SYNTHESIS OF 5-ACETYLAMINO-4-[^{11}C]GUANIDINO-2,6-ANHYDRO-3,4,5-TRIDEOXY-D-GLYCERO- D-GALACTO-NON-2-ENOIC ACID ([^{11}C]GG167) - AN INFLUENZA VIRUS NEURAMINIDASE INHIBITOR

Göran Westerberg,[1] Mark Bamford,[2] Mick J Daniel,[2]
Bengt Långström[1] and Derek R Sutherland[2]

[1]Uppsala University PET Centre, UAS S-751 85 Uppsala, Sweden,
[2]Glaxo Wellcome Research & Development, Gunnels Wood Road,
Stevenage, Hertfordshire SG1 2NY, UK.

Summary

The novel influenza virus neuraminidase inhibitor GG167 (5-acetylamino-4-guanidino-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enoic acid) was labelled with ^{11}C for use in positron emission tomographic studies of drug deposition following intranasal or inhaled administration. [^{11}C]GG167 was obtained within a synthesis time of 50min *via* a two-step procedure, starting from [^{11}C]cyanogen bromide.

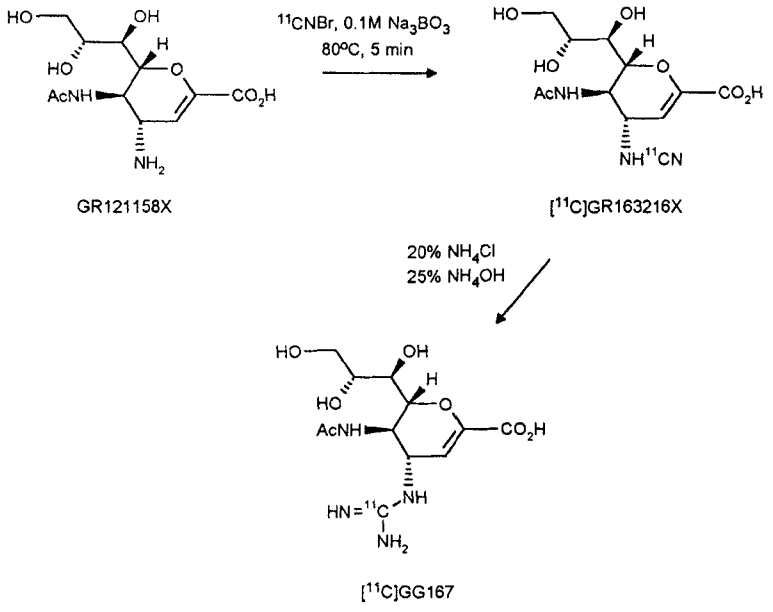
Keywords: [^{11}C]GG167, [^{11}C]guanidine, PET, deposition study.

Introduction

5-Acetylamino-4-guanidino-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enoic acid (GG167) is a potent and selective inhibitor of influenza viral neuraminidase (1,2), an enzyme responsible for the release of newly formed virus particles from infected cells. In animal models of infection (mice and ferrets), GG167 has been shown to be effective with doses as low as 50μg/kg bodyweight, when administered intranasally, whereas it has no siginificant activity when administered by the oral or intraperitoneal routes, due to insufficient distribution to the sites of infection and rapid renal elimination. The drug is currently being developed for the treatment of influenza A and B in man. In order to enable positron emission tomographic studies of drug deposition to be performed, after nasal and inhaled administration, a route to [^{11}C]GG167 was developed. Reaction of the amino acid GR121158X with [^{11}C]cyanogen bromide (3) gave an intermediate cyanamide in high radiochemical yield. The cyanamide was then treated with aqueous ammonia to give [^{11}C]GG167 (Scheme 1).

CCC 0362–4803/96/060585–05

Received 12 January 1996
Revised 22 January 1996



Scheme 1: Synthesis of [^{11}C]GG167

Materials and Methods

General - The labelled precursor for the radiosynthesis, [^{11}C]cyanogen bromide, was produced by an on-line procedure (Westerberg G and Långström B, manuscript in preparation). [^{11}C]Carbon dioxide was prepared by the ^{14}N(p,α)^{11}C reaction using a nitrogen gas (AGA, Nitrogen 6.0) target containing 0.05 % oxygen (AGA, Oxygen 6.0) and 17 MeV protons produced by the Scanditronix MC-17 cyclotron at the Uppsala University PET Centre. [^{11}C]Carbon dioxide was converted to hydrogen [^{11}C]cyanide according to published procedures (4,5), and was carried from the target in a stream of nitrogen and hydrogen gas at a flow rate of *ca* 85ml min^{-1} to a quartz furnace containing nickel on kieselguhr held at 480°C. The [^{11}C]methane produced was mixed with ammonia gas and transferred to a second quartz furnace containing platinum, held at 1050°C. The resulting hydrogen [^{11}C]cyanide was then carried through a drying tower containing Sicapent® in a stream of nitrogen to reduce the excess of ammonia gas, and then converted to [^{11}C]cyanogen bromide by passage through a tube (6.25×150mm) containing pyridinium tribromide (400mg) and antimony powder (200 mesh, 200mg), separated by a plug of quartz wool.

HPLC was performed using a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV detector in series with a β^+-flow detector. Data collection and decay correction were achieved on a personal computer using the Beckman System Gold Chromatography Software Package. Autoradiographic images of TLC plates were obtained using a Molecular Dynamics Phosphorimager (Sunnyvale, California, 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, 3kV capillary voltage, 400V lens voltage and 40V 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 - The precursor for the radiosynthesis, 5-acetylamino-4-amino-2,6-anhydro-3,4,5,-trideoxy-D-glycero-D-galacto-non-2-enoic acid (GR121158X), and 5-acetylamino-4-guanidino-2,6-anhydro-3,4,5,-trideoxy-D-glycero-D-galacto-non-2-enoic acid (GG167) (for use as chromatography reference substance) were supplied by Glaxo Wellcome Research and Development. All other chemicals were of analytical grade purity and used as received

Synthesis of [^{11}C]GG167 - The precursor (GR121158X, 3-4mg, 10-14μmol) was dissolved in 0.1M sodium borate pH8.0 (300μl) in a conical mini-vial (0.9ml) equipped with a septum. The [^{11}C]cyanogen bromide was trapped in this solution at room temperature, and then heated at 80°C for 5min. A solution of 20% ammonium chloride in 25% ammonium hydroxide (300μl) was added, and heating continued for 5min at 120°C. The reaction mixture was then diluted with absolute ethanol (9ml) and passed through a silica solid-phase extraction cartridge (SPE-ED, 1.0g), previously conditioned with water (5ml) and absolute ethanol (10ml). The column was washed with absolute ethanol (5ml), and then eluted with 0.1M sodium borate pH8.0 (2.5ml). The eluate was injected into the semi-preparative HPLC system (Nucleosil NH_2, 5μm, 250×10mm id, eluted with acetonitrile/water, 61/39, flow rate 5ml min^{-1}, column temperature 40°C). The fraction eluting at 5.5-8min was collected and transferred to a rotary evaporator to remove the solvent. The residue was redissolved in aqueous nasal spray solution and sterile filtrated (Dynagard ME, 0.22μm) into a sterile ampoule. A sample (20μl) of this solution was analysed using either of the following HPLC and TLC methods.

Method A - Column: Nucleosil LC-NH_2, 5μm, 250×4.6mm id, eluted with 10mM ammonium dihydrogen phosphate, pH 2.5/ acetonitrile-water, 50-7, 25/75 (v/v), isocratic elution, flow rate 2ml min^{-1}, UV detection at 210nm, injection volume 20μl. The retention time for [^{11}C]GG167 was 6.1min (k' 3.07).

Method B - Column: ASAHIPAK NH_2P-50, 250×4.6mm id eluted with 5mM ammonium sulphate, pH 6.2/ acetonitrile, 40/60 (v/v), flow rate 0.8ml min^{-1}, UV detection at 210nm, injection volume 20 μl. The retention time for [^{11}C]GG167 was 14.1min (k' 3.70).

Thin-layer chromatography (Merck Silica gel F254 plates eluted with water/acetonitrile/methanol, 30/60/9, v/v) showed a single radioactive spot (R_f 0.31), coeluting with authentic GG167.

Mass-spectroscopic analysis

To enable mass-spectroscopic detection of the labelled product, a carrier-added synthesis was performed by adding unlabelled cyanogen bromide (0.5mg, 4.7μmol) to the reaction vessel before trapping the radioactivity, whereafter the reaction mixture was treated as described. The resulting product solution contained 12.1μg ml^{-1} of GG167 and was subsequently used in the LC-MS determination. Samples (40μl) were analysed on a Nucleosil LC-NH_2 RP, 5μm, 250×4.6mm id held at 40°C, mobile phase water/acetonitrile/ 100mM trifluoroacetic acid, 69/29/2, (v/v), flow rate 1.5ml min^{-1}, UV detection at 210 nm. The retention time (radiochannel) for [^{11}C]GG167 was 2.86min, k' 1.8, m/z 333.14 $[M+H]^+$, calculated mw 332.13.

Results and Discussion

A previous report has described the synthesis and applications of the electrophilic labelling precursor [^{11}C]cyanogen bromide (3). In the present study, an improved solid-phase method for the production of [^{11}C]cyanogen bromide was applied, affording shorter synthesis times and a simpler experimental setup (Westerberg G and Långström B, to be reported). The production of [^{11}C]cyanogen bromide, as well as the entire synthesis, formulation and quality control of [^{11}C]GG167 was performed by an automated procedure (6).

The key step in the synthesis of [^{11}C]GG167 was the addition of ammonia to the intermediate [^{11}C]cyanamide. The yield of [^{11}C]GR163216X was near-quantitative with respect to [^{11}C]cyanogen bromide, whereas the conversion to [^{11}C]GG167 proceeded with a low yield on account of pressure buildup and uncontrolled evaporation of reaction mixture when penetrating the septum.

Also, because of the very polar nature of the compounds, losses were encountered in the purification procedure. Thus, 200-1000MBq (5-30mCi) of [^{11}C]GG167 was obtained in a synthesis time of 46-50 min from the end of bombardment, including purification and quality control. The mean yield was 228±64MBq (6.2±1.7mCi, mean value ± SD, n=16). Work is now in progress to develop a system allowing the use of supercritical ammonia (P_c 112.5atm, T_c 132.5°C) for the synthesis of [^{11}C]guanidines from [^{11}C]cyanamides, and for on-line preparative supercritical fluid chromatography of the reaction mixture.

Compound identity was assessed using two independent HPLC systems and thin-layer chromatography by co-injection of authentic reference compound and by LC-MS analysis. In all cases, a single radioactive component was obtained, co-eluting with authentic reference substance. The LC-MS analysis showed an M+1 signal corresponding to GG167. The radiochemical purity was in all cases higher than 95%, as determined using two independent analytical HPLC systems and thin-layer chromatography. Re-analysis of samples after 80min showed an unchanged radiochemical purity. The concentration of GG167 in the product solution was in all cases lower than the detection limit of 0.07μg ml^{-1}, implying a specific radioactivity higher than 260GBq μmol^{-1} (7Ci μmol^{-1}). The product was found to be sterile and free from pyrogens, according to controls by an independent laboratory (Department of Clinical Microbiology, Uppsala University Hospital).

The labelled compound is now being used in human *in vivo* PET studies of drug deposition and disposition. Details of these studies and the syntheses of other isotopically labelled versions of GG167, by alternative labelling procedures (Carr RM, Newman RJJ and Sutherland DR, manuscript in preparation), will be reported elsewhere.

References

1 Woods RM, Bethell RC, Coates JAV, Healy N, Hiscox SA, Pearson BA, Ryan DM, Ticehurst J, Tilling J, Walcottand SM and Penn CR - *Antimicrob Agents Chemother* 37: 1473 (1993).

2 Chandler M, Bamford MJ, Conroy R, Lamont B, Patel B, Patel VK, Steeples IP, Storer R, Weir NG, Wright M and Williamson C - *J Chem Soc, Perkin Trans 1*, 1173 (1995).

3 Westerberg G and Långström B - *Acta Chem. Scand* 47: 974 (1993).

4 Christman DR, Finn RD, Karlstrom K and Wolf AP - *Int. J Appl Radiat Isot* 26: 435 (1975).

5 Iwata R, Ido T, Takahashi T, Nakanishi H and Iida S - *Int. J Appl Radiat Isot* 34: 97 (1983).

6 Bjurling P, Reineck R, Westerberg G, Schultz J, Gee A, Sutcliffe J and Långström B - *Proc VIth Workshop on Targetry and Target Chem, Vancouver, BC, Canada, August 1995.*