Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 121-126 (2001)

SYNTHESIS OF [*N*-METHYL-^{11}C]MIANSERIN: A TETRACYCLIC, ATYPICAL ANTIDEPRESSANT

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Summary

As part of our program to develop PET tracers for investigating monoaminergic processes in the brain, mianserin, a tetracyclic, atypical antidepressant, was selected as a candidate for labelling with ^{11}C for *in vivo* evaluation. [*N*-methyl-^{11}C]Mianserin was produced by the alkylation of *N*-desmethyl mianserin with [^{11}C]methyl iodide followed by HPLC purification and formulation. [*N*-methyl-^{11}C]Mianserin was obtained with a radiochemical purity >93% in a 16% decay corrected radiochemical yield. For a typical production starting with 40 GBq [^{11}C]CO_2, 1.9 GBq [*N*-methyl-^{11}C]mianserin was obtained as a formulated solution in a synthesis time of 35 min (counted from EOB).

Key words: ^{11}C, antidepressant, mianserin, PET

Received 21 August 2000
Revised 22 September 2000
Accepted 1 October 2000

Introduction

The monoaminergic theory of affective disorders states that both serotonin and noradrenaline are involved in the etiology of depression and in its successful treatment (1,2). As a result, there is much interest in finding appropriate radiopharmaceuticals for examining monoaminergic processes in the living brain (3-9). Mianserin (1,2,3,4,10,14b-hexahydro-2-methyldibenzo[c,f]pyrazino[1,2-a] azepine) is an antidepressant drug that affects both serotonergic and noradrenergic processes in the brain (10-12). Mianserin is viewed as atypical, however, because its antidepressant properties cannot be attributed to inhibition of monoamine uptake. Our interest in studying the role of monoaminergic processes in the pathophysiology of affective disorders has induced us to prepare mianserin radiolabelled with C-11 for use in positron emission tomography.

Experimental

General- All chemicals were obtained from Aldrich Chemical Co. Ltd. unless otherwise stated. Mianserin hydrochloride and *N*-desmethyl mianserin hydrochloride were kindly provided by Akzo Nobel, N.V. Organon, Oss, The Netherlands. Solvents were 'HPLC grade' unless otherwise stated, supplied by Aldrich Chemical Co. Ltd. or Merck. Labelling reactions were performed using anhydrous Sure/Seal™ solvents supplied by Aldrich Chemical Co. Ltd. Lithium aluminium hydride (LAH) was obtained from Fluka and was transferred, under argon, to 5 mL vials which were sealed and stored in a desiccator under argon until required. Fresh, saturated solutions of LAH were made before each experiment by the addition of about 5 mL anhydrous tetrahydrofuran (THF, Fluka) to these vials in an inert atmosphere, followed by a 10 fold dilution with THF.

[^{11}C]Carbon dioxide was prepared by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a nitrogen gas target pressurised to 150 psi and 16 MeV protons produced by the General Electric Medical Systems PETtrace 200 cyclotron at Aarhus University Hospital. Irradiations of 30 min with a beam current of 40 μA were typically used. The labelling procedure, including preparation of [^{11}C]methyl iodide, methylation, HPLC purification, rotary evaporation and formulation was performed using a fully automated apparatus (13).

Preparative HPLC was performed using an isocratic pump (Perkin Elmer model 200) equipped with a 1 mL injection loop and connected in series with a Spherisorb 5μ ODS(2) column (250 x 8 mm, Phenomenex, column A), a variable wavelength UV detector (Applied Biosystems model 759A, λ=254 nm), and a photodiode radiodetector of in-house design. Analytical HPLC was performed using a Perkin Elmer model 250 pump with a 20 μL injection loop connected in series with a Spherisorb S5 ODS2 column (250 mm x 4.6 mm, Mikrolab, Aarhus, column B), a variable wavelength detector (Perkin Elmer model LC295, λ=254 nm), and a sodium iodide radiodetector of in-house design.

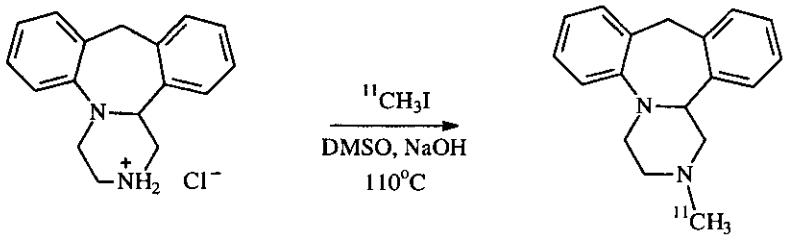
[^{11}C]Methyl Iodide [^{11}C]Carbon dioxide was purged from the target in a stream of nitrogen gas and trapped on 4Å molecular sieves. On heating, the ^{11}C-CO_2 was released and was passed through a solution of LAH (300μl) in a stream of nitrogen gas (25 ml/min). On completion of ^{11}C-CO_2 transfer, the THF was evaporated and 1 ml hydriodic acid was added. The formed ^{11}C-methyl iodide was transferred in a stream of nitrogen gas (20 ml/min) to a solution of the precursor.

[N-methyl-^{11}C] mianserin-(2) ^{11}C-Methyl iodide was distilled in a stream of nitrogen gas to a reaction vial containing 1 mg (3.5 μmol) *N*-desmethyl mianserin hydrochloride (**1**) dissolved in 300 μl DMSO and 4 μl of 3M NaOH solution. After heating for 5 min at 110°C, the crude product was purified on column A using acetonitrile:70mM NaH_2PO_4 solution (pH=3.5, 0.003% EDTA) (40:60) as eluent at a flow rate of 8 ml/min. The fraction containing the [*N*-methyl-^{11}C] mianserin (**2**) (Rt=6.8 min) was collected and evaporated at 120°C to dryness and thereafter it was formulated in a water solution containing 5% ethanol. The radiochemical purity and product identity were determined by analytical HPLC on column B using acetonitrile:70mM NaH_2PO_4 solution (pH=3.5, 0.003% EDTA) (50:50) as eluent at a flow rate of 2 ml/min.

Results and Discussion

[*N*-methyl-^{11}C] mianserin-(**2**) was synthetized by the alkylation of *N*-desmethyl mianserin hydrochloride (**1**) (Scheme 1). ^{11}C-Methylation of **1** in DMSO at 110°C for 5 min, using NaOH as base, produced **2** as one of the major products (>55%).

Reverse-phase semi-preparative HPLC was used to purify the [*N*-methyl-^{11}C]



1 2

Scheme 1. The synthesis of [*N*-methyl-^{11}C] mianserin.

mianserin (Figure 1). The labelling precursor (1) and two unidentified radioactive by-products (eluting at 2.6, 1.3 and 2.2 min, respectively, see Fig. 1.) were well-separated from [*N*-methyl-^{11}C] mianserin which eluted at 6.8 min.

For a typical production starting with 40 GBq [^{11}C]CO_2, 1.9 GBq [*N*-methyl-^{11}C] mianserin was obtained in a synthesis time of 35 min (counting from EOB). Analytical HPLC showed the product to be >93% radiochemically-pure and to co-elute with the authentic sample of mianserin hydrochloride. No detectable levels of the precursor 1 were found in the formulated product. The specific radioactivity is estimated to be higher than 20 GBq/μmol (EOS). The product (pH=3.5) can be regarded as stable in time since purity determination performed one hour after the end of synthesis showed no decomposition.

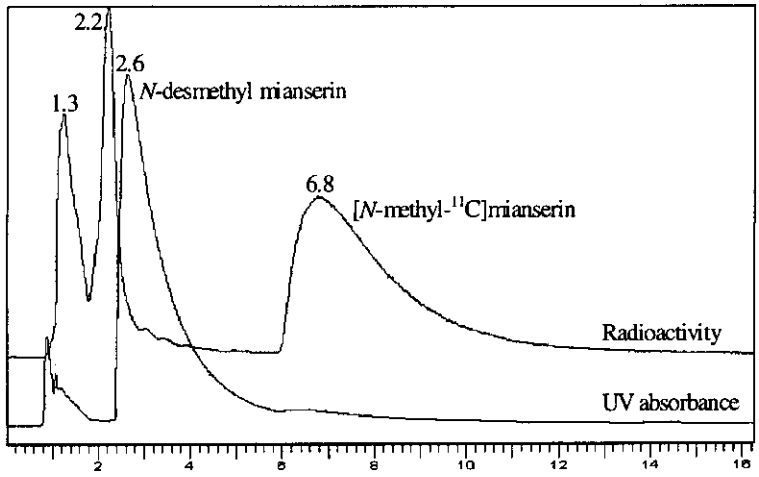


Figure 1. A typical semi-preparative HPLC chromatogram obtained from the purification of [*N*-methyl-^{11}C] mianserin.

Conclusions

[*N*-methyl-^{11}C] Mianserin can be produced with high radiochemical purity and high specific activity in sufficient quantities for *in vivo* biodistribution studies. PET investigations are currently in progress to study the biodistribution of [*N*-methyl-^{11}C] mianserin.

Acknowledgements

The authors would like to thank AkzoNobel, N.V. Organon for kindly supplying samples of mianserin and *N*-desmethyl mianserin, Jens Kristian Graverholt and Torben Lund for their technical assistance and Dr. Nic Gillings for valuable discussions.

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