Journal of Labelled Compounds and Radiopharmaceuticals
*J Label Compd Radiopharm* 2007; **50**: 1211–1214.
Published online 19 September 2007 in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1448

JLCR

Research Article

# The synthesis of tritiated (*R*)-2-methoxy-*N*-*n*-propyl-nor-apomorphine (MNPA)

**JONAS MALMQUIST***, **SUSANNE OLOFSSON** and **PETER STRÖM**

AstraZeneca R&D Södertälje, Medicinal Chemistry, S-151 85 Södertälje, Sweden

*Received 11 June 2007; Revised 13 July 2007; Accepted 20 July 2007*

**Abstract:** A method for the preparation of [$^3$H]-MNPA ((*R*)-2-methoxy-*N*-*n*-propyl-norapomorphine) has been developed addressing the regioisomer problem as well as the oxidation problem. (*R*)-2, 10, 11-trihydroxy-*N*-*n*-propyl-norapomorphine was protected with 10, 11-dibenzyl or 10, 11-acetonide. The pure precursor was then methylated using [$^3$H]-methyliodide. The product was isolated after deprotection and high-pressure liquid chromatography (HPLC) purification. Ascorbic acid was used as an antioxidant in the HPLC eluent and the stock solution. Characterization of the intermediates and products with $^3$H- and $^1$H-NMR was performed. A specific activity of 3.1 TBq/mmol (83.8 Ci/mmol) and 98.9% purity was obtained. Copyright © 2007 John Wiley & Sons, Ltd.

**Keywords:** [$^3$H]-MNPA; ascorbic acid; $^3$H NMR

## Introduction

There is a current interest in the $D_2$ receptor agonist (*R*)-2-methoxy-*N*-*n*-propyl-norapomorphine (MNPA) to quantify dopamine (DA) receptor levels within the brain. MNPA has recently been evaluated as the $^{11}$C-labeled derivative.[1,2] The method used for the incorporation of the radiolabel was direct methylation on (*R*)-2, 10, 11-trihydroxy-*N*-*n*-propyl-norapomorphine (TPNA, **2**) using [$^{11}$C]-methyl iodide. Repeating that protocol with methyl iodide and [$^3$H]-methyl iodide has been unfruitful. The only isolable mono-methyl derivative was methylated on the catechol-ring in the 10-position (Figure 1) differing very little from MNPA, with methyl in the 2-position, in chromatographic systems (0.2 min on a C-18 column with a 20 min gradient). The di- and tri-methylated derivatives were formed to a large extent, although only 0.1 equivalents of the methylating agent was used. Performing the synthesis according to the original synthesis of MNPA[3] failed in the deprotection of 10,11-methylene-MNPA using boron trichloride. On a small scale (2 μmol) the *O*-methyl in the 2-position was lost, as well, to give **2**. Our original plan was to protect the 10- and 11-positions of 2 as *O*-benzyl ethers; however, deprotection of **4** yielded [$^3$H]-MNPA **(1)**, which after purification was only 86–90% pure. Alternatively, the use of the acetonide **5**[4] allowed the preparation of [$^3$H]-MNPA in adequate purity.

## Results and discussions

TNPA·HBr (**2**) was benzylated with two equivalents benzyl bromide and excess potassium carbonate in

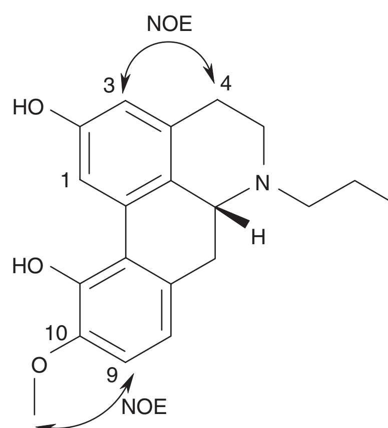


**Figure 1** $^1$H NMR (600 MHz, $CD_3OD$, 0.6 mM, TFA-salt) δ ppm 1.12 (t, $J = 7.40$ Hz, 3 H, $-CH_3$) 1.80 (m, 1 H) 1.96 (m, 1 H) 2.77 (t, $J = 13.3$ Hz, 1 H) 3.06 (m, 3 H) 3.40 (dd, $J = 13.2$, 4.0 Hz, 2 H) 3.61 (td, $J = 12.3$, 5.3 Hz, 1 H) 3.88 (m, 1 H) 3.91 (s, 3 H, O–CH3) 4.29 (dd, $J = 14.1$, 3.4 Hz, 1 H) 6.60 (d, $J = 2.4$ Hz, 1 H) 6.88 (m, 2 H, H-8 and H-9) 7.98 (d, $J = 2.4$ Hz, 1 H).

*Correspondence to: J. Malmquist, AstraZeneca R&D Södertälje, Medicinal Chemistry, S-151 85 Södertälje, Sweden. E-mail: Jonas.Malmquist@AstraZeneca.com

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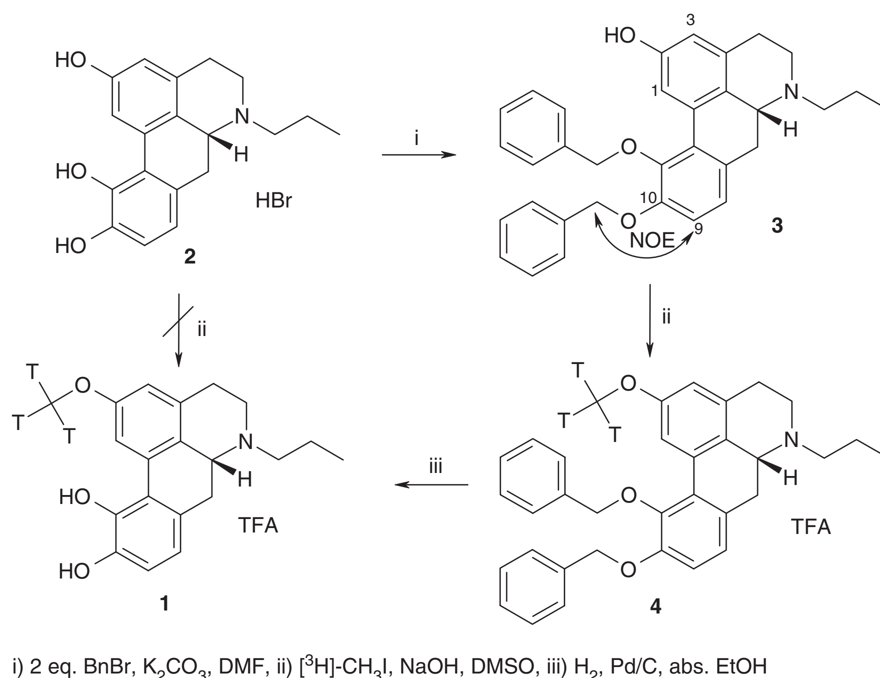
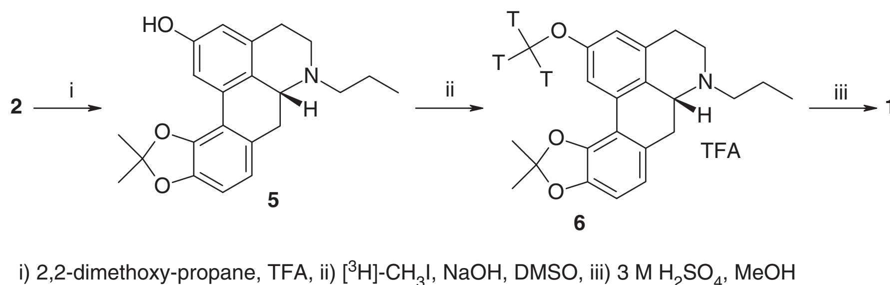



**Scheme 1**

DMF to give **3** in 49% yield. Final purification was performed to obtain pure material. This was crucial to eliminate methylation in an unwanted position. The structure of **1** was confirmed by NMR-experiments where nOe was detected from H-9 to the benzylic protons in the 10-position (Scheme 1). There were no nOes detected from H-1 and H-3. On a 20 μmol scale trial experiment several benzylated derivatives were detected. The chromatographic properties of **3** and 10,*N*-dibenzyl-TNPA were similar, but the individual structures were easily identified by nOe-experiments. The 2-position was very unreactive in these reactions.

Methylation of **3** was performed in dimethyl sulfoxide (DMSO) using 5 M sodium hydroxide as a base and [$^3$H]-methyl iodide at room temperature for 100 min. Compound **4** was cleanly methylated in the 2-position and no quarternization of the nitrogen was observed at any significant amount. The remaining **3** was separated by chromatography on high-pressure liquid chromatography (HPLC). The final deprotection of **4** was performed by hydrogenolysis in ethanol over 1 h, by the action of 10% palladium on charcoal at atmospheric pressure. The target compound **1** was isolated in good yield. The incorporation of tritium was 3.1 TBq/mmol, and the purity was >90% according to $^3$H-NMR and 86% according to HPLC analysis. The presence of an impurity close to [$^3$H]-MNPA prevented a further increase in the purity. Alternatively, acetonide **5**[4] was used for the methylation with [$^3$H]-methyl iodide.

Methylation of very pure **5** was performed in the same way as in the preparation of **4** (Scheme 2). Compound **6** was cleanly methylated in the 2-position and no quarternization of the nitrogen was observed at any significant amount. The remaining **5** was separated by chromatography on HPLC. The final deprotection of **5** was performed by hydrolysis in 3 M $H_2SO_4$.




**Scheme 2**

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DOI: 10.1002.jlcr

The target compound **1** was isolated in good yield with a specific activity of 3.1 TBq/mmol. Ascorbic acid, 0.01% added in the HPLC-eluent, was used as an antioxidant. During storage, **1** remained at the original purity for more than 1 month and dropped to 25% after 1 year of storage at $-20^\circ$C.

## Experimental

### General methods

All solvents used were of analytical grade and commercially available. Anhydrous solvents were routinely used for reactions. Reactions were typically run under an inert atmosphere of nitrogen or argon.

$^3$H and $^1$H spectra were recorded on a Bruker DRX600 NMR Spectrometer, operating at 640 MHz for tritium and at 600 MHz for proton, equipped with a 5 mm $^3$H/$^1$H SEX probehead with Z-gradients. $^1$H decoupled $^3$H spectra were recorded on samples dissolved in $CD_3OD$. For $^3$H NMR spectra referencing, a ghost reference frequency was used, as calculated by multiplying the frequency of internal TMS in a $^1$H spectrum with the Larmor frequency ratio between $^3$H and $^1$H (1.06663975), according to the description in Al-Rawi *et al.*[5] $^1$H spectra were referenced to TMS which was set to 0 ppm.

Mass spectra were recorded on a Waters LCMS consisting of an Alliance 2795 (LC), Waters PDA 2996, and ELS detector (Sedex 75) and a ZMD single quadrupole mass spectrometer. The mass spectrometer was equipped with an electrospray (ES) ion source operated in a positive or negative ion mode. The capillary voltage was 3 kV and cone voltage was 30 V. The mass spectrometer was scanned between $m/z$ 100 and 600 with a scan time of 0.7 s. The column temperature was set to 40°C. The Diode Array Detector was scanned from 200 to 400 nm. The temperature of the ELS detector was adjusted to 40°C and the pressure was set to 1.9 bar. For LC separation a linear gradient was applied starting at 100% A (A: 10 mM $NH_4OAc$ in 5% MeCN) and ending at 100% B (B: MeCN) after 4 min. The column used was a X-Terra MS C8, $3.0 \times 50$; 3.5 µm (Waters) run at 1.0 ml/min.

High-resolution mass spectra were recorded on an LCMS composed of an Agilent HP1100 LC system and a Micromass QTOF micro-quadrupole orthogonal acceleration time-of-flight tandem mass spectrometer. The Agilent HP1100 system consisted of G1379A Micro Vacuum Degasser, G1312A Binary Pump, G1367A Well plate auto-sampler, G1316A Thermostatted Column Compartment and G1315B Diode Array Detector. The Diode Array Detector was scanned from 210 to 300 nm, step and peak widths were set to 2 nm and 0.05 min, respectively. The column used was a Gemini $3.0 \times 50$ mm 3 um (Phenomenex) run at a flow rate of 1.0 ml/min. The column temperature was set to 40°C. A linear gradient was applied starting at 100% A (A: 10 mM HCOOH) and ending at 100% B (B: ACN) after 8 min. The flow was split 1:4 prior to the ion source. A volume of 10 µl of the sample was injected on the column. The sample was analyzed in full scan ES+ continuum mode. The ion source parameters such as capillary voltage and cone voltage were set to 3300 and 20 V, respectively. The mass range was $m/z$ 90–1050 using a scan time of 0.48 s. Leucine Enkephaline $m/z$ 556.2771 (0.3 ng/ml) was used as Lock mass. The collision energy in the MS/MS experiment operated in the centroid mode was set to 20, 30 V, $m/z$ 90–1050 and a scan time of 1 s.

HPLC analyses were performed on an Agilent HP1100 system consisting of a G1379A Micro Vacuum Degasser, a G1312A Binary Pump, a G1367A Well plate auto-sampler, a G1316A Thermostatted Column Compartment and a G1315B Diode Array Detector. The column used was an X-Terra MS, Waters ($3.0 \times 100$ mm, 3.5 µm). The column temperature was set to 40°C and the flow rate to 1.0 ml/min. The Diode Array Detector was scanned from 210 to 300 nm, step and peak widths were set to 2 nm and 0.05 min, respectively. A linear gradient was applied, starting at 100% A (A: 10 mM $NH_4OAc$ in 5% MeCN) and ending at 100% B (B: MeCN), in 6 min. Alternatively HPLC analyses were performed on an Agilent 1100 HPLC system with a binary pump, auto-injector, DAD and column oven, coupled in series with a Packard Radiomatic Flow Scintillator 525TR, equipped with a solid scintillator (SolarScint) cell with a volume of 33 µl. The column used was a Xterra MS Waters(C8, $3 \times 100$ mm, 3 µm). The column temperature was set to 40°C and the flow rate to 0.5 ml/min. A linear gradient was applied, starting at 100% A (A: 0.1% TFA) and ending at 95% B (B: MeCN), in 12 min.

Preparative chromatography was run on a Gilson 305 with a Gilson UV/VIS-151 and a RAYTEST Ramona equipped with a Kromasil column (C18, 5 µm, $10 \times 250$ mm or C8, 7 µm, $20 \times 250$ mm) using isocratic systems with MeCN/0.1% trifluoroacetic acid in MilliQ Water.

Liquid scintillation analysis was performed on a PACKARD TRI-CARB 2900TR.

Thin layer chromatography (TLC) was performed on Merck TLC-plates (Silica gel 60 $F_{254}$) and UV-light (254 nm) was used to visualize the spots. Flash column chromatography was performed on Silica gel 60. Typical solvents used for flash column chromatography were a mixture of dichloromethane, methanol and ammonia.

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DOI: 10.1002.jlcr

**(*R*)-2-hydroxy-10, 11-dibenzyl-*N*-*n*-propyl-nor-apomorphine (3)**. TPNA·HBr (88 mg, 0.22 mmol) was mixed with potassium carbonate (270 mg, 2 mmol) in DMF (2 ml, degassed with Ar). Benzyl bromide (53 ul, 0.44 mmol) was added and the mixture was stirred for 17 h at room temperature. The reaction mixture was acidified with HCl (2 M, aq.) and purified on a preparative HPLC. This afforded **3** as an off-white solid (57 mg, 49%). $^1$H NMR ($CD_3OD$, 1.6 mM) δ 0.99 (t, $J = 7.4$ Hz, 3 H, $CH_3$) 1.57 (m, 1 H) 1.69 (m, 1 H) 2.39 (t, $J = 13.4$ Hz, 1 H) 2.47 (m, 1 H) 2.72 (d, $J = 16.3$ Hz, 1 H, H-5) 2.95 (m, 1 H) 3.02–3.11 (m, 2 H) 3.17 (dd, $J = 13.3$, 3.1 Hz, 1 H) 3.21 (dd, $J = 11.6$, 5.5 Hz, 1 H) 4.72 (d, $J = 10.5$ Hz, 1 H, 11-CH2) 4.83 (d, $J = 10.5$ Hz, 1 H, 11-CH2) 5.12 (d, $J = 11.5$ Hz, 1 H, 10-CH2) 5.17 (d, $J = 11.5$ Hz, 1 H, 10-CH2) 6.54 (d, $J = 2.1$ Hz, 1 H, H-3) 7.00 (m, 2 H, H-8 and H-9) 7.20 (m, 5 H, 11-Ph) 7.34 (d, $J = 7.3$ Hz, 1 H, 10-Ph) 7.38 (t, $J = 7.4$ Hz, 2 H, 10-Ph) 7.50 (d, $J = 7.3$ Hz, 2 H, 10-Ph) 7.74 (d, $J = 2.4$ Hz, 1 H, H-1). HRMS ($[M+1]^+$) calc. $m/z$ 492.2460, found $m/z$ 492.2485.

**(*R*)-2-[$^3$H]-methoxy-10, 11-dibenzyl-*N*-*n*-propyl-nor-apomorphine (4)**. Compound **3** (1.3 mg, 2.6 μmol) was mixed with sodium hydroxide (7 μl, 5 M) in DMSO (400 μl). [$^3$H]-methyl iodide (1.85 GBq, 3.0 TBq/mmol, 37 GBq/ml toluene) was added and the reaction was stirred for 100 min at room temperature. The mixture was flash chromatographed on a silica-column with dichloromethane/methanol/ammonia (38%, aq.) 100/10/1. The solvents were stripped off except for residual DMSO. The solution was purified on a semi-preparative HPLC using 80% MeCN in 0.1% trifluoroacetic acid to give 900 MBq of **4**. LC-MS $m/z$ 512.08 ($[M+1]^+$).

**(*R*)-2-[$^3$H]-methoxy-10, 11-acetonido-*N*-*n*-propyl-nor-apomorphine (6)**. Compound **5** [4] (0.88 mg, 2.5 μmol) was treated in the same way as in the preparation of **4**. The flash-chromatographed product was purified on a semi-preparative HPLC using 65% MeCN in 0.1% trifluoroacetic acid to give an almost quantitative yield of **4**. LC-MS $m/z$ 372.05 ($[M+1]^+$).

**(*R*)-2-[$^3$H]-methoxy-*N*-*n*-propyl-nor-apomorphine (1, $^3$H-MNPA)**. Route 1: **4** (900 MBq) was mixed with 10% Pd/C (0.7 mg) in ethanol (1 ml). The mixture was connected to hydrogen (1 atm.) and stirred for 1 h. The catalyst was filtered off and the solution was concentrated by a stream of $N_2$. The residue was purified on a semi-preparative HPLC using 22.5% MeCN in 0.1% TFA(aq) as eluent to give 615 GBq of **1** at 3.1 TBq/mmol and a purity of 86%. Route 2: **6** (1.8 GBq) was mixed with methanol (500 μl) and sulfuric acid (250 μl, 3M). The mixture was heated to 60°C for 17 h. The mixture was concentrated by a stream of $N_2$ (g) and dissolved in the HPLC-eluent (250 μl, 35% MeCN in 0.1% trifluoroacetic acid with 0.01% ascorbic acid) and purified on a semi-preparative HPLC. The pooled fractions were concentrated by a stream of $N_2$ (g), dissolved in DMSO and repurified using 25% MeCN in 0.1% trifluoroacetic acid with 0.01% ascorbic acid as the eluent. An amount of 1.0 GBq of **1** was isolated in a specific activity of 3.1 TBq/mmol and 98.9% purity. The product was stored in absolute ethanol at 39 MBq/ml and remained at the same purity for more than 1 month. $^1$H NMR ($CD_3OD$, 370 MBq/ml) δ 1.12 (t, $J = 7.3$ Hz, 3 H) 1.79 (m, 1 H) 1.96 (m, 1 H) 2.75 (t, $J = 13.4$ Hz, 1 H) 3.05 (m, 1 H) 3.91 (dd, $J = 12.8$, 6.2 Hz, 1 H) 4.32 (d, $J = 13.5$ Hz, 1 H) 6.74 (m, 3 H, Ar-H) 8.09 (d, $J = 2.3$ Hz, 1 H, H-1). Some signals are obscured by solvent signals. Impurity signals are not reported. $^3$H NMR ($CD_3OD$, 370 MBq/ml) δ 3.77 (s, 3 H, $OCT_3$). LC-MS $m/z$ 332.00 ($[M+1]^+$).

## Conclusions

A working method for the preparation of [$^3$H]-MNPA has been developed addressing the regioisomer problem as well as the oxidation problem.

## REFERENCES


1. Finnema SJ, Seneca N, Farde L, Shchukin E, Sóvágó J, Gulyás B, Wikström HV, Innis RB, Neumeyer JL, Halldin C. *Nucl Med Biol* 2005; **32**: 353–360.
2. Seneca N, Finnema SJ, Farde L, Gulyás B, Wikström HV, Halldin C, Innis RB. *Synapse* 2006; **59:** 260–269.
3. Gao Y, Baldessarini RJ, Kula NS, Neumeyer JL. *J Med Chem* 1990; **33**: 1800–1805.
4. (*R*)-10,11-acetonido-*N*-*n*-propylnorapomorphine (**5**) was a kind gift from Prof. Christer Halldin, Karolinska Institute.
5. Al-Rawi JMA, Bloxsidge JP, O'Brien C, Caddy DE, Elvidge JA, Jones JR, Evans EA. *J Chem Soc Perkin Trans II* 1974; 1635.


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DOI: 10.1002.jlcr