Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVIII, No. 2

Solid-Phase Radiosynthesis of [^{11}C]WAY 100635

Alan A. Wilson, Jean N. DaSilva, and Sylvain Houle

PET Centre, The Clarke Institute of Psychiatry
Toronto, Ontario M5T 1R8 Canada.

Summary

An efficient, fast and simple method is described for the radiosynthesis of the potent and selective 5-HT$_{1A}$ antagonist [*O-methyl*-^{11}C]-N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane carboxamide ([^{11}C]WAY 100635). [^{11}C]Iodomethane was effectively trapped on a C$_{18}$ reverse-phase cartridge at ambient temperature where it reacted rapidly with the normethyl precursor, N-[2-[4-(2-hydroxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane carboxamide. Following high performance liquid chromatography purification and formulation, [^{11}C]WAY 100635 was obtained in high radiochemical yields (40%, uncorrected from [^{11}C]Iodomethane) in a synthesis time of 25 min with an average specific actvity of (at end-of-synthesis) 33 GBq/μmole (900 mCi/μmole).

Key Words: carbon-11, WAY 100635, 5-HT$_{1A}$

Introduction

Since the discovery that the novel non-benzodiazepine anxiolytic, buspirone, displays high affinity for the 5-HT$_{1A}$ receptor [1] the role of this receptor sub-type has been the focus of much research effort. The 5-HT$_{1A}$ receptor has been linked to such disorders as anxiety, dementia, and depression (for a review see Fletcher et al[2]). The recent synthesis of new selective, high affinity antagonists for the 5-HT$_{1A}$ receptor [3] has further stimulated this field in addition to spurring the development of

CCC 0362–4803/96/020149–06

Received 12 July 1995
Revised 29 August 1995

radioligands for studying the 5-HT_{1A} receptor by positron emission tomography (PET) and single photon emission computed tomography(SPECT).

The title compound, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane carboxamide (WAY 100635), is the most potent (Kd 0.1 nM), selective and pure antagonist for the 5-HT_{1A} receptor reported to date [4,5]. WAY 100635 has been labelled with ^{3}H [6] and ^{11}C [7,8] and analogues with ^{123}I [9] and ^{18}F [10]. Evaluation studies including ex vivo studies in mice and rats and in vivo studies in monkeys demonstrate that [^{11}C]WAY 100635 is a most promising PET radiotracer [6-8,11]. A recent abstract has reported its successful use in human PET studies [12]. We report here a simple and efficient method for the radiosynthesis of [^{11}C]WAY 100635 using a commercially available disposable cartridge which offers significant advantages over previously reported methods.

Results and Discussion

The precursor, normethylWAY 100635 (**2**), was prepared from WAY 100635 [7-9] by demethylation using boron tribromide in good yield. The synthetic scheme and a simplified schematic of the apparatus used for the radiosynthesis of [^{11}C]WAY 100635 (**1**) is depicted in the Figure below. A small disposable C_{18} cartridge is loaded with a solution of the phenol precursor in DMF containing tetrabutylammonium hydroxide and inserted between two 3-way valves. [^{11}C]Iodomethane is then swept by a flow of nitrogen into the cartridge where it rapidly reacts with the precursor anion at ambient temperature generating [^{11}C]WAY 100635. The valves are switched and the contents of the cartridge are then eluted onto an HPLC injection loop using HPLC buffer for purification in the conventional manner. Greater than 90% of [^{11}C]iodomethane is trapped on the cartridge at a flow of 20 mL/min (higher flows resulted in greater breakthrough) while residual radioactivity in the cartridge after elution was less than 5% of that trapped. Radiochemical yields of the final purified, formulated and filtered product (based on [^{11}C]iodomethane produced) were on average 40% (uncorrected for decay, range 33-45%) in a total synthesis time of 25 min from end-of-bombardment, corresponding to yields of final product of > 1GBq/μAhr on target (>27 mCi/μAhr). Specific activities (at end-of-synthesis) averaged 33 GBq/μmole (900 mCi/μmole).

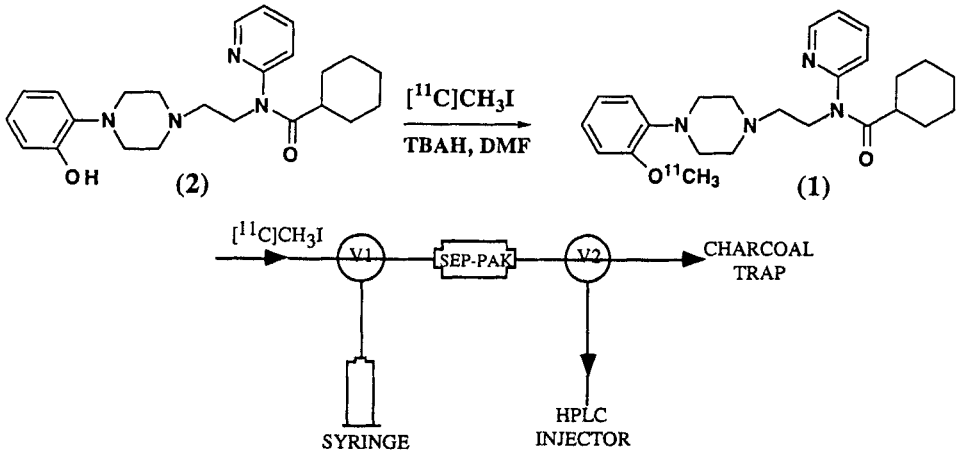

Figure. Solid-phase radiosynthesis of [^{11}C]WAY 100635

Radiosynthesis of PET radiotracers using solid-phase/captive solvent techniques was pioneered by the PET group at Michigan [13,14] and most recently explored, with [^{11}C]iodomethane, by Iwata and Schmitz [15,16]. The attractiveness of the method described herein lies in its simplicity. No cooling of the cartridge is required for efficient trapping of the [^{11}C]iodomethane and no heating is required to effect this reaction in a period of time consummate with the half-life of the radionuclide. In addition, the cartridge is commercially available, requiring no packing by the research workers, which enhances the reproducibility of the method. Finally, readiness for the next production run simply involves cleaning the valves and lines with ethanol using the syringe, and replacing the cartridge. Both previously reported radiosyntheses of [^{11}C]WAY 100635 by conventional solution reactions proceed with much lower isolated radiochemical yields (10-12%), and in one case, a longer synthesis time (50 min) [7,8].

We have applied this method to the radiosynthesis of another phenol methyl ether, [*O-methyl*-^{11}C]-1-(2-Methoxyphenyl)-4-(2-(2-pyridylamino)ethyl)piperazine ([^{11}C]WAY 100634) , the purported major metabolite in humans of WAY 100635 [8], and also to the dopamine transporter ligand [*O-methyl*-^{11}C]RTI-32 from its carboxylic acid precursor [17], with equally good results (data not shown). It may therefore be a general method for the [^{11}C]methylation of reactive nucleophiles with [^{11}C]iodomethane.

Experimental

DMF was stirred overnight with BaO, then distilled under reduced pressure from BaO and stored over 4 Å molecular sieves. Purification and analyses of radioactive mixtures by HPLC were performed with an in-line uv (254 nm) detector in series with a NaI crystal radioactivity detector. The HPLC columns used were either Alltech Econosil C_{18} (250 mm x 10 mm, 10 μ) for purification, or an Alltech CAP C_8 (250 mm x 4.6 mm, 10μ) for analysis and quality control. Peak areas were measured using Hewlett-Packard 3396 and Waters 746 recording integrators. Isolated radiochemical yields were determined with a dose-calibrator (Capintec CRC-712M). Sterility and pyrogenicity testing were performed using standard procedures. Samples of the radiolabelled product prepared according to the procedure described below were determined to be sterile and pyrogen-free in all cases.

N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexane carboxamide (WAY 100635). Compound (**1**) was prepared by literature methods in four steps from 2-aminopyridine and 1-(2-methoxyphenyl)piperazine [9].

N-[2-[4-(2-Hydroxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexane carboxamide (2). A solution of boron tribromide (100 mL, 1N) in dichloromethane was stirred under argon at -70 °C whilst a solution of WAY 100635 (8.0 g, 18.9 mmol) in dichloromethane (50 mL) was added dropwise over 20 min. The solution was stirred at ambient temperature for 24 hr then cautiously quenched with saturated aqueous sodium bicarbonate solution (150 mL). The resultant mixture was stirred vigorously for 2 hr and filtered through a short plug of diatomaceous earth. The organic phase was separated and extracted with sodium hydroxide solution (3 x 150 mL, 0.5 N). The combined aqueous extracts were neutralized to pH 8 with hydrochloric acid (2N) and extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and the solvent removed to leave a brown oil. Silica gel chromatography using EtOAc:hexane:Et_3N (48:48:4) gave a colourless oil which solidified upon drying under high vacuum overnight. Recrystallisation from isopropyl ether gave 5.24 g

(68%) of (**2**) as a white solid: mp 95-96 °C; Anal. Calc. for $C_{24}H_{32}N_4O_2$; C, 70.56; H, 7.90; N, 13.71. Found C, 70.51; H, 7.87; N, 13.67. HPLC analysis could not detect any WAY 100635 (< 0.01%) in the recrystallised product.

[^{11}C]WAY 100635. [^{11}C]iodomethane [17], produced from $^{11}CO_2$, was swept by a stream of nitrogen (20 mL/min) through a Sep-Pak tC18L cartridge (Waters). The cartridge, loaded with a freshly prepared solution of (**2**) (1.5 mg) in DMF (100 μL) containing tetrabutylammonium hydroxide (5 μL, 1M in methanol) prior to the start of the run, was positioned between two three-way valves using standard luer fittings and 1/16 in OD PTFE tubing (Figure). Breakthrough [^{11}C]iodomethane was trapped in a charcoal tube downstream. Upon trapping maximal activity in the column (ca. 4 min) the valve positions were switched and the cartridge flushed with 1 mL of HPLC buffer into the loop of the HPLC injector, then purified by semi-preparative HPLC (50% CH3CN:50% H2O + 0.1N NH4HCO2, 10 mL/min, $R_{T_{product}}$10 min). The desired fraction was collected, evaporated to dryness, and the residue taken up in 10 mL of sterile saline. This was passed through a sterile 0.22 μm filter (Anatop 10) into a sterile, pyrogen free bottle containing aqueous sodium bicarbonate (1 mL, 8.4%). The radiochemical purity and specific activity of the final solution was determined by analytical HPLC (50% CH3CN:50% H2O + 0.1N NH4HCO2, 4 mL/min, $R_{T_{product}}$3.5 min). Confirmation of the identity of the radiolabelled product was achieved by co-injection with authentic (**1**) using a further four different HPLC columns (Alltech C_{18} Econosil, Waters C_{18} Novapak, Phenomenex Ultracarb 7 and Alltech silica Econosil).

Acknowledgements

The authors wish to thank Mr. Stephen Dobbin for his assistance with radiochemical and cyclotron operations. This work was financially supported by the Ontario Ministry of Health and the Medical Research Council of Canada (Grant # MT-12723).

References

(1) New J.S. -Med. Res. Rev. 10: 283 (1990).

(2) Fletcher A., Cliffe I.A., and Dourish C.T. -Trends Pharmacol. Sci. 14: 441 (1993).

(3) Cliffe I.A., and Fletcher A. -Drugs Future 18: 631 (1993).

(4) Fletcher A., Bill D.J., Cliffe I.A., Forster E.A., and Reilly Y. -Br. J. Pharmacol. 112: 91P (1994).

(5) Gozlan H., Thibault S., Laporte A.-M., Lima L., and Hamon M. -Eur. J. Pharmacol. 288: 173 (1995).

(6) Hume S.P., Ashworth S., Opackajuffry J., Ahier R.G., Lammertsma A.A., Pike V.W., Cliffe I.A., Fletcher A., and White A.C. -Eur J Pharmacol 271: 515 (1994).

(7) Mathis C.A., Simpson N.R., Mahmood K., Kinahan P.E., and Mintun M.A. -Life Sci. 55: 403 (1994).

(8) Pike V.W., McCarron J.A., Hume S.P., Ashworth S., Opacka-Juffry J., Osman S., Lammertsma A.A., Poole K.G., Fletcher A., White A.C., and Cliffe I.A. -Med. Chem. Res. 5: 208 (1994).

(9) Zhuang Z.-P., Kung M.-P., and Kung H.F. -J. Med. Chem. 37: 1406 (1994).

(10) Wilson A.A., DaSilva J.N., and Houle S. -XIth International Symposium on Radiopharmaceutical Chemistry, Vancouver D14 (1995).

(11) Laporte A.M., Lima L., Gozlan H., and Hamon M. -Eur J Pharmacol 271: 505 (1994).

(12) Pike V.W., Hume S.P., McCarron S., Ashworth S., Opacka-Juffry J., Lammertsma A.A., Osman S., Bench C.J., Poole K.G., Grasby P., Cliffe I.A., Fletcher A., and White A.C. -J. Nucl. Med. 36: 58P (1995).

(13) Jewett D., Ehrenkaufer R., and Ram S. -Int. J. Appl. Radiat. Isot. 36: 672 (1985).

(14) Watkins G., Jewett D., Mulholland G., Kilbourn M., and Toorongian S. -Appl. Radiat. Isot. 39: 441 (1988).

(15) Iwata R., Pascali C., Yuasa M., Yanai K., Takahashi T., and Ido T. -Appl. Radiat. Isot. 43: 1083 (1992).

(16) Schmitz F., Plenevaux A., Delfiore G., Lemaire C., and Comar D. -J. Labelled Compd. Radiopharm. 35: 94 (1994).

(17) Wilson A.A., DaSilva J.N., and Houle S. -J. Labelled Compd. Radiopharm. 34: 759 (1994).