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Research Article

^{11}C labelling of AG957—a potential tyrphostin radiotracer for PET

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Summary

Carbon-11 labelled 4-(*N*-2,5-dihydroxybenzyl)amino methyl benzoate (AG957), a potential radiotracer for imaging bcr–abl receptors was synthesized. [^{11}C]AG957 was prepared by labelling 4-aminobenzoic acid using [^{11}C]CH_3I, which affords the corresponding [^{11}C] methyl ester in excellent yields. Subsequent condensation of the amino group with 2,5-dihydroxybenzaldehyde formed the respective Schiff base. Reduction of this compound with $NaBH_3CN$ gave [^{11}C]AG957 in overall decay corrected radiochemical yield of 65–75% (based on $^{11}CH_3I$) with an average specific radioactivity of 40 GBq/μmol (1.1 Ci/μmol). The total synthesis time from EOB including formulation was 45 min. At physiological pH, the compound was found to be sufficiently stable for *in vitro* and *in vivo* investigations. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: PET; tyrphostins; AG957; chronic myeloid leukaemia; carbon-11

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Introduction

Chronic myeloid leukemia (CML) is a cancer of one of the two main types of white blood cells, the neutrophils. CML is a disease that progresses slowly and is difficult to detect in its early stages.

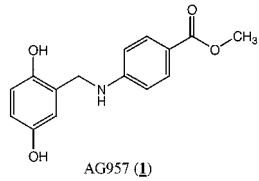
A useful prognostic classification in CML is of considerable importance in order for patients to receive the appropriate therapeutic procedure. So far, clinical criteria such as spleen size, hematocrit and platelet count have been used to classify patients into high-risk and low-risk groups. However, by employing molecular criteria, it may be possible to define low-risk patients with increased accuracy.

The Philadelphia (Ph) chromosome is the most prominent cytogenic abnormality in CML. The existence of this chromosome is due to a translocation, involving the bcr-gene on chromosome 22 and the abl-gene on chromosome 9.[1] The expression of the tyrosine kinase $p210^{bcr-abl}$, a 210 kDa fusion protein, is the result of this translocation. Although the exact function of this protein in the cell is not known, it has been demonstrated that $p210^{bcr-abl}$ induces a myeloproliferate disorder in mice, which is similar to CML. Tyrphostins are inhibitors of this enzyme and have been synthesized as potential drugs.[2,3] The chemical structure of the tyrphostin AG957 (**1**) is shown in Figure 1. This compound is of particular interest because of its small size, which enables the molecule to permeate cells,[4] and because of its selectivity for $p210^{bcr-abl}$ over the normally expressed $p140^{c-abl}$.

AG957 has previously been radiolabelled with ^{3}H and ^{14}C to collect pharmacological data.[5] In this study, we have labelled AG957 with the PET isotope ^{11}C in order to be evaluated as a potential agent for clinical PET studies of chronic myeloid leukaemia.[6]

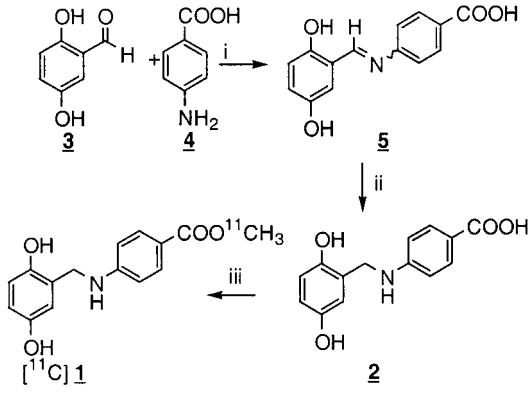
Results and discussion

Two different synthetic pathways leading to [^{11}C]AG957 have been explored. As outlined in Scheme 1, we have initially synthesised 4-(*N*-2,



AG957 (**1**)

Figure 1. Chemical structure of AG957

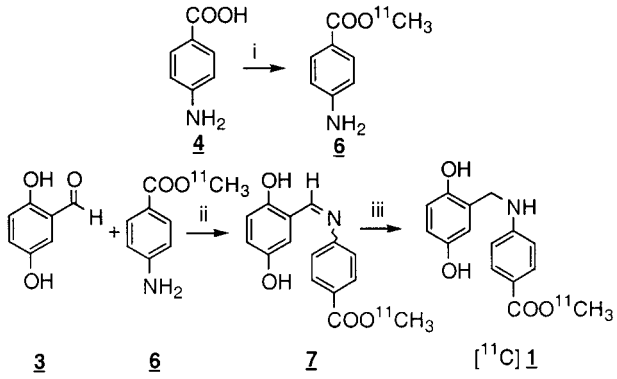


Scheme 1. Synthesis and radiolabelling of AG957 (first pathway). Reagents and conditions: (i) methanol, reflux 9 h; (ii) $NaBH_3CN$, ethanol/water 1 : 1, 1 h, RT; (iii) [^{11}C]CH_3I, $BnNMe_3OH$, 70°C, 5 min

5-dihydroxybenzyl)amino benzoic acid (**2**), assuming that this compound would be a suitable desmethyl precursor for labelling with [^{11}C]CH_3I. The procedure for the synthesis of **2** was based on a published method for the preparation of structurally similar compounds[7] and involves a condensation reaction between 2,5-dihydroxybenzaldehyde (**3**) and 4-amino benzoic acid (**4**) to form the Schiff base **5**. The imino function is subsequently reduced using $NaBH_3CN$ to afford the desired precursor **2**. This reaction sequence is a 'one pot' reaction and does not involve the isolation of the unstable imine **5**.

However, the labelling of **2** with [^{11}C]CH_3I in the presence of benzyl trimethylammonium hydroxide as base produces a complex mixture of radiolabelled products, from which only small amounts of [^{11}C]AG957 could be isolated. The formation of many different radioactive compounds is most likely due to the instability of AG957 in basic medium as well as the labelling of other functional groups in the desmethyl precursor **2**. The use of different bases such as NaH or $NaHCO_3$ did not improve the yield of [^{11}C]AG957.

As outlined in Scheme 2, we have therefore decided to change our synthesis strategy and performed the labelling of 4-amino benzoic acid (**4**) as the first synthesis step.[8] This gave the corresponding [^{11}C] methyl ester **6** in 85–95% decay corrected radiochemical yield, without the formation of any unwanted *N*-methylated side products. Crude **6** was subsequently reacted with 2,5-dihydroxybenzaldehyde (**3**) to give the radiolabelled imine **7**, which was then reduced with $NaBH_3CN$ to form the desired [^{11}C] **1**.



Scheme 2. Synthesis and radiolabelling of AG957 (second pathway). Reagents and conditions: (i) [^{11}C]CH_3I, $BnNMe_3OH$, DMF, 5 min, 70°C; (ii) DMF, TosOH, 5 min, RT; (iii) $NaBH_3CN$, RT, 30 s

This route involves two additional synthesis steps after the labelling reaction and is only possible because of the very fast formation of the imine **7** in the presence of *p*-toluene sulphonic acid and because of the instantaneous reduction of **7** with $NaBH_3CN$. Without *p*-toluene sulphonic acid as catalyst the condensation reaction is too slow to be utilized in PET radiochemistry.

The imine **7** is an unstable compound and cannot be isolated. It undergoes retro-cleavage upon injection into the HPLC system and only starting compounds **3** and **6** can therefore be detected.

The HPLC chromatogram of the crude reaction mixture after addition of 2,5-dihydroxy benzaldehyde and subsequent reduction with $NaBH_3CN$ is shown in Figure 2. The upper trace represents radioactivity, the bottom trace shows UV absorbance.

The radioactive peak at 9.6 min co-elutes with an authentic sample of AG957, which we have synthesized following a published procedure.[7] The radioactive peak at 7.5 min corresponds to the methyl ester **6**. Because of the instability of **7** we could not establish, whether the presence of **6** in the crude reaction mixture was due to incomplete reduction of **7** or incomplete condensation of **3** and **6** from the previous synthesis step.

[^{11}C]AG957 was isolated by semi-preparative HPLC and formulated in 10% ethanol and 0.01 mg/ml of ascorbic acid. The synthesis time without formulation from EOB was 40 min, formulation took an additional 5 min. The formulation was found to be stable at room temperature over a period of >2 h.

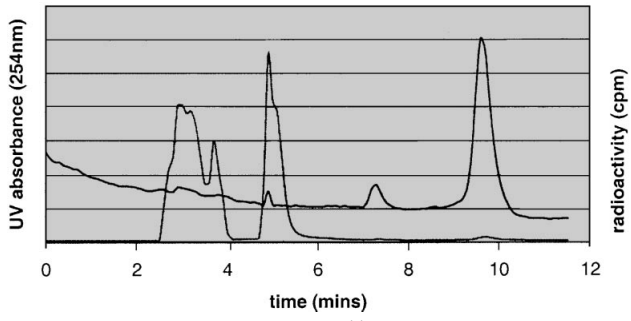
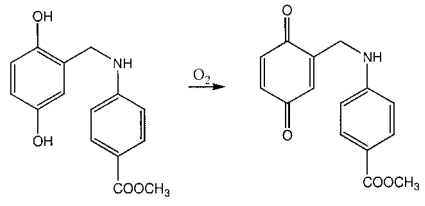


Figure 2. HPLC chromatogram of crude [^{11}C] 1



1 8

Figure 3. Oxidation of AG957

This reaction sequence enabled us to produce [^{11}C] **1** in high radiochemical yields of 65–75% (decay corrected for [^{11}C]CH_3I) and high average specific radioactivity of 40 GBq/μmol (1.1 Ci/μmol). High specific activity should, however, not be required for future biological studies, because of the high density of the target protein.

Previous studies[5] on the stability of AG957 have shown that the molecule decomposes at room temperature when exposed to air and light. However, despite their importance for future metabolite studies, no decomposition products have thus far been identified. Our stability tests on the non-formulated radioligand revealed that AG957 undergoes oxidation to its corresponding quinone **8** if exposed to atmospheric oxygen, as shown in Figure 3.

The oxidation of **1** in the presence of atmospheric oxygen is pH dependent and is faster in basic medium than under acidic conditions, but does not occur within 2 h in the presence of ascorbic acid. In order to ensure reproducibility of our results the addition of ascorbic acid to the final formulation was therefore necessary.

For independent quality control, as well as for future metabolite studies, it was necessary to chemically synthesize and characterize this quinone. We achieved this by reacting AG957 with an aqueous solution

of iodine and extraction of the quinone with ethyl acetate. After characterization by NMR and accurate mass spectroscopy, methyl 4-{[(3,6-dioxocyclohexa-1,4-dien-1-yl)methyl]amino}benzoate (**8**) was used as HPLC standard for independent quality control in order to ensure that oxidation of the formulated radioligand had not occurred.

Experimental

General

$[^{11}C]CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a target gas that consisted of 98.03% nitrogen and 1.97% oxygen. A 10 MeV proton beam was generated using the IBA Cyclone 10/5 cyclotron at the Austin & Repatriation Medical Centre.[9] Typical irradiation parameters were 40 μA/h for 30 min, which produced 22.2–25.9 GBq (600–700 mCi) of $[^{11}C]CO_2$. Except for $LiAlH_4$, all chemicals and solvents were purchased from Sigma-Aldrich $LiAlH_4$ (1 M in THF) was obtained in 1 ml vials from ABX advanced biochemical compounds.

A cold standard of AG957 was synthesized according to a literature procedure.[7] The synthesized compound showed NMR data, which were consistent with the published values.

The labelling procedure including semi-preparative HPLC purification, formulation and sterile filtration was carried out using an in-house built automated system.

Semi-preparative HPLC was performed using a Shimadzu LC-10AS isocratic pump equipped with a 1.2 ml injection loop, a reversed phase column (Exsil C-18, 250 mm, I.D. 10 mm), a Shimadzu SPD-6AV UV detector (254 nm) and a Geiger–Müller tube as radiodetector.

Independent quality control was performed by HPLC, using an Alltech Alltima C-18 reverse phase column (C-18, 53 mm × 7 mm, 20 μl injection loop), with 40 : 60 acetonitrile:water as mobile phase at a flow rate of 1 ml/min. Detection of chemical compounds was achieved with a Shimadzu SPD-6AV UV detector (254 nm) and a Berthold LB506 C-1 monitor for radiochemical detection.

4-(N-2,5-dihydroxybenzyl)amino benzoic acid (**2**)

A solution of 4-amino benzoic acid (0.41 g, 3 mmol) and 2,5-dihydroxybenzaldehyde (0.41 g, 3 mmol) in 20 ml of methanol were

refluxed for 16 h. The reaction was cooled to room temperature and $NaBH_3CN$ (0.22 g, 3.5 mmol) was added. After stirring at room temperature for 2 h the reaction mixture was hydrolysed with 100 ml of 0.1 M HCl and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and the solvent evaporated to give a light yellow oil which was then triturated with $CHCl_3$ and filtered to give 0.2 g (26% yield) of **2** as a light yellow solid.

^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.62 (2H, d, *J* 11.8 Hz), 6.84 (1H, m), 6.61 (1H, d, *J* 11.2 Hz), 6.53 (2H, d, *J* 11.8 Hz), 6.42 (1H, dd, *J* 11.2, 4 Hz), 4.15 (2H, s).

Accurate Mass (ESI) calc (M + H) 260.0917. Found 260.0919.

[^{11}C] Methyl iodide

[^{11}C]CO_2 was purged from the target, trapped in a cryogenic loop using liquid nitrogen and then passed through a vessel, containing a 1 M solution of $LiAlH_4$ in THF (150 µl). After completion of the transfer, the THF was evaporated and non-stabilized HI (300 µl, 55–58%) was added. The vessel was heated up to 125°C to form [^{11}C] methyl iodide.

[^{11}C]AG957 (first pathway)

[^{11}C] methyl iodide was distilled in a stream of nitrogen gas into a reaction vial containing 4-(*N*-2,5-dihydroxybenzyl)amino benzoic acid (**2**) (7 mg, 27 µmol) and benzyl trimethylammonium hydroxide (6.5 µl of a 40 wt% solution in water, 16 µmol) in 300 µl of DMF. After complete transfer of the methyl iodide, the vial was kept at 70°C for 5 min. For injection into the HPLC, 800 µl of mobile phase was added and the crude product was then purified by semi-preparative HPLC using the above-mentioned set-up with 70% 0.1 M ammonium formate/30% acetonitrile as mobile phase at a flow rate of 4 ml/min. Only 185 MBq (5 mCi) of [^{11}C]AG957 could be isolated.

[^{11}C]AG957 (second pathway)

[^{11}C] methyl iodide was distilled in a stream of nitrogen gas into a reaction vial containing 4-amino benzoic acid (3.5 mg, 25 µmol) and benzyl trimethylammonium hydroxide (6.5 µl of a 40 wt% solution in water, 16 µmol) in 300 µl of DMF. After complete transfer of the methyl iodide, the vial was kept at 70°C for 5 min. A solution of

2,5-dihydroxybenzaldehyde (8 mg, 58 μmol) and *p*-toluene sulphonic acid monohydrate (40 mg, 210 μmol) in 300 μl of DMF was then added and the vial was left at room temperature for 5 min.

Sodium cyanoborohydride in tetrahydrofuran (15 μl of a 1 M solution, 15 μmol) was added to the reaction vial containing the red solution of imine **7**. The red colour disappeared immediately upon addition of the cyanoborohydride and the mixture was kept at room temperature for 30 s. For injection into the HPLC, 600 μl of mobile phase was added and the crude product was then purified by semi-preparative HPLC using the above-mentioned set-up with 70% 0.1 M ammonium formate/30% acetonitrile as mobile phase at a flow rate of 4 ml/min.

The radioactive peak at approximately 12 min was collected and the radioligand subsequently formulated in 10% ethanol and 0.01 mg/ml of ascorbic acid using the SepPak method.[10] In the final step, the formulation was filtered through a sterilized 0.22 μm non-vented Millipore GS filter unit. With this method, we produced a decay corrected radiochemical yield of 65–75% (2.8–3.1 GBq, 75–84 mCi) of [^{11}C]AG957 based on [^{11}C]CH_3I. Synthesis time from EOB was 40 min. Formulation using the SepPak method took an additional 5 min.

Methyl 4-{[(3,6-dioxocyclohexa-1,4-dien-1-yl)methyl]amino}benzoate

To a solution of AG957 (273 mg, 1 mmol) in 10 ml of acetonitrile was added a solution of iodine (50 mg/ml, 5 ml) in water. Then, 50 ml of water was added and the aqueous phase extracted with EtOAc. The organic phase was dried over $MgSO_4$ and the solvent removed to form 195 mg (70% yield) of red crystals.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.88 (2H, d, *J* 11.8 Hz), 6.82–6.69 (3H, m), 6.60 (2H, d, *J* 11.8 Hz), 4.28 (2H, d, *J* 2.2 Hz), 3.85 (3H, s).

Accurate Mass (ESI) calc (M + H) 272.0917. Found 272.0920.

Conclusions

In conclusion, [^{11}C]AG957 was synthesized in high yields via a three step synthesis starting from commercially available compounds.

In vivo studies are currently being conducted to determine the potential of this radioligand as a PET radiotracer.

Acknowledgements

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