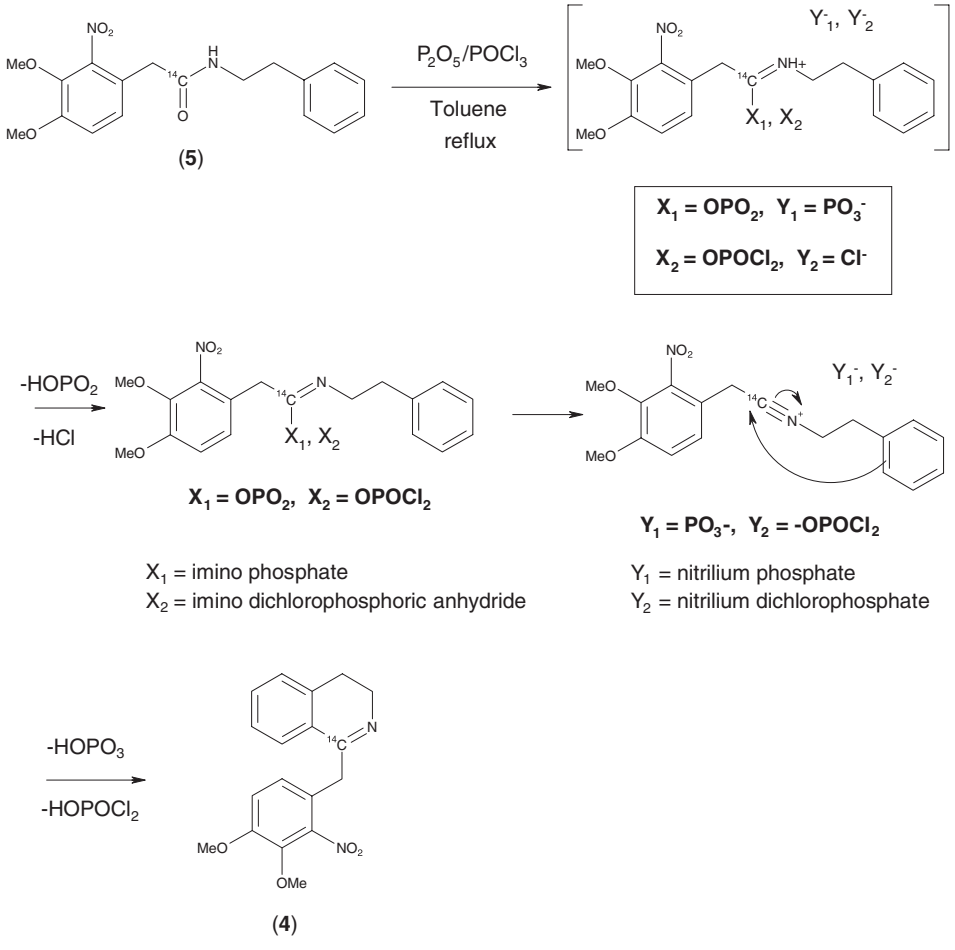

Scheme 4. Mechanistic view of the Bischler–Napieralski cyclodehydration

ion $[MH]^+$ bundle at $m/z = 309/311$ and ^{1}H NMR (d_6-DMSO, 200 MHz) $\delta = 7.74$ (d, 1H, $J = 6.9$ Hz), 7.66 (d, 1H, $J = 9.4$ Hz), 7.57 (t, 1H, $J = 6.9$ Hz), 7.50 (t, 1H, $J = 6.4$ Hz), 7.42 (d, 1H, $J = 8.3$ Hz), 7.23 (d, 1H, $J = 9.4$ Hz), 4.04 (s, 3H), 3.94 (s, 3H), 3.94 (t, 2H, $J = 7.0$ Hz), 2.81 (t, 2H, $J = 7.0$ Hz).

The above data is consistent with structure (**12**) having a substituted anthranil ring from the cyclodehydration of the nitrophenyl moiety with the reactive methylene group[15] in (**5**) to give the intermediate 6,7-dimethoxy-*N*-phenethylanthranil-3-[carboxy-^{14}C]amide (**11**).

The amide moiety was further dehydrated to initiate the intramolecular cyclization via the phenethylamino group to give 3-(6,7-dimethoxyanthranil)-dihydro[1-^{14}C]isoquinoline (**12**).

Hey and Palluel[16] have carried out the Bischler–Napieralski cyclodehydration on unlabelled (**5**) and reported the by-product to be unlabelled (**11**).

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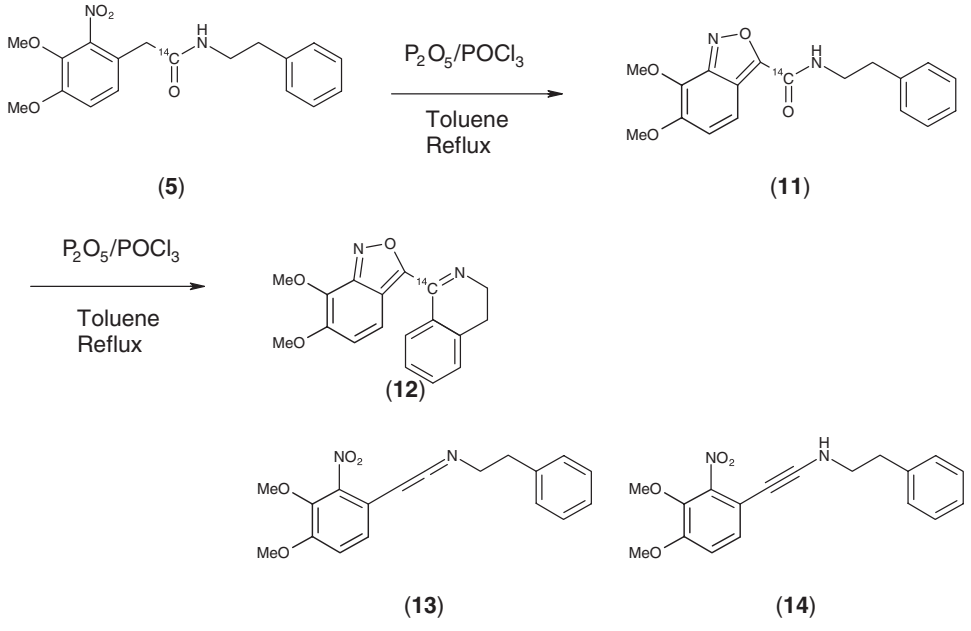

Scheme 5. Bischler–Napieralski dehydration products

This compound would have a molecular mass of 326 compared to (**12**) which has a molecular mass of 308 when unlabelled. The Hey and Palluel[16] conclusion must be dismissed based on the MS and ^{1}H NMR analysis. Previous co-workers[17] had assigned the by-product to be the ketamine (**13**) or acetylene (**14**). No *von Braun* amide degradation products, i.e. 3,4-dimethoxy-2-nitrobenzyl[^{14}C]cyanide (**9**) and styrene were observed under any of these conditions.

When the cyclodehydration of (**5**) was carried out with $POCl_3$ in refluxing acetonitrile (Scheme 6) it gave only the non-basic dehydration product in the *exocyclic* form, 1-(3,4-dimethoxy-2-nitrobenzal)-1,2,3,4-tetrahydro[1-^{14}C]isoquinoline (**15**).[18]

This product was identified by ^{1}H-NMR ($CDCl_3$, 200 MHz) $\delta = 7.75$ (d, 1H, $J = 9.4$ Hz), 7.40–7.20 (m, 5H), 7.10 (d, 1H, $J = 9.4$ Hz), 6.75 (bt, 1H, NH), 4.20 (s, 3H), 4.00 (s, 3H), 3.80 (q, 2H, $J = 7.3$ Hz), 3.00 (t, 2H, $J = 7.3$ Hz) and LRMS analysis by positive ion EI produced the expected ion bundle [M^+] at $m/z = 326/328$ as expected for a single carbon-14 label. This data is consistent with structure (**15**) having a 'stilbene' with extended conjugation between the two aromatic rings. The enamine proton in the tetrahydroisoquinoline gave a triplet and does not exchange with D_2O indicating (**15**) was non-basic. This product did not undergo *N*-methylation with methyl iodide under various conditions.

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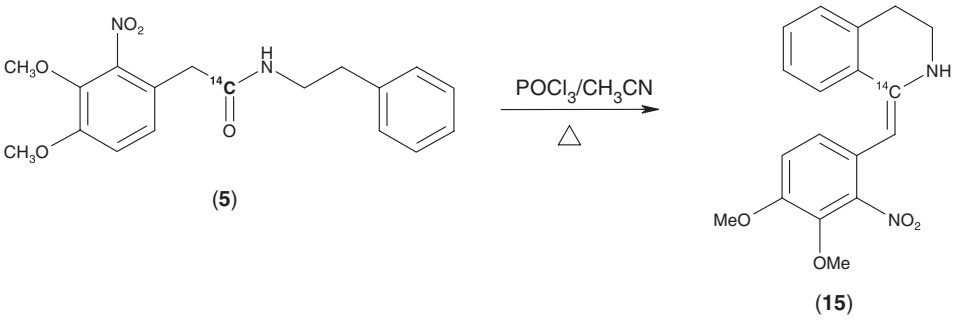

Scheme 6. The Bischler–Napieralski exocylic product (15)

N-methylation and reduction

The Bischler–Napieralski product 1-(3,4-dimethoxy-2-nitrobenzyl)dihydro[1-^{14}C]-isoquinoline (**4**) was then converted into the quaternary salt with methyl iodide (Scheme 7). On isolation this gave 1-(3,4-dimethoxy-2-nitrobenzyl)dihydro[1-^{14}C]isoquinoline methiodide (**16**). MS by negative ion fast atom bombardment (FAB) analysis produced the expected ion bundle $[M–I]^-$ at $m/z = 127$ for the iodide ion. Positive ion electrospray (ES) produced the ion bundle $[M^+]$ at $m/z = 341/343$ as expected for a single carbon-14 label. The 3,4-dihydroisoquinolinium ring was reduced by sodium borohydride in ethanol to give 1-(3,4-dimethoxy-2-nitrobenzyl)-2-methyl-1,2,3,4-tetrahydro[1-^{14}C]isoquinoline (**3**). The strategy employed for the reduction of (**16**) prevented the carbon–carbon bond cleavage between the 3,4-dihydroisoquinolinium ring and the 3,4-dimethoxy-2-nitrophenyl moiety. This reductive cleavage occurs readily in *Reissert* 1,2-dihydroisoquinoline derivatives.[19,20] This subsequent reduction would have given 2-methylisoquinoline and 3,4-dimethoxy-2-nitrotoluene.

Reductive Pschorr cyclization[21,22]

The reduction of the aryl nitro group in 1-(3,4-dimethoxy-2-nitrobenzyl)-2-methyl-1,2,3,4-tetrahydro[1-^{14}C]isoquinoline (**3**) to the aryl amino group was achieved using zinc dust/hydrochloric acid (Scheme 7). Diazotization of the amino group to the corresponding arenediazonium salt followed by the addition of copper facilitated an intramolecular Pschorr coupling (probably via an aryl radical intermediate)[23,24] to generate a new six-membered ring to form the aporphine (*R*/*S*)-(±)-[6a-^{14}C]apomorphine dimethyl ether (**17**). The enantiomers of (**17**) were separated by preparative chiral HPLC using a Chiralcel-OD column to give (*R*)-(-)-[6a-^{14}C]apomorphine dimethyl ether (**2**) and (*S*)-(+)-[6a-^{14}C]apomorphine dimethyl ether (**18**).[25] Assignment of the enantiomers was established by reacting authentic (*R*)-(-)-apomorphine

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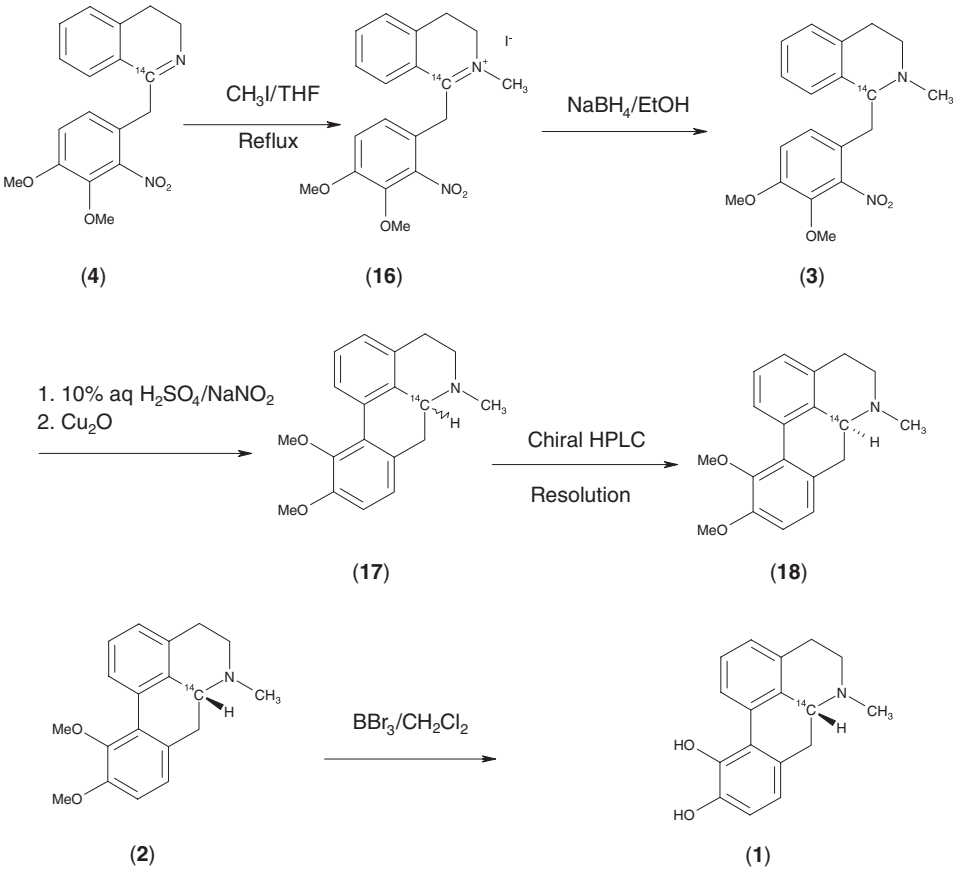

Scheme 7. Radiosynthesis of (*R*)-(-)-[6a-^{14}C]apomorphine (1)

hydrochloride hemihydrate with diazomethane to give (*R*)-(-)-apomorphine dimethyl ether.

O-demethylation

The *O*-demethylation of (*R*)-(-)-[6a-^{14}C]apomorphine dimethyl ether (**2**) was accomplished with boron tribromide in dichloromethane (Scheme 7) to give the non-selective D_1/D_2 dopamine receptor agonist: (*R*)-(-)-[6a-^{14}C]apomorphine (**1**) as the hydrochloride hemihydrate [RCP = 98.4%, optical purity = 99.6%, SA = 55 mCi/mmol].

The structure of the final product was confirmed by comparison (HPLC, NMR, MS) with authentic (*R*)-(-)-apomorphine hydrochloride hemihydrate [41372-20-7] from Aldrich Chemical Company. The other enantiomer (*S*)-(+)-apomorphine hydrochloride hemihydrate [41035-30-7] is also available from Aldrich. (*R*)-(-)-apomorphine

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hydrochloride hemihydrate is commercialized as apomorphine SL (Ixense®, and Uprima®).

Experimental procedures

Materials and methods

All radioactive experiments were carried out in the custom labelling special synthesis laboratories in specially designed hoods dedicated to carbon-14 only. Proton NMR spectra were recorded on a Bruker AC 200 or Bruker Avance 400 FT-NMR spectrometer. Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in either d_6-DMSO or $CDCl_3$ and coupling constants (*J*) are reported in Hertz. All reagents were purchased from commercial sources and were used without further purification. 3,4-dimethoxy-2-nitrobenzylchloride (**8**) was prepared using reported methods.[5,11] Thin layer chromatography (TLC) was carried out using Merck Kieselgel silica gel 60 F_{254} and Whatman LKC18F octadecyl-silica gel ODS glass plates. Radio-TLC was carried out on a BIOSCAN System AR 2000. Compounds were detected with UV light at 254 nm. Final products were dried in a vacuum desiccator over phosphorus pentoxide. The radiochemical yield was determined by liquid scintillation counting using the WALLAC 1409 DSA. Analytical HPLC was performed on the Hewlett Packard series 1100 using a Berthold Scintillator Pump equipped with a Berthold LB507A monitor used for the detection of radiochemical compounds. Radiochemical and chiral purity by HPLC of the final compound was performed on a Chiralcel OD-R column (250×4.6 mm)[25] with a flow of 0.5 ml/min and 0.05 M sodium perchlorate buffer pH 2.0:CH_3CN (65:35) as the mobile phase at UV 273 nm. Low resolution mass spectrometry [LRMS] was performed on the following instruments; Jeol JMS-DX300, Kratos MS25RF and Q-Tof2 spectrometers. Sample introduction was by DCI, Direct Insertion or De-absorption Probes, LC or infusion with the instruments set to detect positive or negative ions to obtain electron impact (EI), electrospray (ES), chemical ionization (CI) and fast atom bombardment (FAB) spectra.

Carbon-14 radiosynthesis

*Synthesis of 3,4-dimethoxy-2-nitrobenzyl[^{14}C]cyanide (**9**).* To a solution of 3,4-dimethoxy-2-nitrobenzylchloride (**8**) (8.1 g, 26 mmol) in DMF (273 ml) was added a solution of potassium[^{14}C]cyanide (1450 mCi, 55 mCi/mmol, 26 mmol) in water (27 ml). The reaction was heated to 80°C and held for 90 min with stirring. HPLC (Genesis AQ column, CH_3CN/H_2O) analysis of the reaction mixture gave 87% product conversion. The reaction mixture was quenched with water (273 ml) and the product extracted into ether (10×130 ml). The combined organic extracts were washed with 1 N sodium hydroxide (150 ml),

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separated, dried [$MgSO_4$], filtered and evaporated *in vacuo* to give a pale orange oil. This was purified by column chromatography on flash silica eluting with a mixture of ether/pentane. The appropriate fractions were combined and concentrated *in vacuo* to afford 3,4-dimethoxy-2-nitrobenzyl[^{14}C]cyanide (**9**) (1310 mCi, 55 mCi/mmol, 90%) as a white solid. Analytical HPLC (Genesis AQ column, CH_3CN/H_2O) analysis gave a single radioactive peak at $RT \sim 21$ min and no starting material was observed. TLC (silica gel, ether/hexane, 50:50) $R_f = 0.38$ gave a single spot.

*Synthesis of 3,4-dimethoxy-2-nitrophenyl[carboxyl-^{14}C]acetic acid (**10**).* 3,4-Dimethoxy-2-nitrobenzyl[^{14}C]cyanide (**9**) (1310 mCi, 23.8 mmol, 55 mCi/mmol) was added to 50% hydrochloric acid (200 ml) and the heterogeneous reaction mixture was heated to 126°C for 5 hours. Analytical HPLC (Genesis AQ column, CH_3CN/H_2O) of the reaction mixture gave 98% product conversion. The reaction mixture was cooled to ambient temperature and quenched with ice water (200 ml) and the product was extracted into ether (5×100 ml). The ether extracts were then combined and extracted with saturated sodium bicarbonate (5×100 ml). The basic extract was acidified with concentrated hydrochloric acid and the product extracted back into ether (3×250 ml). The ether extracts were combined, dried over [Na_2SO_4], filtered and concentrated *in vacuo* to give the crude product. This was purified by column chromatography on silica eluting with a mixture of ether/hexane/acetic acid. The appropriate fractions were combined and concentrated *in vacuo* to give 3,4-dimethoxy-2-nitrophenyl[carboxyl-^{14}C]acetic acid (**10**) (1130 mCi, 55 mCi/mmol, 20.5 mmol, 86%) as a yellowish solid. TLC RCP = 99.5%, $R_f = 0.73$ [silica gel, ether/acetic acid (100:1)].

*Synthesis of 3,4-dimethoxy-2-nitrophenyl-N-phenethyl[carboxyl-^{14}C]acetamide (**5**).* 3,4-Dimethoxy-2-nitrophenyl[carboxyl-^{14}C]acetic acid (**10**) (20.5 mmol, 1130 mCi, 55 mCi/mmol) was dissolved in anhydrous dichloromethane (200 ml). Excess oxalyl chloride (15 g, 118 mmol) was added to the stirred yellow solution, followed by one drop of DMF. The reaction mixture was stirred at ambient temperature for one hour. After complete consumption of the starting material by TLC analysis, the excess oxalyl chloride was removed *in vacuo* to give the yellow oil 3,4-dimethoxy-2-nitrophenyl[carboxyl-^{14}C] acetylchloride (**6**). The oil was taken up into anhydrous dichloromethane (200 ml) and the solution was cooled down in an ice water bath. 2-Phenethylamine (**7**) (12.2 g, 101 mmol) was added dropwise to the cold solution. After complete addition the reaction mixture was allowed to warm to ambient temperature and left stirring overnight. The mixture was quenched with 2 N hydrochloric acid (200 ml) and dichloromethane (200 ml). The organic layer was separated and washed with 2 N hydrochloric acid

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(3×500 ml) followed by 2 N sodium hydroxide (500 ml) and brine (500 ml). The organic layer was dried over [Na_2SO_4], filtered and concentrated *in vacuo* to give the crude product as brown oil (1052 mCi). The product was purified by flash column chromatography on silica eluting with a mixture of hexane/ether. The product fractions were combined and concentrated *in vacuo* to afford an off-white solid 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl[-carboxyl-^{14}C]acetamide (**5**) (6.2 g, 968 mCi, 55 mCi/mmol, 86%): TLC RCP = 99.5%, $R_f = 0.74$, [silica gel, ethyl acetate/acetic acid (100:1)]; TLC RCP = 99.1%, $R_f = 0.74$, [ODS, CH_3CN/H_2O (8:2)]; LRMS positive ion EI [M^+]:344/346; specific activity 55 mCi/mmol; ^{1}H-NMR (d_6-DMSO, 400 MHz) $\delta = 8.11$ (bt, 1H, NH), 7.33–7.17 (m, 6H), 7.12–7.08 (d, 1H, $J = 8.3$ Hz), 3.88 (s, 3H), 3.70 (s, 3H), 3.38 (s, 2H), 3.23 (t, 2H, $J = 6.4$ Hz), 2.69 (t, 2H, $J = 7.8$ Hz).

*Synthesis of 1-(3,4-dimethoxy-2-nitrobenzyl)-dihydro[1-^{14}C]isoquinoline (**4**).* A solution of 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl[carboxyl-^{14}C]acetamide (**5**) (906 mCi, 55 mCi/mmol, 16.5 mmol) in toluene (200 ml) was added dropwise over 20 min to a suspension of phosphorous pentoxide (49 g) and phosphorous oxychloride (12 ml) in toluene (120 ml). On complete addition, the reaction was refluxed for a further 30 min and then allowed to cool to ambient temperature. The active orange-coloured toluene solution was decanted off and the residual solid was washed with toluene (3×100 ml). The residual solid was then decomposed with ice and the product was extracted into chloroform (7×50 ml). The combined chloroform extracts were shaken with 2 N sodium hydroxide, dried over [Na_2SO_4], filtered and concentrated *in vacuo* to give a dark oil. The crude product was purified by flash chromatography on silica eluting with a mixture of ether/hexane to give the Bischler–Napieralski product 1-(3,4-Dimethoxy-2-nitrobenzyl)-dihydro[1-^{14}C]isoquinoline (**4**) (396 mCi, 43%) as an oil. TLC $R_f = 0.46$ (silica gel, ethyl acetate), ^{1}H-NMR (d_6DMSO, 400 MHz) $\delta = 7.55$ (d, 1H, $J = 6.8$ Hz), 7.39–7.10 (m, 5H), 4.05 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.48 (t, 2H, $J = 7.3$ Hz), 2.59 (t, 2H, $J = 7.8$ Hz); Positive ion LC–MS–ES: $[MH]^+$ 327/329; specific activity 55 mCi/mmol.

*Synthesis of 1-(3,4-dimethoxy-2-nitrobenzyl)dihydro[1-^{14}C]isoquinoline methiodide (**16**).* 1-(3,4-Dimethoxy-2-nitrobenzyl)dihydro[1-^{14}C]isoquinoline (**4**) (396 mCi, 55 mCi/mmol) was taken up in anhydrous tetrahydrofuran (100 ml) and methyl iodide (5 ml, 80.3 mmol) was added. The reaction mixture was heated under reflux for 12 hours. TLC analysis (ODS, CH_3CN/H_2O 8:2) indicated complete consumption of the starting material. The cooled reaction mixture was evaporated *in vacuo* to give a yellow solid 1-(3,4-dimethoxy-2-nitrobenzyl)dihydro-[1-^{14}C]isoquinoline methiodide (**16**). LRMS positive ion ES: $[MH]^+$ 341/343 and negative ion FAB: 127 for iodide counter ion.

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*Synthesis of 1-(3,4-dimethoxy-2-nitrobenzyl)-2-methyl-1,2,3,4-tetrahydro[1-^{14}C] isoquinoline (**3**).* 1-(3,4-Dimethoxy-2-nitrobenzyl)dihydro-[1-^{14}C]isoquinoline methiodide (**16**) was taken up in ethanol (125 ml) and treated with sodium borohydride (1.33 g, 35.2 mmol). The reaction was stirred at ambient temperature for 1.5 hours. The excess sodium borohydride was destroyed by the addition of 2 N hydrochloric acid and the reaction was basified with 2 N sodium hydroxide. The basic solution was extracted with ether and the combined ether extracts were dried over [$MgSO_4$], filtered and concentrated *in vacuo* to give the crude product. This was purified by reverse phase HPLC using a Phenomenex Prodigy column eluting the product off with a mixture of acetonitrile/water to afford a pale yellow solid 1-(3,4-dimethoxy-2-nitrobenzyl)-2-methyl-1,2,3,4-tetrahydro[1-^{14}C]isoquinoline (**3**) (306 mCi, 55 mCi/mmol). RCP TLC 98.2% at $R_f = 0.31$ (silica gel, ethyl acetate) and 97.5% at $R_f = 0.50$ [ODS, acetonitrile:water (8:2)]; ^{1}H-NMR ($CDCl_3$, 200 MHz) $\delta =$ 7.10 (m, 3H), 6.86 (d, 1H, $J = 8.7$ Hz), 6.85 (s, 1H), 6.79 (d, 1H, $J = 8.7$), 3.93 (s, 3H), 3.88 (s, 3H), 3.77 (t, 1H), 3.30–2.50 (m, 6H), 3.02 (s, 3H).

*Synthesis of (R/S)-(±)-[6a-^{14}C]apomorphine dimethyl ether (**17**).* A solution of 1-(3,4-dimethoxy-2-nitrobenzyl)-2-methyl-1,2,3,4-tetrahydro[1-^{14}C]isoquinoline (**3**) (306 mCi, 55 mCi/mmol) in ethanol (140 ml) and concentrated hydrochloric acid (63 ml) was cautiously treated with zinc dust (7.9 g, 120 mmol) and the reaction heated under reflux for 1 hour. On complete consumption of the starting material (by TLC analysis on silica eluting with ethyl acetate), the reaction was cooled and basified with 20% aqueous sodium hydroxide and then extracted with dichloromethane (500 ml). The organics were washed with water, saturated sodium bicarbonate solution and brine then dried over [$MgSO_4$], filtered and evaporated *in vacuo* to give a dark residue. The residue was dissolved in 10% aqueous sulphuric acid (47.5 ml) and cooled to 0°C. A solution of sodium nitrite (638 mg, 9.38 mmol) in water (24 ml) was then added dropwise. The reaction was stirred for 30 min at −3°C to give the diazonium salt suspension. Urea was added to destroy the excess nitrous acid. Copper(I)oxide powder (638 mg, 9.38 mmol) was added to the diazonium salt suspension and the reaction was left to warm to ambient temperature. The reaction was basified with 2 N sodium hydroxide and the product extracted into ethyl acetate (2×200 ml) and dichloromethane (1×200 ml). The combined organic extracts were dried over [$MgSO_4$], filtered and concentrated *in vacuo* to give the crude product. This was purified by preparative HPLC on a Dynamax silica column eluting with 2% methanol in dichloromethane to afford a pale yellow oil (*R*/*S*)-(±)-[6a-^{14}C]apomorphine dimethyl ether (**17**). ^{1}H-NMR ($CDCl_3$, 200 MHz) $\delta = 8.24$ (d, 1H, $J = 7.9$ Hz), 7.28 (t, 1H, $J = 7.8$ Hz), 7.08 (d, 1H, $J = 7.5$ Hz), 7.02 (d, 1H, $J = 8.2$ Hz), 6.85 (d, 1H, $J = 8.2$ Hz), 4.99 (s, 3H), 3.90 (s, 3H), 3.70 (s, 3H), 3.35–2.40 (bm, 7H).

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*Chiral resolution of (R/S)-(±)-[6a-^{14}C]apomorphine dimethyl ether (**17**).* (*R*/*S*)-(±)-[6a-^{14}C]Apomorphine dimethyl ether (**17**) was separated by normal phase preparative HPLC using a cellulose-based chiral column (Chiralcel OD) eluting with hexane/propan-2-ol/dimethylamine (950:50:5). This gave (*R*)-(-)-[6a-^{14}C]apomorphine dimethyl ether (**2**) (49 mCi, 55 mCi/mmol) which co-eluted with authentic (6a*R*)-(-)-apomorphine dimethyl ether and the other unwanted enantiomer (*S*)-(+)-[6a-^{14}C]apomorphine dimethyl ether (**18**) (34 mCi, 55 mCi/mmol).

*Synthesis of (R)-(-)-[6a-^{14}C]apomorphine (**1**).* To a solution of (*R*)-(-)-[6a-^{14}C]apomorphine dimethyl ether (**2**) (42 mCi, 55 mCi/mmol) in anhydrous dichloromethane (42 ml), boron tribromide (1.0 ml, 1.0 mmol, 1.0 M solution in dichloromethane) was added. The reaction was stirred at ambient temperature for 90 min. Methanol (3 × 50 ml) was added and the solvent removed by evaporation *in vacuo*. The crude product was purified on a reverse phase PLRP-S column eluting the product with a mixture of acetonitrile/aqueous hydrochloric acid. The solvent was evaporated *in vacuo* to give the final product (*R*)-(-)-[6a-^{14}C]apomorphine (**1**) (32 mCi, 76% yield) as a white crystalline hydrochloride salt. Radiochemical purity: 98.4%, Chiral purity: 99.6% [Chiralcel OD-R, 0.05 M sodium perchlorate buffer pH 2.0/acetonitrile (65:35)]; LRMS positive ion EI: [M^+] 36/38 as expected for the hydrochloride counter ion and by DCI-MNH_4^+ gave a pseudo molecular ion $[MH]^+$ bundle at $m/z = 268/270$ confirming the structure as a single carbon-14 label. Specific activity 55 mCi/mmol; ^{1}H-NMR (D_2O, 400 MHz) $\delta = 8.10$ (d, 1H, $J = 7.7$ Hz), 7.32 (t, 1H, $J = 7.8$ Hz), 7.07 (d, 1H, $J = 7.6$ Hz), 6.61 (m, 2H), 3.75 (m, 1H), 3.57 (bm, 1H), 3.16–3.09 (m, 2H), 2.98 (s, 3H), 2.88–2.81 (m, 2H), 2.49 (m, 1H).

Conclusion

A wide array of experimental conditions (i.e. temperature and duration of heating) and condensing agents have been used to facilitate the Bischler–Napieralski cyclodehydration on 3,4-dimethoxy-2-nitrophenyl-*N*-phenethyl [carboxyl-^{14}C]acetamide (**5**). The dehydrating agent phosphorous oxychloride, either alone or in an inert solvent (i.e. toluene), was found to be a mild reagent and gave exclusively 1-(3,4-dimethoxy-2-nitrobenzal)-1,2,3,4-tetrahydro [1-^{14}C]isoquinoline (**15**). In our case we found utilizing a mixture of phosphorous pentoxide and phosphorous oxychloride in refluxing toluene gave better results in generating 1-(3,4-dimethoxy-2-nitrobenzyl)dihydro [1-^{14}C]isoquinoline (**4**) than would be when used separately. This cyclodehydration step is important in the synthesis of the aporphine alkaloids. When the substituted aromatic ring to which ring closure is to occur is not strongly activated as in (**5**) neutral compounds have been isolated. These neutral

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products have been assigned various structures containing an acetylene (**14**), ketamine (**13**) and anthranil (**11**) moieties. Leading from the work of Neumeyer,[5] Hey and Lob [11] we have modified the synthesis to enable the incorporation of a single carbon-14 radiolabel in (*R*)-(-)-[6a-^{14}C]apomorphine (**1**) and in the course of this work we have identified the neutral compound to be 3-(6,7-dimethoxyanthranil)-dihydro[1-^{14}C]isoquinoline (**12**). This synthetic methodology can be applied to the synthesis of other carbon-14 aporphines which would be useful in ADME and pharmacokinetic studies on neurodegenerative diseases.

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