Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIX, No. 6

SYNTHESIS OF *meta*-IODOBENZYL [^{11}C]GUANIDINE

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SUMMARY

An efficient and fully automated one-pot method for the synthesis of 3-iodobenzyl [^{11}C]guanidine ([^{11}C]MIBG) was developed. Starting from [^{11}C]cyanogen bromide, [^{11}C]MIBG was obtained in 62 % radiochemical yield and a radiochemical purity higher than 98 % *via* the intermediate 3-iodobenzyl [^{11}C]cyanamide. The synthesis time was 40 min counted from the end of bombardment and the product was obtained with a specific radioactivity of 3.5 Ci/μmol (130 GBq/μmol) at the end of synthesis.

Keywords: [^{11}C]MIBG, PET, [^{11}C]cyanogen bromide

Introduction

Radiolabelled *m*-iodobenzylguanidine (MIBG) has since its introduction (1) as an adrenomedullary imaging agent become a widely used tool both in the diagnosis and therapy of tumours of neural crest origin, such as neuroblastoma and pheochromocytoma (2,3). MIBG is a functional analogue of norepinephrine, competing with biogenic amines for uptake and storage in chromaffin granules, either by passive diffusion or *via* a sodium-dependent pump.

Most commonly, MIBG is labelled with γ-emitters such as ^{123}I or ^{131}I for use in single photon emission computed tomography (SPECT). While such studies may give tomographic image information, the possibility of gaining quantitative or dynamic information is limited. If, however, compounds labelled with short-lived positron emitters such as ^{11}C (half-life 20.3 min) are used, positron emission tomography (PET) offers the possibility to accurately quantitate tissue uptake and distribution as well tracer dynamics. For this purpose, several groups have developed methods for labelling MIBG or MIBG analogues with positron emitters suitable for PET, *e.g.* [^{124}I]MIBG (4), [^{76}Br]MBBG (5) and ([4-^{18}F]fluoro-3-*meta*-iodobenzyl)guanidine (6). In some

CCC 0362–4803/97/060525–05$17.50

Received 20 February 1997
Revised 6 March 1997

instances, it may be of interest to perform repeated studies of uptake and biodistribution during a short time interval, in which case the shorter half-life of ^{11}C would be more convenient. The purpose of the present study was thus to develop a method to synthesise ^{11}C-labelled MIBG for use in both *in vitro* and *in vivo* experiments.

Labelling of guanidines may be achieved by treatment of a labelled cyanamide with an amine or ammonia. Benzyl[^{11}C]guanidine has been prepared by proton irradiation of calcium nitride to give [^{11}C]cyanamide, followed by reaction with benzylamine (7). This method, however, afforded relatively low yields and required an elaborate purification procedure for [^{11}C]cyanamide. Recently, an alternative route to [^{11}C]cyanamide was described (8). An attractive alternative is reaction of the appropriate amine with [^{11}C]cyanogen bromide, followed by treatment with ammonia. We have recently reported on the use of this method for the synthesis of the novel antiviral compound [^{11}C]GG167 (9), the σ-receptor ligand 1,3-di(2-tolyl)-[^{11}C]guanidine (10) and a system for use with supercritical ammonia in the synthesis of a series of ^{11}C-guanidines (11).

Materials and Methods

General

[^{11}C]Carbon dioxide was prepared by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a nitrogen (AGA 6.0) gas target containing 0.05 % oxygen (AGA 6.0) and 17 MeV protons produced by the Scanditronix MC-17 cyclotron at the Uppsala University PET Centre. The [^{11}C]carbon dioxide was converted to hydrogen [^{11}C]cyanide according to published procedures (12,13) using the Scanditronix RNP-17 radionuclide production system. Synthesis, solid-phase extraction, semi-preparative HPLC purification, formulation and analysis of the final product was performed using a fully automated system (SYNTHIA, 14). HPLC was performed using a Beckman 126 pump and a Beckman 166 variable wavelength UV-detector in series with a β^+-flow detector (15). Data collection and decay correction were performed on a personal computer using the Beckman System Gold Chromatography Software Package. Thin-layer chromatography (TLC) was performed on Merck Silica gel F_{254} plates. Autoradiographic images of TLC plates were obtained using a Molecular Dynamics PhosphorImager® (Sunnyvale, Cf., USA). LC-MS was performed using a CMA 240 Autosampler (CMA Microdialysis, Stockholm, Sweden) and a Beckman 126 gradient pump and a Fisons VG Quattro Mass Spectrometer equipped with pneumatically assisted electrospray and an RF ion bridge (positive ionisation mode, 3 kV capillary voltage, 400 V lens voltage and 40 V cone voltage). A post-column 1:100 split was used, with 1 % of the total flow delivered to the electrospray tube and 99% to a Beckman 166 variable wavelength UV-detector and a β^+-flow-detector (Bioscan Flow-Count).

Chemicals

3-Iodobenzylamine hydrochloride was obtained from Aldrich Chem. Co., Steinheim, Germany. Authentic 3-iodobenzylguanidine was obtained from EMKA Chemie, Markgröningen, Germany. All other chemicals were of analytical grade quality and used as received.

Synthesis of [^{11}C]cyanogen bromide

[^{11}C]Cyanogen bromide was produced according to a procedure described in detail elsewhere (16). Hydrogen [^{11}C]cyanide was passed through a drying tower filled with phosphorous pentoxide and then through a quartz tube containing pyridinium tribromide and antimony powder. The [^{11}C]cyanogen bromide formed was then trapped in a receiving vessel for further synthesis. Synthesis times were 3 - 5 min counted from the end of bombardment.

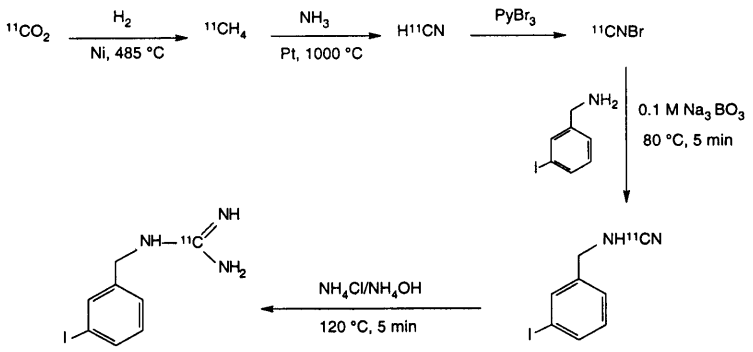
Synthesis of 3-iodobenzyl [^{11}C]guanidine

The [^{11}C]cyanogen bromide was trapped at room temperature in a solution containing 3.0 mg 3-iodobenzylamine hydrochloride in 300 µL 0.1 M sodium borate (pH 8.0). When the radioactivity in the trapping vessel had reached a maximum, the reaction mixture was heated at 80 °C for 5 min, whereupon 300 µL 20 % ammonium chloride in 25 % aqueous ammonia was added and heating continued for 5 min at 120 °C. After heating, the reaction mixture was diluted with 9.0 mL of water and applied to a solid-phase extraction column (Supelco LC-C_{18}, 3 mL), preconditioned with 5 ml absolute ethanol and 9 mL of water. The column was washed with 5 mL of water and then eluted with 2.5 mL 0.1 M NaH_2PO_4:THF, 75/25. The eluate was injected onto the semi-preparative HPLC column (Beckman Ultrasphere Octyl, 5 µm, 250×10 mm i.d.) eluted with 50 mM ammonium formate, pH 3.5 and methanol, 55/45, linear gradient to 20/80 from 3 to 8 min, flow 5 mL/min, UV-detection at 254 nm. The fraction eluting at c:a 8 min was collected and transferred to a rotary evaporator and the solvent removed under vacuum. The residue was redissolved in 4.5 mL 0.1 M phosphate buffered saline (pH 7.4) and passed through a sterile filter (Dynagard ME, 0.22 µm) into a sterile ampoule. A sample (20 µL) of this solution was analysed a Beckman Ultrasphere Octyl, 5 µm, 250×4.6 mm i.d. eluted with 50 mM ammonium formate, pH 3.5 and methanol (55/45, v/v, linear gradient to 20/80 from 2 to 7 min, flow 1.5 mL/min. Thin-layer chromatography (dichloromethane/methanol/ammoniumhydroxide, 3 M, 20/10/1, v/v) showed a single radioactive spot (Rf 0.62), co-eluting with authentic reference compound.

LC-MS analyses were performed using a KromaSil C_{18} column, 100×4.6 mm i.d. held at 40 °C, mobile phase 10 mM acetic acid/10 mM acetic acid in acetonitrile, 85/15 (v/v), linear gradient to 5/95 (v/v) from 0-5 min, flow 1 mL/min, UV-detection at 254 nm. The retention time for [^{11}C]MIBG was 5.0 min, k' 3.2, m/z 378.1 $[M+H]^+$, calculated m.w. 377.2.

Results and Discussion

The synthetic route to [^{11}C]MIBG is outlined in Scheme 1. Commercially available 3-iodobenzylamine hydrochloride was reacted with [^{11}C]cyanogen bromide in an aqueous borate buffer to give 3-iodobenzyl [^{11}C]cyanamide. Treatment with ammonium chloride in aqueous ammonium hydroxide at elevated temperature afforded [^{11}C]MIBG in 62 ± 11 % (mean ± S.D., n = 12) radiochemical yield counted from [^{11}C]cyanogen bromide.



Scheme 1

Purification of the crude [^{11}C]MIBG required a two-step procedure; after addition of ammonia, the reaction mixture had a high pH and ionic strength, making direct HPLC purification difficult. Therefore, the crude product was first desalted using reverse-phase solid-phase extraction. The eluate from the solid-phase extraction cartridge was then further purified by semi-preparative HPLC to give [^{11}C]MIBG with a chemical and radiochemical purity higher than 98 %. The specific radioactivity of the final product was always higher than 3.3 Ci/μmol as determined using HPLC. Starting with 30 GBq (0.8 Ci) of [^{11}C]cyanogen bromide, 3.8 - 5.4 GBq (102 - 146 mCi) of [^{11}C]MIBG was obtained within 40 min from the end of radionuclide production, including purification and quality control.

Examples of applications include assessment of the pharmacokinetics and biodistribution of [^{11}C]MIBG during the first two hours following intravenous administration. This, in turn, enables dosimetry calculations in patients undergoing [^{131}I]MIBG treatment. Fundamental aspects of MIBG biochemistry, such as the mechanism of accumulation and metabolism of drug in cultured neuroblastoma aggregates may be studied with *in vitro* techniques (17).

Acknowledgements

This work was financially supported by the Swedish Natural Sciences Research Council, Grant No. K-KU 3463.

References

1. Wieland, D.M., Brown, L.E., Rogers, W.L., Worthington, K.C., Wu, J.-I., Clinthrone, N.H., Otto, C.A., Swanson, D.P., Beierwaltes, W.H. – J. Nucl. Med. 22: 22 (1981).

2. Sisson, J.C., Frager, M.S., Valk, T.W. – New Engl. J. Med. 305: 12 (1981).

3. Shapiro, B., Copp, J.E., Sisson, J.C., Eyre, P.L., Willis, J., Beierwaltes, W.H. – J. Nucl. Med. 26: 576 (1985).

4. Qureshi, M.A., Sajjad, M., Vora, M.M, Lambrecht, R.M. – Proc. IXth Aust. Symp. Anal. Chem. Sydney, 2: 723 (1987).

5. Loc'h, C., Mardon, K., Valette, H., Brutesco, C., Merlet, P., Syrota, A. and Mazière, B. – Nucl. Med. Biol. 21: 49 (1994).

6. Vaidyanathan, G., Affleck, D.J. and Zalutsky, M.R. – J. Med. Chem. 37: 3655 (1994).

7. Iwata, R., Ido, T. – Int. J. Appl. Radiat. Isot. 34: 973 (1983).

8. Kruijer, P.S., Van Leuffen, P.J., Herscheid, J.D.M. – Appl. Radiat. Isot. 47: 611 (1996).

9. Westerberg, G., Bamford, M., Daniel, M.J., Långström, B and Sutherland, D.R. – J. Lab. Compd's Radiopharm. 38: 585 (1996).

10. Westerberg, G., Kärger, W., Onoe, H. and Långström, B. – J. Labelled Compd's Radiopharm. 34: 691 (1994).

11. Jacobson, G.B., Westerberg, G., Markides, K.E. and Långström, B. – J. Am. Chem. Soc. 118: 6868 (1996).

12. Christman, D.R., Finn, R.D., Karlstrom, K. and Wolf, A.P. – Int. J. Appl. Radiat. Isot. 26: 435 (1975).

13. Iwata, R., Ido, T., Takahashi, T., Nakanishi, H. and Iida, S. – Int. J. Appl. Radiat. Isot. 34: 97 (1987).

14. Bjurling, P., Reineck, R., Westerberg, G., Sutcliffe, J. and Långström, B. – Proc. VIth Workshop Targetry and Target Chemistry,Vancouver, Canada, 282 (1995).

15. Långström, B. and Lundqvist, H. – Radiochem. Radioanal. Lett. 41: 375 (1979).

16. Westerberg, G. and Långström, B. – Appl. Radiat. Isot. (1997). In press.

17. Matsumura, K., Bergström, M., Onoe, H., Takechi, H., Westerberg, G., Antoni, G., Bjurling, P., Jacobson, G.B., Långström, B. and Watanabe, Y. – Neuroscience Res. 22: 19 (1995).

