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Radioiodination of 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-iodobenzene (dRFIB), a putative thymidine mimic nucleoside for cell proliferation studies

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Abstract

Iodine-124 was produced via the ^{124}Te(p,n)^{124}I reaction by 15 MeV proton irradiation of an in-house solid mass tellurium dioxide target, using the Tübingen PETtrace^{TM} (General Electric Medical Systems) cyclotron. 1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-iodobenzene (dRFIB), a stable, non-polar thymidine mimic nucleoside, was synthesized in 5 steps following a literature method, for radioiodination with [^{124}I] iodide via isotope exchange in the presence of copper sulphate and ammonium sulphate in methanol-water. The radiolabelling procedure was optimized with respect to temperature, amount of dRFIB, amount of sodium hydroxide and reaction time, to produce radiochemical yields of up to 85% with a 1-h reaction at 140 °C. With routine I-124 production of 30 MBq/run, relatively high specific activities, approaching 100 MBq/mmol, can be expected. The activation energy for dRFIB radioiodination was calculated from temperature-time RCY data to be approximately 100 kJ/mol using no-carrier-added [^{124}I]iodide.

Keywords: I-124; Radioiodination; Thymidine mimic; Difluorophenyl deoxyribonucleosides; 1-(2-deoxy-β-D-ribofuranosyl)-2; 4-difluoro-5-iodobenzene; dRFIB

1. Introduction

Deoxynucleosides are the building blocks of DNA. During S-phase of the cell cycle, deoxynucleosides are phosphorylated to the respective triphosphate nucleotides, which under the influence of DNA polymerases, are used to build new strands of DNA along single strands of template DNA. Cellular nucleosides are formed de novo from fundamental cell components including amino acids, formate and hydrogen carbonate, and via a salvage pathway that provides nucleosides by recycling intermediate products of nucleic acid catabolism (Kornberg and Baker, 1992).

The in vivo biosynthesis of DNA, and therefore, indirectly, cell proliferation, can be monitored by administering radiolabelled natural deoxynucleosides such as ^{11}C-thymidine (^{11}C-TdR, Goethal et al., 1996). As is frequently the case, the natural substrates (i.e., ^{11}C-TdR) provide complex biodistribution/imaging data because of their numerous and rapidly formed catabolic products (Shaw and MacPhee, 1986). Therefore, chemically altered nucleosides that are substrates for nucleoside transporters and thymidine kinase (TK), but not subject to catabolism, are preferred for imaging cell proliferation. Of the numerous radiolabelled nucleosides reported, 3′-[^{18}F]fluoro-3′-deoxythymidine (FLT) (Shields et al., 1998) is used almost universally for clinical cell proliferation assessments in oncology. Critical elements of effective TK substrates for cell proliferation imaging have been reviewed (Wiebe, 2007).

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Kool and colleagues synthesized 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-methylbenzene (dRFMB), a hydrophobic, non-hydrogen-bonding thymidine analogue based on the 2,4-difluoro-5-methylphenyl aglycone, to examine the importance of hydrogen bonding during DNA replication (Schweitzer and Kool, 1994). This nucleoside has a chemically stable C–C nucleoside bond, and its triphosphate (dRFMB-TP) was incorporated into *Eschreschia coli* DNA with high fidelity opposite adenine by the Klenow fragment of polymerase I. Importantly, incorporation efficiency was similar to that of thymidine triphosphate (TdR-TP) (Schweitzer and Kool, 1995; Moran et al., 1997, 1997a). On the other hand, a series of difluorophenyl nucleoside analogues of dRFMB, designed as antivirals and anti-cancer compounds, were remarkably devoid of biological activity (Wang et al., 2000, 2000a, 2000b, 2001, 2001a; Naimi et al., 2001). Their low toxicity was unexpected given the strong interaction of the 5-iodo analogue [1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-iodobenzene; dRFIB] with a cloned nucleoside transporter (Smith et al., 2004), but can be rationalized at least in part by the weak interaction of dRFIB with TK in vitro. In vitro studies have revealed low phosphorylation rates for dRFIB (TK-1 9.9%; TK-2 73.6%) relative to thymidine (Al-Madhoun et al., 2004). Selectivity for TK-2 over TK-1 may indicate that radiolabelled dRFIB would be better suited for gene therapy imaging with the HSV-1 TK—ganciclovir protocol than for proliferation imaging (Wiebe, 2007).

The radioiodination of dRFIB is now reported, in support of further investigation of the in vivo biological properties of this class of compounds.

2. Materials and methods

Reagent grade chemicals and chromatography grade solvents were purchased from commercial suppliers. ^{1}H-NMR spectra were measured in $CDCl_3$ on a Brucker 250 MHz spectrometer; ^{1}H chemical shifts (δ) were recorded relative to TMS (internal reference 0.00 ppm). Silica gel 60 (40–60 μm; Merck) was used for silica gel flash chromatography. HPLC with water:methanol (45:55 v/v; 4 mL/min) eluent and a Partisil ODS 3 column (10 μm; 50 mm × 8 mmϕ) gave retention times of 6.9 and 2 min, respectively, for dRFIB and iodide. dRFIB was detected on line by UV (254 nm) and radioactivity (NaI/Tl crystal 2 in) detectors in sequence. C18 Reversed phase cartridges (Waters SepPak) were used to condition reactions prior to HPLC analysis.

Iodine-124 was produced via the ^{124}Te(p,n)^{124}I reaction on an in-house solid mass tellurium dioxide target, using the Tübingen PETtrace^{TM} (General Electric Medical Systems) cyclotron (16.5 MeV protons). The target consisted of a mixture (300 mg) of 94% tellurium dioxide (>99% ^{124}Te; Chemotrade, Germany) and aluminium oxide (6%) melted onto a platinum/iridium plate, to produce a target density of ~200 mg/cm^2. The target plate was placed into the target holder at an angle of 10° to the incident beam in order to substantially increase beam path-length through the target material, thereby enabling a reduction in beam current for more efficient cooling without loss of yield; in addition, there is an increase in the effective beam strike area on the target (Finn et al., 1995).

The tellurium oxide targets were irradiated with 15 MeV protons at 5–13 μA for 10–120 min. After irradiation, the iodine was recovered from the target by dry distillation at 720 °C (Knust et al., 2000). Volatile iodine was trapped in a stainless steel capillary which was pre-coated with NaOH by rinsing with aqueous NaOH (4 mM). The trapped radioactivity was collected by repeated washing of the capillary with NaOH solution (4 mM; 300 μL). I-124 radioactivity yield and purity were determined by γ-ray spectroscopy (Weinreich and Knust, 2000).

1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-iodobenzene (dRFIB) was synthesized in 5 steps following the literature method shown in Scheme 1. dRFIB was recovered as a white solid (55 mg, 0.15 mmol; mp 70 ± 2 °C); NMR data matched literature values (Wang et al., 2001).

2.1. General procedure for radioiodination

1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-iodobenzene (dRFIB) was radioiodinated with [^{124}I]iodide via isotope exchange as illustrated in Scheme 2. dRFIB (1.4 mg in 100 μL methanol) was mixed with a solution of copper sulphate and ammonium sulphate in water, and the required amount of radioiodine in sodium hydroxide was added. The vial caps were fitted with inlet and venting needles, the latter fitted with activated carbon cartridges to trap any volatile radioactivity (radioiodine and/or hydriodic acid). The reaction vials were closed, the radioactivity was measured and then the solvent was evaporated under a stream of dry nitrogen. The radioactivity was measured again to determine loss of radioactive iodine during the drying procedure. After heating, the vial contents were taken up in solvent for recovery of radioiodinated dRFIB using a C-18 cartridge. The C-18 cartridges were pre-activated with methanol (10 mL) and washed with water (10 mL). The reaction solutions (nominally 320 μL) were loaded onto the cartridge, followed by a water flush (10 mL) and elution with methanol (4 mL) and a flush with air (10 mL). The aqueous fraction contained iodide while the methanol fraction contained the labelled product, which was assayed by radio-HPLC. Reaction conditions were optimized for radiochemical yield (RCY) by varying temperature, amounts of the precursor and NaOH in the reaction vial, and reaction time.

2.2. Effect of reaction temperature on RCY

Aliquots of dRFIB (1.4 mg in 100 μL methanol) solution were placed in individual reaction vials (1 mL), and solutions