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Synthesis of [Methoxy-^{11}C]PD153035, a Selective EGF Receptor Tyrosine Kinase Inhibitor

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Summary

[Methoxy-^{11}C]PD153035, a potent and specific inhibitor of the EGF receptor tyrosine kinase, was prepared by *O*-alkylation of *O*-desmethyl PD153035 with [^{11}C]methyl iodide in DMF. The radiochemical incorporation of [^{11}C]CH_3I was on the order of 45%. The mean specific activity obtained at end-of-synthesis (EOS) was 26 GBq/μmol (n=3; range 20-36 GBq/μmol) and total synthesis time was 45-50 minutes including formulation.

Key Words: Carbon-11, Positron emission tomography, PET, EGF receptor, Tyrosine kinase inhibitor, PD153035

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Introduction

Differentiation and proliferation of mammalian cells are controlled by biochemical reactions initiated by the binding of polypeptide factors, such as the epidermal growth factor (EGF), to their receptors on the cell surface (1,2). The EGF receptor is a transmembrane receptor with an extracellular ligand binding domain and an intracellular tyrosine kinase domain (2). Binding of EGF to the receptor activates the tyrosine kinase activity, resulting in autophosphorylation of tyrosine in the receptor. The receptor can now associate with and phosphorylate various substrates in the cell, leading to a cascade of signalling and cellular activities affecting growth (2,3,4). Genetic deviations affecting this signal-transduction pathway are commonly associated with the generation of a malignant cell (2). Overexpression of the EGF receptor has been found in numerous cancers and the extent of overexpression has correlated well with poor clinical prognosis (5).

The diverse nature of the signal-transduction pathway for the EGF receptor yields several possible routes of pharmacological intervention as a means of antitumour treatment (6). One approach has been to use inhibitors for the receptor tyrosine kinase. Recently a very potent and specific inhibitor of the EGF receptor tyrosine kinase, PD153035 (4-(3-bromoanilino)-6,7-dimethoxyquinazoline), **1**, has been synthesized (4,7). The K_i and IC_{50} for PD153035 are 5.2 pM and 29 pM, respectively (4). It has been shown that PD153035 at low nanomolar concentration rapidly suppressed autophosporylation of EGF receptors in fibroblasts and in human epidermoid carcinoma cells.

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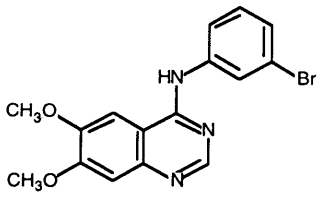
PD153035 also selectively blocked EGF-mediated cellular processes including mitogenesis, early gene expression and oncogenic transformation.

In *in vitro* studies a radioiodinated analogue, 4-(3-iodoanilino)-6,7-dimethoxyquinazoline, **2**, has been shown to bind selectively to the EGF receptor in MDA-468 cells, a human tumour line rich in EGF receptors (8). Furthermore the binding could be allosterically activated by EGF. These *in vivo* results indicates that this type of compound could be potentially interesting as tumour imaging agents.

To explore the *in vivo* characteristics of this family of compounds with PET, two labelling strategies directed toward the aromatic alkoxy substituents, producing **3** and **4**, were recently developed (presented in preliminary communications 9 and 10). Here we report the details of mono-alkylation with $[^{11}C]CH_3I$.



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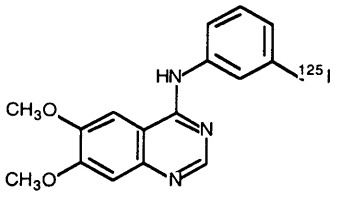

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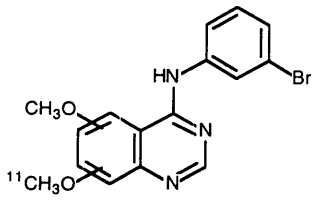

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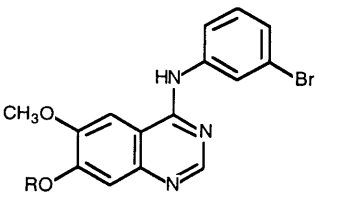


R = $^{11}CH_3$ or $^{18}FCH_2CH_2$

4

Materials and Methods

General

2-Amino-4,5-dimethoxybenzoic acid, **5**, 3-bromoaniline and dibenzyl diselenide were purchased from Aldrich. Phosphorus oxychloride ($POCl_3$), chlorotrimethylsilane ($ClSi(CH_3)_3$), sodium iodide (NaI), hydrobromic acid (HBr), sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$) and sodium borohydride ($NaBH_4$) were obtained from Merck. Boron tribromide (BBr_3) and formamide (H_2NCHO) were obtained from Fluka. Iodotrimethylsilane ($ISi(CH_3)_3$) was obtained from Janssen Chimica.

Sodium benzeneselenoate ($NaSeC_6H_5$) was generated *in situ* from dibenzyl diselenide and $NaBH_4$. Dimethylformamide (DMF), Analar, was distilled from barium oxide and stored over activated molecular sieves (4Å) under N_2 at 4°C. All solvents used were of analytical grade and commercially available.

^{1}H-NMR was recorded using a JEOL FX90Q spectrometer and chemical shifts are reported in ppm downfield from internal tetramethylsilane (0.00 ppm). Melting points were determined using an Electrothermal IA9200 digital melting point apparatus and are uncorrected. Analytical TLC was

performed using Merck 60 F_{254} silica plates with chloroform/ethanol 9:1 as eluent. Column chromatography was performed using silica gel 60 (70-230 mesh) purchased from Merck.

Analytical radio-HPLC was performed using a Shimadzu LC 6A pump, with a Shimadzu SPD-6A UV-spectrophotometer and a Beckman model 170 β-flow radiodetector to monitor the UV-absorption (λ = 254 nm) and the radioactivity, respectively. The column used was a μBondapak C18 (Waters 300 x 3.9, 10 μm) and as eluent CH_3CN/H_3PO_4 (0.01 M) 30:70, flow 4.0 mL/min.

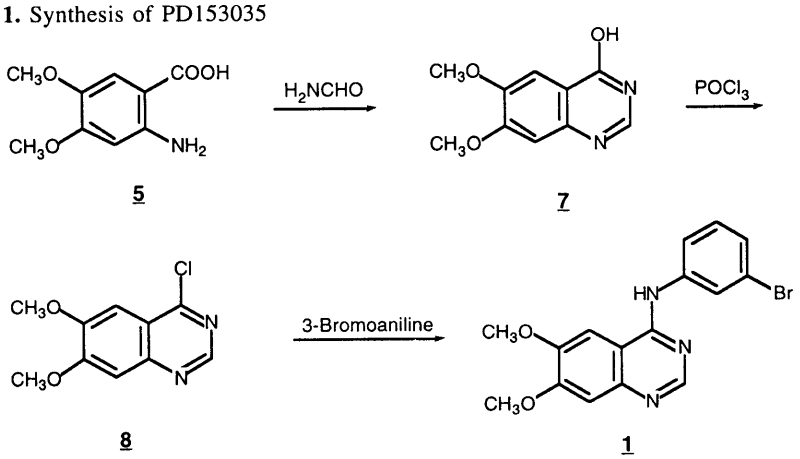
Semi-preparative HPLC was performed using a Shimadzu LC-6A pump with an LDC Spectromonitor II and a GM tube to monitor the UV-absorption (λ = 254 nm) and the radioactivity, respectively. The column used was a μBondapak C18 (Waters 300 x 7.8, 10 μm) and as eluent CH_3CN/H_3PO_4 (0.01 M) 30:70, flow 4.0 mL/min.

Synthesis of PD153035, **1**

PD153035 (4-(3-bromoanilino)-6,7-dimethoxyquinazoline), **1**, was synthesized according to the method of Rewcastle *et al* (7) (Scheme 1). Briefly, 2-amino-4,5-dimethoxybenzoic acid, **5**, (5.0 g, 25.4 mmol) was heated (150°C) with H_2NCHO (4.5 g, 100 mmol) for 7 h. Following isolation by filtration and drying, the brown solid (1.17 g, 5.68 mmol, 22%) corresponding to **7** was refluxed with $POCl_3$ for 4 h. The $POCl_3$ was removed using a rotary evaporator, the residue redissolved in $CHCl_3$ and washed with aqueous NaOH (1 M). The product **8** was isolated using silica chromatography, yielding a creamy white solid (0.93 g, 4.15 mmol, 73%).

Compound **8** (0.93 g, 4.15 mmol), 3-bromoaniline (1.43 g, 8.3 mmol) and a small amount of 3-bromoaniline hydrochloride (0.05 g, 0.23 mmol) in a mixture of CH_2Cl_2 and 2-propanol were heated (60°C) for 2 h. The solution was concentrated on a rotary evaporator, a few drops of concentrated aqueous ammonia was added and the resulting solution was partitioned between water and $CHCl_3$. The phases were separated and the aqueous phase was extracted three times with $CHCl_3$. The pooled organic phases were dried over anhydrous $MgSO_4$, evaporated and the product was isolated using silica chromatography (chloroform/ethanol 99:1), yielding a white solid (1.22 g, 3.39 mmol, 82%).

Scheme 1. Synthesis of PD153035



Melting point: 188-189°C (free amine) (Lit. value: 264-266°C (as hydrochloride) (7)).

^{1}H-NMR [$(CD_3)_2SO$] δ (ppm) 9.5 (br s, 1H), 8.52 (s, 1H), 8.2-8.1 (m, 1H), 7.97-7.75 (m, 2H), 7.45-7.15 (m, 3H), 3.97 (s, 3H), 3.94 (s, 3H)

Synthesis of desmethyl PD153035, **6**

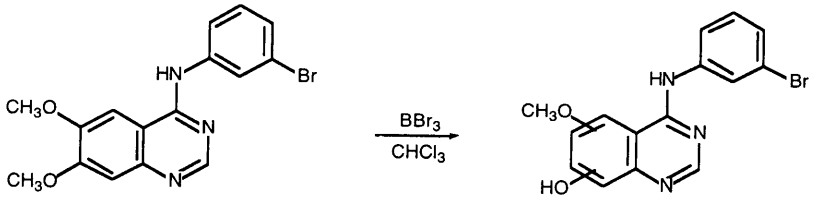
Starting material for the carbon-11 labelling, desmethyl PD153035, **6**, was prepared by mono-demethylation of **1** (Scheme 2). BBr_3 (114.5 µL, 1.19 mmol) in $CHCl_3$ (5 mL) was added drop-wise under nitrogen atmosphere to a solution of **1** (213.9 mg, 0.59 mmol) in $CHCl_3$ (25 mL) at 0°C. The solution was refluxed overnight. No new product had been formed, according to TLC analysis. More BBr_3 (1.0 mL, 10.4 mmol) was added and the solution was refluxed for an additional 24 h, yielding a product (R_f = 0.33) that was more polar than the starting material (R_f = 0.5). This product could be extracted into aqueous NaOH (1 M) but not into saturated aqueous $NaHCO_3$ indicating a weak acid (phenol).

The reaction mixture was cooled and saturated aqueous $NaHCO_3$ (20 mL) was added. The phases were separated and the aqueous phase was extracted twice with $CHCl_3$. The pooled $CHCl_3$ fractions were washed with water twice prior to drying with anhydrous $MgSO_4$. After filtration, the solvent was evaporated and the resulting solid was purified on silica column (chloroform/ethanol 97:3), yielding a white solid (11.1 mg, 0.032 mmol, 5%).

Melting point: decompose 195-200°C.

^{1}H-NMR [$(CD_3)_2SO$] δ (ppm) 9.7-9.5 (very br s, 1H), 9.4 (br s, 1H), 8.48 (s, 1H), 8.3-8.2 (m, 1H), 7.97-7.72 (m, 2H), 7.40-7.15 (m, 3H), 3.98 (s, 3H)

Scheme 2. Demethylation of PD153035



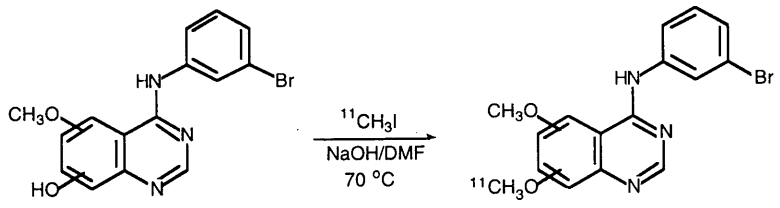
1 **6**

Radiotracer production (Scheme 3)

[^{11}C]Carbon dioxide was produced at the Karolinska Hospital/Institute with a Scanditronix MC 16 cyclotron using 17 MeV protons in the $^{14}N(p, \alpha)^{11}C$ reaction. [^{11}C]CH_3I was prepared by the one-pot method in (11) and trapped in a solution of **6** (0.9 mg, 2.6 µmol), NaOH (10 µL, 5 M) and DMF (0.5 mL). At the end of trapping, the reaction mixture was heated at 70°C for 7 min. Subsequent addition of mobile phase (0.4 mL) quenched the reaction and the labelled PD153035 was isolated using semi-preparative HPLC. The radioactive fraction corresponding to [methoxy-^{11}C]PD153035, **3**, eluting between 13.5 - 15.0 min, was collected and the mobile phase was evaporated using a

rotary evaporator. The residue was redissolved in phosphate buffer (5 mL, 12.3 mM) prior to sterile filtration using a Millipore filter (0.22 μm).

Scheme 3. Synthesis of [methoxy-^{11}C]PD153035



6 **3**

Results and Discussion

The structure of PD153035 suggests two feasible routes for obtaining a radiotracer for *in vivo* imaging applications: replace either the *m*-bromo substituent on the aniline ring with an appropriate radiohalogen or one of the two methyl groups with a labelled alkyl group. Without prior knowledge about the metabolic lability of the substituents, either of the two methoxy groups in PD153035 could be demethylated to obtain the corresponding nor-compound. Subsequent realkylation with [^{11}C]CH_3I by standard procedures would yield [methoxy-^{11}C]PD153035 for biodistribution screening studies.

Methyl-aryl ethers may be demethylated to phenols using a number of reagents (for a review of synthetically useful dealkylation techniques see (12)). Introduction of two [^{11}C]-methyl groups in the same molecule is, of course, not feasible due to the small amounts of alkylating reagent relative to the nor-compound. Therefore, the demethylation technique used should remove only one methyl group. Some factors which may affect the selectivity of dealkylations are: the stereoelectronic characteristics of the ether-cleaving reagent, structural differences between the groups cleaved and differences in the molecular environments of the C-O bonds. For example, hindered ethers are more selectively cleaved by $ISi(CH_3)_3$ or BBr_3 and ethers adjacent to electronegative carbonyls are preferentially cleaved by acidic reagents such as HCl, $AlCl_3$, MgI and boron halides (12).

A number of reagents (BBr_3, $ClSi(CH_3)_3$/NaI, HBr, $ISi(CH_3)_3$ and $NaSeC_6H_5$) were used here in attempts to selectively demethylate PD153035. Only two of the reagents (BBr_3 and $NaSeC_6H_5$) led to the mono-demethylated product **6** at all, albeit in low yields. Reaction with BBr_3 gave **6** in yields ≈ 5% after work-up and column purification. That mono-demethylation had occurred was supported by comparison of the ^{1}H-NMR of the isolated **6** (δ 3.98 (s, 3H, MeO-)) with that of the starting material (δ 3.97 (s, 3H, MeO-) and δ 3.94 (s, 3H, MeO-)). However, which of the aromatic methoxy groups (6- or 7-) was removed has not yet been resolved. Demethylation with $NaSeC_6H_5$ gave several new products. One of these new compounds demonstrated elution behaviour (TLC, HPLC) consistent with the demethylated product obtained using BBr_3. However, its isolation from the complex product mixture proved to be difficult and the yield of the isolated, but not quite pure, product was even lower than that obtained with BBr_3.

Removal of an alkyl group in potentially interesting ligands is a technique commonly used to generate small amounts of precursors to be radiolabelled with [^{11}C]CH_3I for the PET screening studies. It is not quite clear why this particular demethylation proved to be so difficult. Analyses of product mixtures indicated that the di-methoxy starting material did not appreciably react with most of the demethylation reagents under the conditions tested (i.e. **1** could be recovered). PD153035 was rather insoluble, which could have hindered adequate mixing with the reagents. Furthermore, the three amines in the molecule could at least partially scavenge the acidic reagents and thereby aggravate the maintenance of appropriate molar ratios. Salts so formed would decrease even further the amount of substrate in solution. Based on these experiences, we conclude that the synthesis of larger amounts of **6** will benefit from an alternative synthetic strategy.

Radiolabelling was successfully performed by alkylation of the phenol group with [^{11}C]CH_3I using standard methylation conditions. The radiochemical conversion of [^{11}C]CH_3I to **3** after 7 min at 70°C was on the order of 45%, according to analytical radio-HPLC. The total synthesis time including production of [^{11}C]CH_3I, alkylation, HPLC-purification, formulation and sterile filtration was 45-50 min. The radiochemical purity, according to radio-HPLC, was >99% and the identity of radioactive product was confirmed by co-elution with a reference sample of PD153035. The specific activity obtained at end-of-synthesis ranged from 20 to 36 GBq/μmol (n=3; mean 26 GBq/μmol). This value is of comparable magnitude to those we have previously obtained in alkylations with [^{11}C]CH_3I synthesized from [^{11}C]CO_2 obtained from the MC 16 here at Karolinska. These values are consistent with our additional analytical evidence that **6**, prepared and isolated as described here, contained no or very little contaminations of PD153035.

The distribution of [methoxy-^{11}C]PD153035, synthesized according to this method, has been examined *in vivo* in healthy and tumour-implanted rats with PET and is the subject of separate reports (preliminarily in (9) and manuscript in preparation). Based on the promising initial biodistribution studies, we are currently pursuing an alternative route for synthesizing the labelling precursor (in which the position of mono-demethylation is known) for continued evaluation of the potential of [methoxy-^{11}C]PD153035 as a tumour imaging agent.

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