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Applied Radiation and Isotopes 62 (2005) 49–53
Applied Radiation and Isotopes
www.elsevier.com/locate/apradiso

# Tritium labelling of dopaminergic ligands domperidone and (+/−)-7-hydroxy DPAT

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Received 20 October 2003; received in revised form 21 May 2004; accepted 29 June 2004

**Abstract**

Methods are presented for tritiating the D2 specific dopaminergic antagonist domperidone and D3 specific dopaminergic agonist (+/−)-7-hydroxy DPAT. Techniques to characterize the products of the tritiation are also given.


*Keywords:* Domperidone; (+/−)-7-Hydroxy DPAT; Tritium; Tritium NMR

## 1. Introduction

The function of abnormal dopaminergic transmission in many neurological disorders, such as Parkinson's disease and schizophrenia, has been aggressively pursued over the past several decades. As the understanding of this receptor class has clarified, it was found (on the basis, in large part, of pharmacological and biochemical criteria), that the dopamine receptor is actually a family of multiple binding sites (Kebabian and Calne, 1979; Seeman, 1990). To better define the location and role of these dopamine receptor subclasses and potentially identify new and useful drug candidates, investigators have tried to find both high affinity agonists and antagonists for them. Our laboratories have had a long-standing interest in radiolabelling dopaminergic ligands by producing tools for receptor binding assay (Filer and Ahern, 1980; Orphanos and Filer, 2002), and now we report on the tritiation of the dopaminergic ligands domperidone **1** and (+/−)-7-hydroxy DPAT **2**, as well as on the characterization of the products of this process (Scheme 1).

## 2. Experimental

Evaporations were carried out on a Buchi rotary evaporator (Model RE 111) at bath temperatures below 40 °C. Analytical thin layer chromatography (TLC) was performed on Analtech plates coated with silica gel (250 μm). Autoradiography was performed at 0 °C, after the plates were sprayed with 2,5-diphenyl-1,3-oxazole (PPO) and an X-ray film was exposed to them. The TLC plates were also scanned for radioactivity (~370 kBq) with a Vangard Autoscanner. Analytical and preparative high performance liquid chromatography (HPLC) separations were performed on a Waters instrument (model 510 pump) with a simultaneous UV (280 nm, Waters 440 UV detector) and radioactivity (IN/US Systems Beta RAM Model 3) detection. Solution radioassays were conducted with a Beckman Model LS 3801 instrument. The tritium NMR spectra were recorded on a Bruker 300 MHz instrument, and the chemical shifts are reported as parts per million (ppm) downfield from internal standard tetramethylsilane

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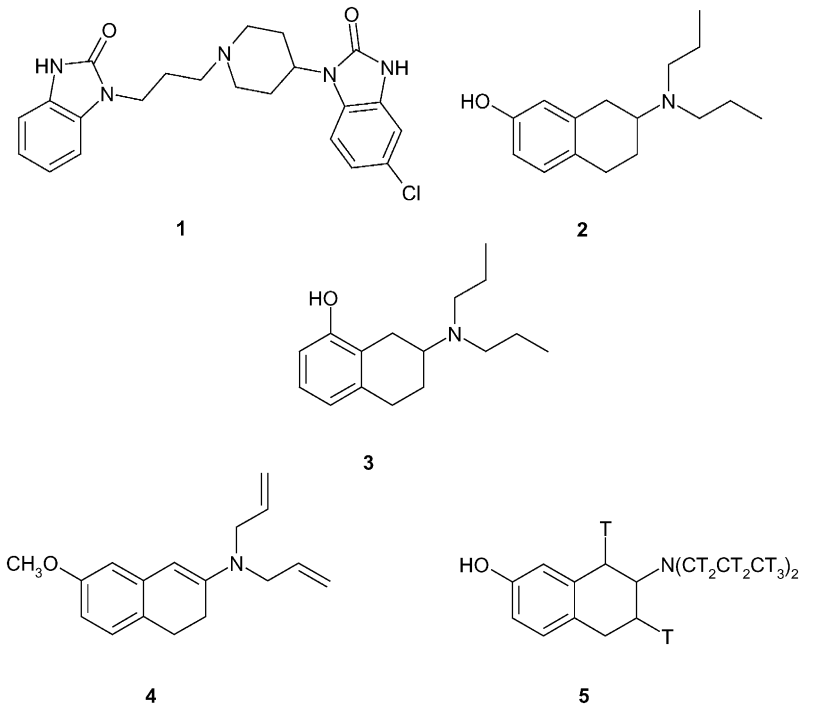
0969-8043/$ - see front matter 
doi:10.1016/j.apradiso.2004.06.010




Scheme 1.

(TMS). The mass spectra were obtained on a Kratos Model MS25 RF instrument with direct injection. All chemicals used were reagent grade.

### 2.1. Bromination of domperidone

A solution of 67 mg (0.42 mmol) of bromine in 2 ml of acetic acid was added dropwise to a solution of domperidone **1** (42.5 mg, 0.1 mmol, Sigma-RBI Cat. ♯D-122) in 2 ml of acetic acid with rapid stirring at ambient temperature over the course of several minutes. A precipitate occurred, and the solution was stirred overnight. The precipitate was then collected by vacuum filtration, washed with several 1 ml portions of cold diethyl ether, and dried to afford 60 mg of the product. A TLC analysis (ethyl acetate:methanol:cyclohexane:-concentrated ammonium hydroxide (14:3:3:1)) showed no precursor **1** ($R_f$~0.76), but it revealed several higher, closely running spots ($R_f$~0.83). This brominated precursor mixture was directly used for the subsequent tritiation without a further repurification.

### 2.2. [Phenyl -$^3$H] Domperidone

A solution of 24.5 mg (~0.04 mmol) of the above polybrominated domperidone mixture in 3 ml of ethyl acetate, which also contained 5 mg of 5% $Pd/Al_2O_3$ and 25 μl of triethylamine, was vigorously stirred with 2.22 TBq of tritium gas for 2 h at ambient temperature. The catalyst was then filtered off, the labile tritium was removed by several evaporations of sequentially added portions of ethanol, and a crude product (57 GBq) in 10 ml of ethanol was obtained. The product was purified by preparative TLC on three 500-μm silica gel plates, which were developed with the mixture ethyl acetate :cyclohexane:methanol:concentrated ammonium hydroxide (14:3:3:1). A sample of pure compound **1** was also put on each plate to migrate separately on its side and, thus, to facilitate the product location by means of a UV lamp. After UV visualization, the appropriate bands were scraped and eluted with a minimum amount of ethanol, which resulted in 18.7 GBq of the product (an approximate 25% radiochemical yield based on the polybrominated precursor mixture). The product was >97% radiochemically pure; it completely co-chromatographed with **1** on TLC (the same system as above), as well as HPLC (Zorbax cyano column elution with a mixture of 0.01 N aqueous potassium phosphate (pH 3): acetonitrile (65:35)). The specific activity of the product was found to be 1.7 TBq/mmol (a UV ethanol assay; compound **1** had $\varepsilon_{288} = 12{,}278$), and the UV spectrum of the product superimposed the spectrum of compound **1**. The product also provided a proton decoupled tritium NMR spectrum ($CD_3OD$) displaying a complex multiplet at $\delta$7.15 ppm (Fig. 1).

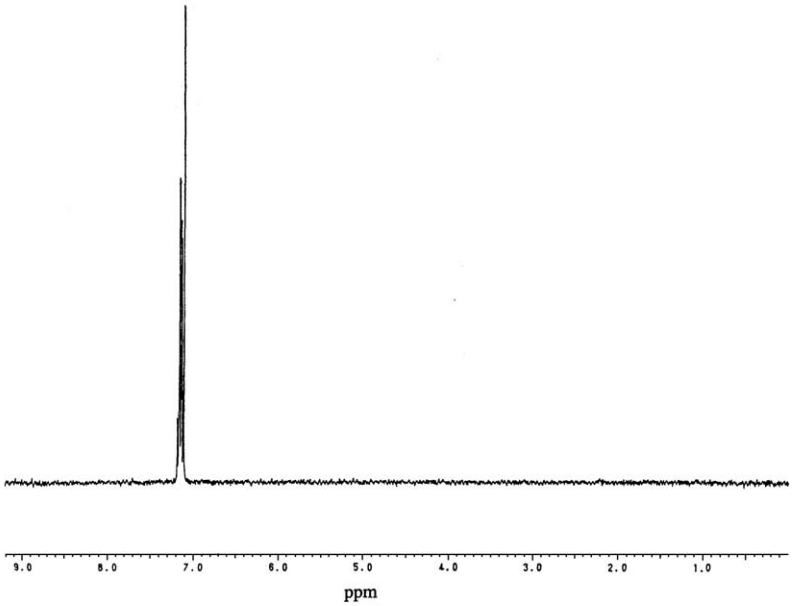



Fig. 1. Proton decoupled tritium NMR ($CD_3OD$) of [phenyl-$^3$H] domperidone.

### 2.3. *Enamine intermediate* (**4**)

A solution of 24 mg (0.14 mmol) of 7-methoxy-2-tetralone (Aldrich Cat. #16,418-6) in 0.1 ml (78.7 mg, 0.81 mmol) of diallylamine was refluxed with a solution of 2 mg of toulenesulfonic acid in 5 ml of benzene for 4 h with Dean-Stark water removal. The reaction mixture was then cooled, and the solvent was removed by rotary evaporation, which produced 30 mg of a tan oil. The product exhibited a complex $^1$H NMR spectrum ($CDCl_3$) with the key resonances at $\delta$7.75 (d), 7.20 (m), 6.75 (m), 5.90 (m), and 5.30 (m) ppm. This intermediate was directly used in the reaction described below without a further repurification.

### 2.4. *[$^3$H] (+/−)-7-Hydroxy DPAT* (**5**)

A mixture of 15 mg (0.06 mmol) of the olefin precursor **4**, 5 ml of ethyl acetate, and 50 mg of 10% Pd/C was vigorously stirred with 2.22 TBq of gaseous tritium overnight at ambient temperature. The catalyst was then filtered off, and the labile tritium was removed by several evaporations of sequentially added portions of ethanol. This operation produced a crude intermediate (113 GBq) in 50 ml of ethanol. A 30 GBq portion of this product was divided into two parts, and each part was purified by preparative TLC on a $20 \times 20\,cm^2$ plate with 500 μm silica gel. One of the plates was developed with chloroform:methanol:concentrated ammonium hydroxide (10:1:0.1), and the other with hexane:ethyl acetate:triethylamine (50:50:1).

A sample of pure intermediate (+/−)-7-methoxy DPAT was also put on each plate to migrate separately on its side and, thus, to facilitate the product location by means of a UV lamp. After development, the appropriate bands were scraped, combined, and the compound was eluted with ethanol. The ethanol solution was evaporated to near dryness, and the residue was transferred into a 2.5 ml portion of acetic acid containing 10 mg of sodium bisulfite and 5 mg of EDTA. The mixture was stirred at ambient temperature for five minutes, after which 3.5 ml of a 48% aqueous solution of HBr was added, and the resulting mixture was refluxed under argon for two hours. It was then neutralized with a saturated aqueous solution of potassium bicarbonate, and the product was extracted with five 10 ml portions of chloroform. The chloroform extracts were evaporated, and the residue was dissolved in 5 ml of ethanol to yield 2.9 GBq of the crude product **5**. The product was then purified by reverse phase HPLC with a mobile phase consisting of a solution of 0.01 M aqueous triethylammonium acetate (pH 4):acetonitrile (75:25) to give 1 GBq of pure **5** (a 1.2% radiochemical yield based on precursor **4**). The product was 97% radiochemically pure and completely co-chromatographed with **2** on reverse HPLC (same system as above). According to a mass spectral analysis, the specific activity of product **5** was 5.55 TBq/mmol, and it exhibited a proton decoupled tritium NMR ($CD_3OD$) spectrum shown in Fig. 2. Multiple sites of aliphatic tritium incorporation can be seen.

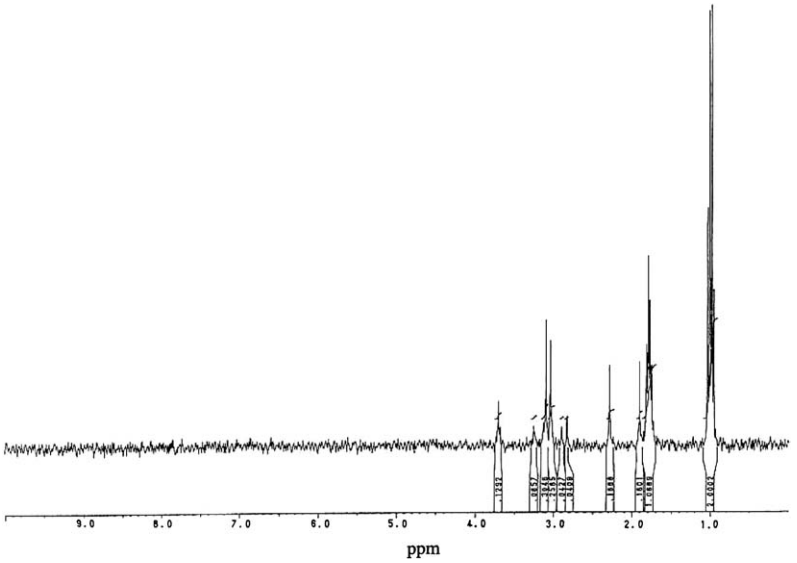



Fig. 2. Proton decoupled tritium NMR ($CD_3OD$) of **5**.

## 3. Results and discussion

A consideration of compounds **1** and **2** illustrates the exquisite diversity of structures present within the dopaminergic ligand family. Also, as is so often the case, different strategies are required to successfully label such ligands with tritium at a specific activity of at least 0.74 TBq/mmol for use in receptor binding assay. Domperidone **1**, a bis benzimidazolinone derivative, is a D2 dopamine receptor antagonist. Compound **1** was easily brominated under mild conditions to give a mixture of the polybromo analogues. This mixture of precursors was smoothly tritiated with catalyst to produce radiochemically pure [phenyl-$^3$H] domperidone at a high specific activity. This robust method repeatedly provided the product at a specific activity of 1.5–2.2 TBq/mmol. A proton decoupled tritium NMR ($CD_3OD$) spectrum of the product (Fig. 1) showed that tritium was incorporated exclusively in the aromatic locations. [Phenyl-$^3$H] Domperidone has proven to be a very useful radiolabelled ligand in elucidating details of the D2 dopamine receptor system (Seeman et al., 2003; Gelbard et al., 1989).

The case of (+/−)-7-hydroxy DPAT (**2**) illustrates that a seemingly small structural change can exert a remarkable effect on the neurochemistry of a compound. The analogue (+/−)-8-hydroxy DPAT (**3**) is a high affinity 5-$HT_{1A}$ serotonin agonist (Bjork et al., 1989), while compound **2** is a potent and selective D3 dopamine receptor agonist. The structure of compound **2** and the method of its synthesis provided an opportunity for its tritiation at even higher specific activities than those achieved with compound **1**. Our tritiation of compound **2** utilized the technique that had been used by us and other researchers (Gozlan et al., 1983) to tritiate compound **3**. The enamine precursor **4** was prepared from the commercially available compounds 7-methoxy-2-tetralone and diallylamine. NMR spectra suggested that compound **4** was probably a mixture of the structure shown for this label and of the enamine whose olefin was not conjugated with the phenyl group. A catalytic tritiation of compound **4**, followed by a removal of the methoxy group, produced a radiochemically pure compound **5** at very high specific activity. A proton decoupled tritium NMR spectrum ($CD_3OD$) (Fig. 2) has proven the indicated positions of the tritium incorporation. Radiolabelled ligand **5** has also emerged as a very valuable agent in clarifying elements of the D3 dopamine receptor (Maj et al., 1998; Ricci et al. 1997).

## Acknowledgements

We would like to thank Drs. Puliyur Srinivasan and Mahendra Suryan of PerkinElmer Life and Analytical Sciences Inc. for the NMR and mass spectra, respectively.

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