Radiosynthesis of [^{11}C]-N-Methylacyclovir

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Summary

A selective and potent analogue of the anti-herpes agent acyclovir has been labelled with ^{11}C in good yield and at high specific activities. 1-[^{11}C]-Methyl-9-[(2-hydroxyethoxy)methyl)guanine may be useful for detecting HSV encephalitis using positron emission tomography.

Key Words: carbon-11, acyclovir, HSV encephalitis, PET

Following the discovery that the guanosine analogue 9-[(2-hydroxyethoxy)-methyl]guanine (acyclovir) was a potent and selective anti-herpes drug (1), many derivatives have been synthesized and tested as anti-viral agents (2, 3). One of these, 1-methyl-9-[2-hydroxyethoxy)-methyl]guanine (methylacyclovir, MAC), has demonstrated equivalent efficacy and selectivity to acyclovir in inhibiting HSV-1 and HSV-2 herpes simplex viruses (2) and is suitable for labelling with the positron emitting radionuclide, ^{11}C. The development of radiolabelled antiviral agents which selectively localize in infected cells may permit the non-invasive detection of HSV encephalitis using positron emission tomography (PET), and perhaps preclude the need for invasive tissue biopsy for definitive diagnosis. We report here the radiosynthesis and purification of [^{11}C]-MAC (4) which may prove to be a useful radiotracer for such studies.

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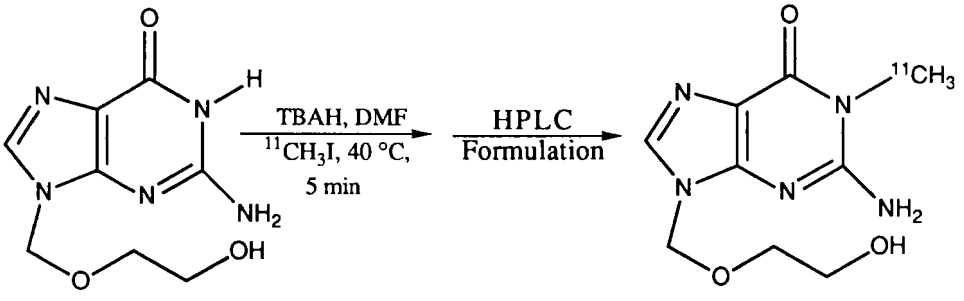
Unlabelled MAC was prepared by modification of a literature procedure (2). Using acyclovir as precursor, the radiosynthesis of [^{11}C]-MAC (Scheme 1) is a facile procedure . Treatment of the conjugate base of acyclovir, generated at -70 °C in DMF with tetrabutylammonium hydroxide, with [^{11}C]-iodomethane effected [^{11}C]-methylation to give the desired product. In the absence of added base methylation occurs more slowly and primarily at N_7 rather than the desired N_1 (2). In consideration for the rapid decay of ^{11}C ($t_{1/2}$ 20.3 min), the reaction was conducted at 40 °C to ensure completion in five min.



Acyclovir **[^{11}C]-MAC**

Scheme 1.

Rapid purification of [^{11}C]-MAC was achieved by semi-preparative HPLC. Upon isolation of the product-containing fraction from the HPLC, the product was formulated suitable for animal or human studies. The average (n = 6) time of synthesis was 21 min from end-of-bombardment to formulated product ready for biological studies. The average isolated radiochemical yield was 20% based on [^{11}C]-iodomethane (uncorrected for decay) and the average specific activity was 955 mCi/μmole at end-of synthesis. Radiochemical purities were greater than 98%.

Experimental

NMR spectra were obtained on an IBM NR/80 using $(CH_3)_4Si$ as an internal standard. DMF was stirred overnight with BaO, then distilled under reduced pressure from BaO. Purification and analyses of radioactive mixtures by HPLC were performed with a previously described system (5). Peak areas were measured using Hewlett-Packard 3390A recording integrators. Isolated radiochemical yields were determined with a dose-calibrator (Capintec CRC-12). All formulated radiochemical preparations tested sterile and pyrogen-free.

N-Methylacyclovir (MAC) A solution of 9-[(2-hydroxyethoxy)methyl]guanine (50 mg, 0.21 mmol) and tetrabutylammonium hydroxide (0.22 mL of a 1M solution in methanol) in DMF (4.6 mL) was stirred at room temperature whilst a solution of iodomethane (32.6 mg, 0.23 mmol) in DMF (150 µL) was added over 5 min. The mixture was stirred for a further 20 min and the crude product collected by vacuum filtration upon precipitation with ether (50 mL). Recrystallization from water afforded pure product as white crystals (27 mg, 54%): mp 233-237 °C (dec.) (lit. 253 °C (2)). The ^{1}H NMR spectrum (in DMSO-d_6) was nearly identical to that previously reported (2).

[^{11}C]-MAC [^{11}C]-Iodomethane, produced as previously described (6), was swept by a stream of nitrogen into a freshly prepared solution of acyclovir (1-1.2 mg, 4.3-5.1 µmol) in DMF (50 µL) containing 0.5 equivalents of aqueous tetrabutylammonium hydroxide (0.4 M) at -70 °C. The reaction mixture was heated in a water bath at 40 °C for 5 min then quenched with 0.1N ammonium formate (900 µL) and injected onto the HPLC column (Alltech Econosil C_{18}; 25cm x 10mm; 5% CH_3CN:95% H_2O + 0.1N NH_4HCO_2 10 mL/min; k'$_{MAC}$= 4.5). The appropriate fraction was collected, volatiles removed by rotary evaporation under vacuum, and the residue taken up in sterile pyrogen-free saline (7 mL). After filtration through a Millipore filter (0.22 µ) the solution was adjusted to physiological pH by addition of sterile pyrogen-free 1N sodium bicarbonate. The radiochemical purity and specific activity were determined by analytical HPLC (7) (Alltech Econosil C_{18}; 25cm x 4.5mm; 5% CH_3CN:95% H_2O + 0.1N NH_4HCO_2 4 mL/min; k'$_{MAC}$= 3.5).

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