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Research Article

Radiosynthesis of [*N*-methyl-^{11}C]methylene blue

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Summary

In this paper we present the radiochemical synthesis of the novel compound [*N*-methyl-^{11}C]methylene blue. The synthesis of [*N*-methyl-^{11}C]methylene blue was accomplished by means of ^{11}C-methylation of commercially available Azure B using [^{11}C]methyl trifluoromethanesulfonate ([^{11}C]methyl triflate). Following purification [*N*-methyl-^{11}C]methylene blue was obtained with a radiochemical purity greater than 97% in a 4–6% decay corrected radiochemical yield. The synthesis was completed in an average of 35 min following the end of bombardment. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: [*N*-methyl-^{11}C]methylene blue; [^{11}C]methyl triflate; PET

Introduction

As part of our oncology research programme to develop novel positron emission tomography (PET) tracers, 3,7-bis(dimethylamino)phenothiazine-5-ium chloride (methylene blue) was chosen for labelling with the positron emitting ^{11}Carbon (^{11}C) isotope. Methylene blue is a low molecular weight, water soluble, tricyclic organic compound, which diffuses through the cellular membranes and accumulates selectively in melanoma cells.[1] Melanoma is the most serious form of skin cancer and

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claims around 2000 lives each year in the United Kingdom of Great Britain.[2] According to Cancer Research UK[2] malignant melanoma is the 11th most common cancer in women, and the 12th most common cancer in men with over 5700 new cases of melanoma each year in the UK. Melanoma develops from cells producing melanin, a pigment that protects the deeper layers of the skin from the damaging effects of the sun.[2] Methylene blue possesses a very high affinity to melanin by forming a charge transfer complex with the pigment.[3] Over several years Link *et al.* have carried out clinical research focusing on methylene blue labelled with relatively long lived radioisotopes such as ^{211}Astatine (^{211}At, half-life ($t_{1/2}$) = 7.2 h), ^{123}Iodine (^{123}I, $t_{1/2}$ = 13.2 h) and ^{131}Iodine (^{131}I, $t_{1/2}$ = 8 d).[1] They investigated the α-particle emitter compound [^{211}At]methylene blue as a therapeutic agent and were able to prove that this radioactive compound prevents metastatic spread and controls the growth of melanoma when given to human-melanoma-bearing animals.[1] They also investigated the γ-emitting ^{123}I- and the β-emitting [^{131}I]methylene blue compounds for diagnostic purposes of disseminated melanoma. Using a gamma camera they concluded that in particular the ^{131}I labelled compound was suitable for the detection of melanoma metastases.[1] Our aim was to label the heterocyclic organic compound methylene blue with the short lived positron emitting ^{11}C isotope ($t_{1/2}$ = 20.4 min). This labelled compound would be structurally identical compared to non radioactive methylene blue and hence would show the same biodistribution, which is important for PET studies. Therefore [*N*-methyl-^{11}C]methylene blue may be very useful in particular as an *in vivo* PET tracer for patients suffering from melanoma and probably other diseases. Here we describe the radiosynthesis of the novel positron emitting compound [*N*-methyl-^{11}C]methylene blue.

Experimental

Chemicals and solvents

All reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. All used solvents were purified and degassed according to standard procedures.

Analytical methods

All analyses of the labelled compounds were performed with a Gynkothek HPLC system (P580 pump) and variable Wavelength UV/

VIS detector (at 664 nm) coupled in series with a BIOSCAN NaI detector (B-FC-3200). The HPLC system was operated using a Phenomenex Luna C-18 column (150 × 3.0 mm, particle size: 5 μm). The eluent was produced by adding 0.75% of acetic acid and 0.25% of methane sulfonic acid to a mixture of HPLC grade acetonitrile and distilled water (1:4). The eluent was filtered and degassed with helium before use. The flow rate was set at 1 ml/min.

Preparation of the silver trifluoromethanesulfonate column

The silver trifluoromethanesulfonate (silver triflate) column was prepared according to D.M. Jewett.[4] Coarse silver triflate (1.0 g) and Graphpac-GC 80/100 (2.0 g, Alltech) was ground to a homogenous mixture. An empty stainless steel HPLC C-18 Luna column (250 × 3 mm) was loosely packed (10 cm length) with the mixture into the central region and to restrain the packing material both ends of the column were fitted with glass wool. Before the first reaction the column was inserted into a tube furnace (carbolite furnaces) and conditioned under argon gas flow for 30 min at 300°C.

[^{11}C]Carbon dioxide radiosynthesis

[^{11}C]Carbon dioxide was prepared by proton bombardment of a gas mixture (98% N_2, 2% O_2) by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction. The gas target was pressurised to 270 psi and irradiated with 11 MeV protons produced by the CTI RDS-111 cyclotron at the John Mallard Scottish P.E.T Centre in Aberdeen. Irradiations of 10 min with a beam current of 27 μA were typically used.

[^{11}C]Methyl iodide radiosynthesis

[^{11}C]Methyl iodide was prepared according to the traditional lithium aluminium hydride (LAH)/hydriodic acid (HI) method.[5] At the end of bombardment (EOB) [^{11}C]carbon dioxide was transferred from the target in a stream of helium gas to the remote controlled automated [^{11}C]methyl iodide module where it was passed into 200 μl of a cooled 0.1 M solution of LAH in tetrahydrofuran (THF). The [^{11}C]carbon dioxide reacted with LAH to produce the [^{11}C]methoxide anion. The first reaction vessel was then heated to 130°C to evaporate the solvent. After completing the THF evaporation the contents of the reaction

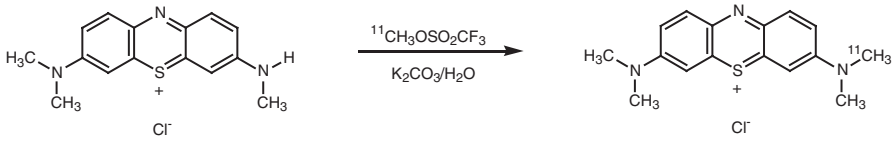
vessel were cooled down to 0°C and 1 ml of 10%-phosphoric acid was added to synthesise [^{11}C]methanol. [^{11}C]Methanol was then distilled to the second reaction vessel containing 600 µl of HI. The second reaction vessel was heated to 135°C to produce on average 4.8 GBq of [^{11}C]methyl iodide. The average specific activity was 780 GBq/mmol.

[^{11}C]Methyl trifluoromethanesulfonate radiosynthesis

[^{11}C]Methyl trifluoromethanesulfonate ([^{11}C]methyl triflate) was prepared according to the method of Jewett.[4] In a stream of helium gas the [^{11}C]methyl iodide was passed through the silver triflate graphpac column which was connected in series to the [^{11}C]methyl iodide module. The column was inserted into a tube furnace operated at 200°C synthesising on average 2.0 GBq of [^{11}C]methyl triflate.

[N-methyl-^{11}C]Methylene blue radiosynthesis

The synthetic scheme for the radiosynthesis of [*N*-methyl-^{11}C]methylene blue, **2**, using [^{11}C]methyl triflate is presented in Figure 1. The [^{11}C]methyl triflate was trapped in a reaction vessel containing a solution of Azure B, **1**, (1 mg, 3.27 µmol) and potassium carbonate (K_2CO_3) (20 mg, 144.72 µmol) in 1.5 ml of sterile water. After the collection of [^{11}C]methyl triflate the solution was stirred at room temperature (RT) for 5 min. The solution was transferred on to a cation exchange cartridge (Waters, Sep-Pak Accell Plus CM) which was washed with 5 ml of ethanol and 15 ml of sterile water. Then the cartridge was rinsed with 10 ml of sterile sodium chloride 0.9% w/v solution to provide **2**. Radiochemical purity and specific activity of the final solution was determined by HPLC. The identity of the radiolabelled product was confirmed via co-injection with a commercial sample of methylene blue. The retention time in the UV-chromatogram was identical to the retention time of **2** in the radioactivity-



1 **2**

Figure 1. Radiochemical synthesis of [*N*-methyl-^{11}C]methylene blue, 2, by means of methylation of Azure B, 1, using [^{11}C]methyl triflate ($^{11}CH_3OSO_2CF_3$)

chromatogram. In all experiments **2** was obtained with a radiochemical purity greater than 97% in an averaged 4–6% radiochemical yield based on [^{11}C]methyl iodide. The average specific activity was 1.5 GBq/μmol.

Results and discussion

It is interesting to point out that [^{11}C]methyl iodide is not only the fastest reacting methyl halide in nucleophilic substitution (S_N2) reactions such as *N*-, *O*- and *S*-methylation procedures[6] but it is also regarded as the most commonly used labelling agent for the preparation of ^{11}C-radiotracers.[7] Hence to achieve our aim, the radiosynthesis of [*N*-methyl-^{11}C]methylene blue, **2**, we decided to carry out preliminary experimental work choosing [^{11}C]methyl iodide as the methylating agent and Azure B, **1**, as the substrate. Several experiments were performed to obtain [^{11}C]methylene blue via a single step reaction by ^{11}C-methylation of **1** using a base and [^{11}C]methyl iodide. However in all our experiments both, radioactive yield and radiochemical purity proved to be unsatisfactory. The highest radiochemical yield we obtained for **2** was less than 0.5% and attempts to optimize this yield were not successful. Therefore it was decided to focus on [^{11}C]methyl triflate instead. [^{11}C]Methyl triflate is superior to [^{11}C]methyl iodide since it shows a higher reactivity and a more selective attack on *N*-methylation.[6] In addition to this it is a less volatile radioactive precursor and is therefore easier to trap.[4,8] There are several radioactive labelling reactions that have been improved by using [^{11}C]methyl triflate instead of [^{11}C]methyl iodide.[7,9,10] This trend was also confirmed in our study. Experiments using [^{11}C]methyl triflate as a methylating agent increased not only the radioactive yield but also the radiochemical purity of **2**. Compound **2** has been successfully prepared in a single step reaction by ^{11}C-methylation of commercially available **1** (Figure 1) using [^{11}C]methyl triflate. For this an argon filled vial equipped with a magnetic stirring bar was filled with a solution of **1** and potassium carbonate in sterile water and subsequently placed on a magnetic stirrer 5 min prior to EOB. The amount of [^{11}C]methyl triflate trapped in the purple solution reached a maximum (on average 2.6 GBq) after usually 15 min (from EOB). The magnetic stirrer was then switched on and the solution stirred for 5 min at RT resulting in the alkylation of **1** with [^{11}C]methyl triflate. To isolate and purify the product a small disposable cation exchange cartridge was chosen. Transferring the deep blue

solution from the reaction vessel on to the cation exchange resin immobilised the product. The cartridge was subsequently rinsed with ethanol and sterile water to remove not only unreacted starting material **1** but also up to 98% of the radioactive [^{11}C]byproducts. Elution with sterile sodium chloride 0.9% w/v solution desorbed the product **2** from the cartridge. Analytical HPLC showed the product to be >97% radiochemically pure in a 4–6% radiochemical yield and to co elute with a commercial sample of methylene blue at the same retention time of 7.8 min (Figure 2). On average only 7–10 μg/ml of precursor **1** could be

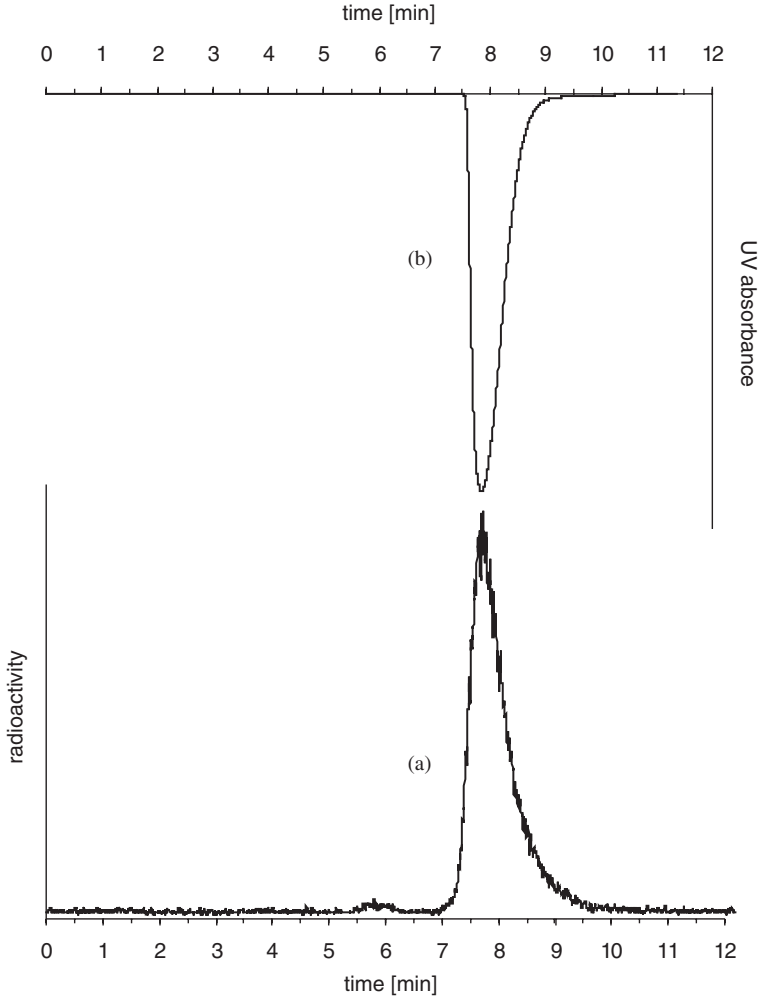


Figure 2. (a) Radioactivity chromatogram of [*N*-methyl-^{11}C]methylene blue (98%, 7.8 min). The minor peak at 5.8 min is unidentified. (b) UV-chromatogram of non radioactive methylene blue (7.8 min)

found in the product rinse, as determined by the UV detection spectrum. The total synthesis time from EOB is 35 min. In all experiments the product was obtained with specific average activities (from end of synthesis) of 1.5 GBq/μmol. Extensive experimental work is currently in progress in order to improve the synthesis yield and purity of the product. For this different solvents and reaction conditions are being investigated.

Conclusions

The novel radiotracer [*N*-methyl-^{11}C]methylene blue was synthesized via a single step reaction with a radiochemical purity greater than 97% in a 4–6% radiochemical yield and with specific average activities of 1.5 GBq/μmol. The labelled compound may be very useful as an in vivo PET tracer for patients suffering from melanoma, the most serious form of skin cancer. The next stage will be to construct and build a fully automated synthesis module for the regular production of [*N*-methyl-^{11}C]methylene blue.

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