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**Research Article**

# Synthesis of $^{3}$H- and $^{14}$C-labeled AR-A000002, a new and selective 5-HT$_{1B/1D}$ ligand

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## Summary

AR-A000002 is a novel and selective high-affinity 5-HT$_{1B/1D}$ receptor antagonist. The compound has been shown to enhance 5-HT turnover in the guinea pig brain *in vivo* and to increase the extracellular concentration of 5-HT and the metabolite 5-hydroxyindoleacetic acid (5-HIAA) in guinea pig frontal cortex. The observed effects suggest that the compound could be used for the treatment of affective disorders.

The syntheses of labeled AR-A000002 analogues as needed for the further pharmacological evaluation of this selective 5-HT$_{1B/1D}$ antagonist, are described. 

**Key Words:** 5-HT$_{1B}$; 5-HT$_{1D}$; $^{14}$C; $^{3}$H; Rosenmund–von Braun

## Introduction

A disturbed serotonergic signaling has been implicated in the pathogenesis of psychiatric diseases such as anxiety and depression. The release of 5-HT from the raphe region in the brain is mainly regulated by the 5-HT$_{1A}$ receptor located on the soma and dendrites of the serotonergic neurons, and by the pre-synaptic 5-HT$_{1B}$ receptor located at the nerve terminal.[1] Inhibition of the pre-synaptic 5-HT$_{1B}$ receptor gives an increased availability of serotonin in the synaptic cleft, and potentially an alleviation of, e.g. symptoms associated with anxiety and depression. The 5-HT$_{1B/1D}$ antagonist AR-A000002 (**1**) is currently being developed for testing of this hypothesis in man. In order to delineate the biological properties of this compound, a labeled version of the material was needed.

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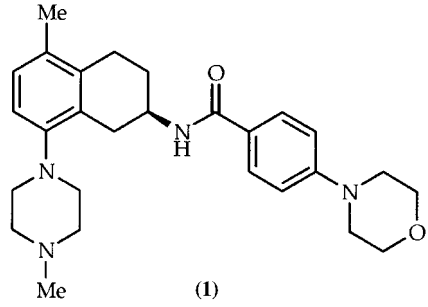
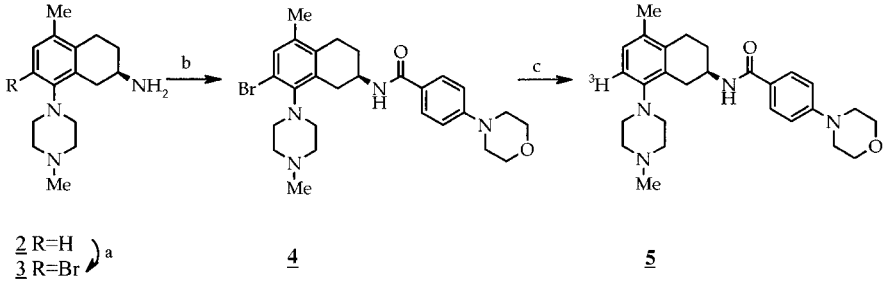



(1)

## Results and discussion

[$^3$H]AR-A000002 (**5**) with a specific activity of 833 GBq/mmol, was obtained in three steps from (*R*)-2-amino-5-methyl-8-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalene.[2] (*R*)-2-Amino-5-methyl-8-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalene was selectively brominated in the 7-position. The primary amine **3** was acylated with 4-morpholinobenzoic acid and 1,1′-carbonyldiimidazole[3] (CDI) in DMF affording the brominated precursor **4**. Catalytic debromination in tritium gas gave the desired product **5** (Scheme 1). $^3$H NMR at 426 MHz, giving a doublet at δ 7.0 ppm ($J = 8$ Hz), confirmed the incorporation of the tritium atom.



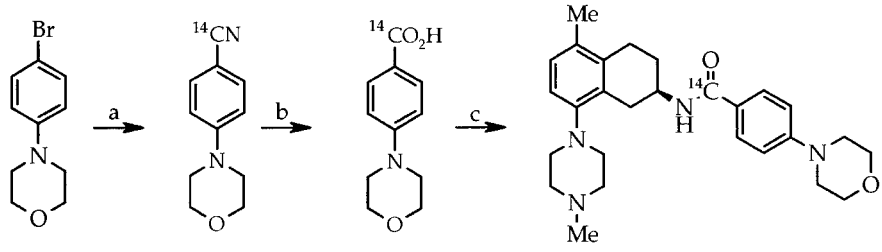

**Scheme 1. (a) $Br_2$ (b) 4-morpholinobenzoic acid/CDI (c) $^3H_2$/PdO**

$^{14}$C-Labeled 4-cyanophenylmorpholine was synthesized via the Rosenmund–von Braun[4] reaction of 4-bromophenylmorpholine[5] with freshly prepared Cu$^{14}$CN in *N*-methylpyrrolidone at elevated temperature (Scheme 2). Acidic hydrolysis of the nitrile afforded labeled 4-morpholinobenzoic acid. The benzoic acid-derivative was then condensed with **2** as above, to give $^{14}$C-labeled AR-A000002 with a specific activity of 2057 MBq/mmol.

## Experimental

Tritium gas was purchased from RC Tritec AG, Switzerland, and potassium [$^{14}$C]cyanide was purchased from Amersham, England. Radiochemical purity




7 8 9

**Scheme 2. (a) $Cu^{14}CN$ (b) HCl/HOAc (c) 2/CDI**

was determined by thin layer chromatography (TLC) using a Bioscan System 200 Imaging Scanner or with a Packard 500 TR Flow Scintillation Analyzer connected to a HPLC instrument. TLC was performed on TLC precoated plates, silica gel60 $F_{254}$ (Merck) and column chromatography were performed on silica gel 60 (230–400 mesh ASTM, Merck). Gas chromatographic analysis was performed on a Hewlett Packard 5890 apparatus equipped with a 10 m × 0.15 mm fused silica capillary column coated with 0.12 μm CP-Sil 5. Radioactivity was measured in a Packard 1000 liquid scintillation spectrometer using Packard Ultima Gold as a counting medium. $^{3}H$ NMR, $^{1}H$ and $^{13}C$ NMR spectra were obtained on either a Varian 300 or a 400 MHz NMR spectrometer. The samples were dissolved in $CDCl_3$ and the $^{14}C$- and $^{3}H$-labeled samples were all run in Teflon®-tubes (Wilmad Glass Co., Inc. USA). Mass spectra were recorded on a Finnigan Mat SSQ 710 instrument operated at an electron energy (EI) of 70 eV and connected to a GC HP5890 inlet or a Finnigan Mat SSQ 7000 TSP instrument. Specific rotations were determined on an AA-10 from Optical Activity Ltd. The organic extracts of crude products were dried over anhydrous sodium sulfate.

*(R)-2-Amino-7-bromo-5-methyl-8-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalene,* **3**

To a solution of (*R*)-2-amino-5-methyl-8-(4-methylpiperazin-l-yl)-1,2,3,4-tetrahydronaphtalene[2] (**2**) (280 mg, l.l mmol) in ethanol (12 ml) was added HCl (2 M, 14 ml) followed by the dropwise addition of bromine (58 μl, 1.1 mmol). The reaction mixture was stirred for 1 h, cooled on an ice-bath, then made alkaline with NaOH (2 M) and extracted with diethyl ether. The organic phase was dried, filtered, evaporated *in vacuo*, and purified by chromatography ($SiO_2$, $CHCl_3$/MeOH/$NH_4OH$ 95:5:0.5) affording the title compound (170 mg, 47%) as colorless oil. $[\alpha]_D^{21} = +2°$(*c* 1, $CHCl_3$), $^{1}H$ NMR (300 MHz), δ 7.19 (s, 1 H), 3.65–3.58 (m, 1 H), 3.53–3.43 (m, 1 H), 3.22 (dd, $J = 17$, 5 Hz, 1 H), 3.14–3.02 (m, 1 H), 2.91–2.80 (m, 2 H), 2.78–2.31 (m, 9 H),

2.35 (s, 3 H), 2.14 (s, 3 H), 2.07–1.95 (m, 1 H), 1.63 (br s, 2 H), 1.68–1.45 (m, 1 H), $^{13}$C NMR (75 MHz) δ 145.2, 137.5, 135.4, 134.8, 132.1, 120.4, 56.0, 55.9, 48.6, 46.9, 46.5, 36.9, 31.9, 26.0, 18.9, MS 337/339 ($M^+$).

*(R)-N-[(7-Bromo-1,2,3,4-tetrahydro-5-methyl-8-(4-methylpiperazin-1-yl)-2-naphthyl]-4-morpholinobenzamide,* **4**

A solution of 4-morpholinobenzoic acid (115 mg, 0.55 mmol) and 1,1′-carbonyldiimidazole (98 mg, 0.603 mmol) in DMF (20 ml) was heated at 80°C for 30 min. The mixture was cooled, and (*R*)-2-amino-7-bromo-5-methyl-8-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalene (**3**) (170 mg, 0.50 mmol) in DMF (5 ml) was added and stirring was continued at room temperature for 24 h. The solvent was then evaporated *in vacuo* and the residue was extracted with $CH_2Cl_2$. The organic phase was dried, filtered, evaporated and the crude solid was purified on a column ($SiO_2$, $CHCl_3$/EtOH/$NH_4OH$ 100:3:0.1) affording the title compound (172 mg, 65%) as white crystals, mp 263–264°C, $[\alpha]_D^{21} = -70°$(*c* 0.25, $CHCl_3$) $^1$H NMR (300 MHz) δ 7.71 (d, $J = 9$ Hz, 2 H), 7.23 (s, 1 H), 6.89 (d, $J = 9$ Hz, 2 H), 6.09 (d, $J = 8$ Hz, 1 H), 4.42–4.37 (m, 1 H), 3.86 (t, $J = 5$ Hz, 4 H), 3.63–3.52 (m, 2 H), 3.34–3.2 (m, 1 H), 3.24 (t, $J = 5$ Hz, 4 H), 2.87–2.81 (m, 2 H), 2.75–2.60 (m, 4 H), 2.4–2.3 (m, 1 H), 2.35 (s, 4 H), 2.17 (s, 4 H), 1.84–1.71 (m, 1 H), $^{13}$C NMR (75 MHz) δ 166.6, 153.4, 145.4, 136.6, 135.7, 134.7, 132.6, 128.4, 125.0, 120.6, 114.1, 66.6, 55.9, 55.8, 48.5, 48.4, 48.0, 46.4, 45.2, 33.4, 28.4, 25.5, 19.0.

*(R)-N-[(1,2,3,4-Tetrahydro-7-[$^3$H]-5-methyl-8-(4-methylpiperazin-1-yl)-2-naphthyl]-4-morpholinobenzamide,* **5**

A solution of (*R*)-*N*-[(7-bromo-1,2,3,4-tetrahydro-5-methyl-8-(4-methylpiperazin-1-yl)-2-naphthyl]-4-morpholinobenzamide (**4**) (2.46 mg, 4.66 μmol) and palladium oxide (4.07 mg) in DMF (0.3 ml) was stirred in an atmosphere of carrier-free $^3H_2$ in a tritium manifold system (RC TRITEC AG, Switzerland). After 18 h the reaction mixture was freeze-degassed, filtered and the solvent distilled *in vacuo*. Labile tritium was removed by repeated lyophilization from ethanol, leaving a residue containing 6.4 GBq of the desired product. The radiochemical purity of the product, determined by TLC ($SiO_2$, $CHCl_3$/EtOH/$NH_4OH$ 95:5:0.1) was 70%. A portion of crude material above (ca 2.2 GBq) was purified by preparative HPLC (Phenomex C18 column 300 × 3.9 mm, phosphate buffer (pH = 3)/acetonitrile 70:30) with UV detection at 290 nm. The product obtained (992 MBq) had a specific radioactivity of 833 GBq/mmol determined by MS and a radiochemical purity of 98% in the HPLC system above. $^3$H NMR (426 MHz) δ 7.0 (d, $J = 8$ Hz).

## 4-[$^{14}$C]Cyanophenylmorpholine, 7

To a stirred aqueous solution of copper(II)sulfate (1.33 M, 1.0 ml) sodium bisulfite solution (1.33 M, 0.75 ml) was added dropwise at 47°C . After 2 min, potassium [$^{14}$C]cyanide (1.85 GBq, 0.91 mmol) in water (1 ml) was added to form a white precipitate. The mixture was stirred at 47–49 °C for 10 min, cooled and centrifuged (10 min, 4000 r/m). The precipitate was washed with water (2 × 1 ml), ethanol (2 × 1 ml), dried *in vacuo* to yield copper(I)[$^{14}$C]cyanide as a white powder (79 mg, 86%). To the powder 4-bromophenylmorpholine (**5**) (243 mg, 1.0 mmol) in *N*-methylpyrrolidone (1.4 ml) was added and the reaction mixture was heated at 170–175 °C for 135 min. After cooling, NaOH (2 M, 3 ml) was added and the mixture filtered. The remainder was extracted in ethyl acetate/diethyl ether (4 × 1.5 ml). The combined organic phase was then washed with water (2 × 1.5 ml), brine (2 × 1.5 ml), dried, filtered and evaporated *in vacuo*, to give the title compound (230 mg) as a yellow oil (GC purity ca 64%) which was used without further purification in the next step.

### 4-*Morpholino-1-[$^{14}$C]-benzoic Acid,* 8

The crude oil from above (230 mg) was dissolved in HCl (20%, 3 ml) and acetic acid (2 ml) and then the mixture was heated at reflux overnight. The solution was taken to dryness and the residue dissolved in $NH_4OH$ (2 M, 6 ml) and washed with diethyl ether (3 × 1.5 ml). The alkaline solution was acidified with HCl (5 M) to pH 2 and the precipitate centrifuged and collected. The precipitate was crystallized from water (0.5 ml) and ethanol (3 ml) to give after drying *in vacuo*, the title compound (66 mg, 35% from **7**) as white crystals. The radiochemical purity determined by TLC ($SiO_2$, $CH_2Cl_2$/MeOH/acetic acid 90:6:3) was 99%. MS/TSP m/z: 208 ($M^+$).

### *(R)-N-[1,2,3,4-tetrahydro-5-methyl-8-(4-methylpiperazin-1-yl)-2-naphthyl]-4-morpholino-1-[$^{14}$C]-benzamide,* 9

A solution of the above acid (66 mg, 0.32 mmol) and 1,1′-carbonyldiimidazole (56 mg, 0.33 mmol) in DMF (0.65 ml) was heated at 70°C for 30 min. The mixture was cooled, and (*R*)-2-amino-5-methyl-8-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalene (**2**) (79 mg, 0.30 mmol) in DMF (0.3 ml) added, and stirring was continued overnight at room temperature. The precipitated product was centrifuged as above, and the residue washed with diethyl ether and dried *in vacuo*, affording 47 mg of crude material. The supernatant was taken to dryness, and NaOH (2 M, 3 ml) added and the mixture extracted with $CH_2Cl_2$ (4 × 1 ml).

The combined organic phase was washed with water (2 × 1 ml), brine (1.5 ml), dried, filtered and evaporated to give another 79 mg of crude **9**. The

solid products were crystallized from methanol (0.9, then 1.2 ml) to give first 35 and then 48 mg of white crystals. The material was combined and recrystallized from methanol to give the title compound (58.9 mg, 42%) as white crystals. The radiochemical purity determined by TLC ($SiO_2$, $CHCl_3$/EtOH/$NH_4OH$ 90:10:0.1) was 98% and by HPLC 97%. The chemical purity (HPLC) was 99%. The specific radioactivity was 2057 MBq/mmol measured by liquid scintillation counting. $^1$H NMR (300 MHz): δ 7.77 (d, $J$ = 8.7 Hz, 2 H), 7.01 (d, $J$ = 8.1 Hz, 1 H), 6.89 (d, $J$ = 8.1 Hz, 1 H), 6.86 (d, $J$ = 8.7 Hz, 2 H), 5.98 (d, $J$ = 7.3 Hz, 1 H), 4.43 (m, 1 H), 3.84 (dd, $J$ = 4.5, 4.5 Hz, 4 H), 3.46 (s, 3 H), 3.21 (dd, $J$ = 4.5, 4.5 Hz, 5 H), 2.87 (m, 3 H), 2.77 (dd, $J$ = 6.3, 6.3 Hz, 2 H), 2.54 (br s, 3 H), 2.32 (s, 3 H), 2.18 (s, 4 H), 1.88 (m, 1 H), $^{13}$C NMR (75 MHz): δ 166.5, 153.3, 149.8, 135.1, 131.8, 129.6, 128.3, 127.9, 125.2, 117.1, 114.1, 66.6, 55.6, 52.1, 48.2, 46.1, 45.1, 31.8, 28.7, 25.4, 19.3, MS 451 ($M^+$).

## References

1. Knobelman D, Kung HF, Lucki I. *J Pharmacol Exp Ther* 2000; **292**: 1111–1117.
2. Patent WO99/05134.
3. Skiles JW, Fuchs V, Leonard SF. *Bioorg Med Chem Lett* 1991; **1**: 69–72.
4. Snieckus V, Onouchi T, Boekelheide VJ. *Org Chem* 1972; **37**: 2845.
5. Reeves WP, King II, RM. In *Synthetic Commun* 1993; **23**: 855–859 4-Bromomorpholinobenzene was synthesized according to the method in *Synthetic Commun* from morpholinobenzene affording material with a mp 115–116°C.