Antitumor Imidazotetrazines. 40.[1] Radiosyntheses of [4-^{11}C-*Carbonyl*]- and [3-*N*-^{11}C-*Methyl*]-8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (Temozolomide) for Positron Emission Tomography (PET) Studies

Gavin D. Brown,*[,†] Sajinder K. Luthra,[‡] Cathryn S. Brock,[†] Malcolm F. G. Stevens,[§] Patricia M. Price,[†] and Frank Brady[‡]

MRC Clinical Sciences Centre, Imperial College of Science, Technology and Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK, Imaging Research Solutions Ltd, Cyclotron Building, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK, and Cancer Research Laboratories, School of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD

Received April 25, 2002

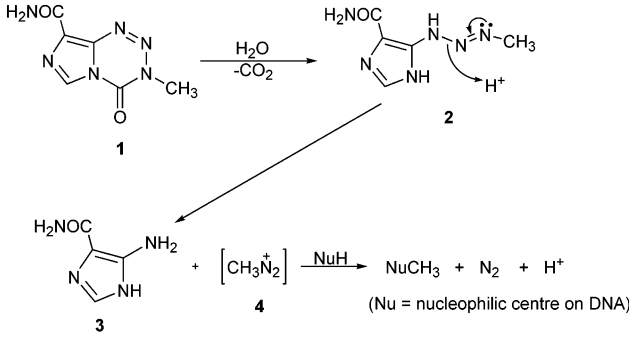
8-Carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (temozolomide, **1**) is an anticancer prodrug. As part of investigations to probe its postulated mode of action using PET we have developed two rapid radiosynthetic routes for the preparation of temozolomide labeled with the short-lived positron emitter, carbon-11 ($t_{1/2}$ = 20.4 min). Reaction of 5-diazoimidazole-4-carboxamide (**7**) with the novel labeling agent [^{11}C-*methyl*]methyl isocyanate (**8**) gave [3-*N*-^{11}C-*methyl*]temozolomide (**9**) in 14−20% radiochemical yield from [^{11}C-*methyl*]methyl isocyanate (**8**) (decay corrected). The position of radiolabeling in the 3-*N*-methyl group was confirmed by [$^{11/13}$C]colabeling and subsequent carbon-13 NMR spectroscopy. Similarly, the reaction of 5-diazoimidazole-4-carboxamide (**7**) with [^{11}C-*carbonyl*]methyl isocyanate (**10**) gave [4-^{11}C-*carbonyl*]temozolomide (**11**) in 10−15% radiochemical yield from [^{11}C-*carbonyl*]methyl isocyanate (**10**) (decay corrected). Apyrogenic samples of [3-*N*-^{11}C-*methyl*]temozolomide (**9**) and [4-^{11}C-*carbonyl*]temozolomide (**11**), with good chemical and radiochemical purities, have been prepared and used in human PET studies.

Introduction

Brain tumors rank among the most aggressive of tumors. Malignant brain tumors such as anaplastic astrocytoma and glioblastoma multiforme are largely incurable despite the attempts of surgical and radiotherapy treatments. Alternative cytotoxic chemotherapy regimens currently used include nitrosoureas such as carmustine and lomustine; the hydrazine derivative, procarbazine and the triazene, dacarbazine; or a combination of procarbazine, lomustine, and vincristine.[2] However, these drugs have several severe side effects and have had little impact on overall survival rates,[3] consequently the prognosis for malignant brain tumors remains poor.

In light of the high mortality rates associated with these cancers, the role of cytotoxic chemotherapeutic drugs continues to be investigated. One such drug which in recent years has emerged as arguably the most effective anticancer agent for the treatment of malignant brain tumors is 8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (temozolomide, **1**). Temozolomide (**1**) initially demonstrated broad-spectrum antitumor activity against murine tumors.[4] In subsequent clinical trials conducted under the auspices of the Cancer Research Campaign, UK, it showed notable antitumor activity against recurrent glioblastoma multiforme,[5−7] recurrent anaplastic astrocytoma,[8] advanced malignant melanoma,[9] and paediatric solid tumors.[10,11] In Europe, temozolomide (**1**) is widely regarded as the preferred drug for the treatment of malignant brain tumors, while in the USA the first comparative studies[12] have shown it gives better clinical results than the drug treatment of choice, procarbazine. In 1998 temozolomide (**1**) was licensed by the European Medicines Evaluation Agency (EMEA) for clinical use in the treatment of glioblastoma multiforme. Then in 1999 it was licensed by both the EMEA and the Food and Drug Administration in the USA for clinical use in the treatment of anaplastic astrocytoma.

Scheme 1

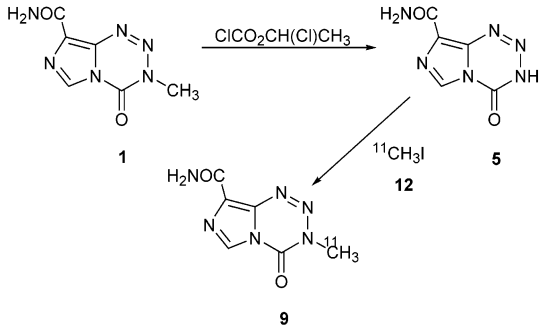


The mechanism of action of temozolomide (**1**) has been investigated and inferred through a number of scientific studies of its crystalline structure,[13] chemical decomposition,[14] and its interaction with DNA using molecular modeling techniques.[15] From these studies it is postulated that temozolomide (**1**) behaves as a prodrug (Scheme 1). Given orally, it is rapidly absorbed systemi-

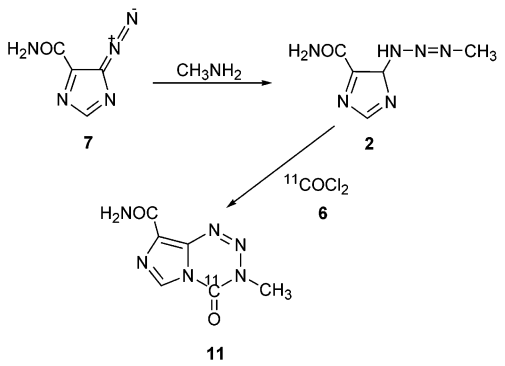
* To whom correspondence should be addressed. Tel: +44-208-3833162. Fax: +44-208-3832029. E-mail: gavin.brown@ic.ac.uk.
† MRC Clinical Sciences Centre.
‡ Imaging Research Solutions Ltd.
§ Cancer Research Laboratories.

10.1021/jm020921f CCC: $22.00
Published on Web 11/05/2002

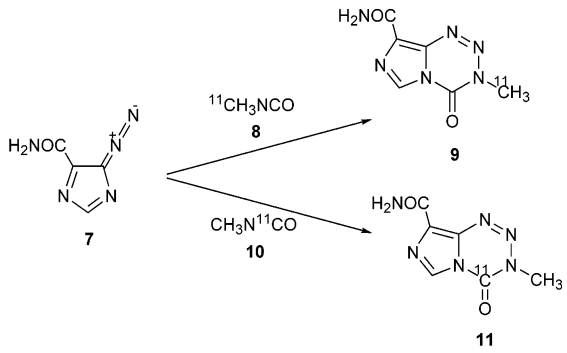
Scheme 2



Scheme 3



Scheme 4



cally and at physiological pH undergoes nucleophilic attack by water at the 4-carbonyl position of the tetrazinone ring to produce 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC, **2**). Under the same conditions, MTIC (**2**) further reacts with water to liberate 5-aminoimidazole-4-carboxamide (**3**) with the generation of the highly reactive methyldiazonium cation **4**.[14] Finally, as the active species, the methyldiazonium cation **4** preferentially methylates DNA at the O^6 position of guanine in guanine-rich regions.[14] All nucleophilic sites on DNA have the potential to become methylated. However, because the sensitivity of tumor cells to temozolomide correlates with levels of the DNA repair protein *O*^{6}-alkylguanine DNA alkyltransferase activity, it is clear that methylation at the O^6 position of guanine is the significant cytotoxic lesion.[16,17]

For any given drug a precise knowledge of its mode of action is an important prerequisite in drug development and the further development of treatment strategies. With this aim, positron emission tomography has been used with [^{11}C]temozolomide to investigate the proposed prodrug activation of temozolomide (**1**) in vivo. To achieve this, we have separately labeled temozolomide (**1**) in two different positions using carbon-11. The positions of labeling were specifically chosen in order to monitor the retention or loss of the radiolabel following activation of the prodrug in accordance with the proposed mechanism (Scheme 1). It was anticipated that retention of the label in tumor would occur if temozolomide (**1**) was labeled in the 3-*N*-methyl position since the *N*-methyl group is believed to eventually alkylate DNA. On the other hand the label in the 4-carbonyl position should be lost as [^{11}C]carbon dioxide. Here we report suitable radiochemistry strategies developed to label temozolomide (**1**) in each of these positions.

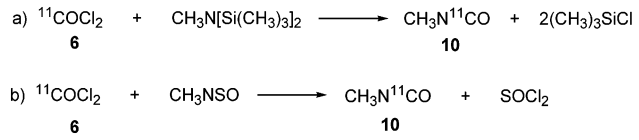
Results and Discussion

Radiolabeling Strategies. The structure of temozolomide (**1**) severely restricts possible strategies for direct radiolabeling with carbon-11 due to the short half-life ($t_{1/2}$ = 20.4 min) of the radionuclide. Two direct approaches were considered for introducing carbon-11 in the 3-*N*-methyl and 4-carbonyl positions, respectively. The first approach was to [^{11}C]methylate the *N*-desmethyl analogue **5**[18] of temozolomide (**1**) to give temozolomide labeled with carbon-11 in the 3-*N*-methyl position (Scheme 2). The *N*-desmethyl analogue **5** required for this reaction might be obtained from temozolomide (**1**) itself by removal of the 3-*N*-methyl group attached to the tetrazinone ring using an *N*-dealkylating agent. However, attempts to achieve this by reaction of temozolomide (**1**) with 1-chloroethyl chloroformate, a known *N*-dealkylating agent[19] proved unsuccessful.[1,20]

A second direct approach was the cyclization of MTIC (**2**) with [^{11}C]phosgene (**6**) (Scheme 3), this time with a view to introducing carbon-11 in the 4-carbonyl position. This seemed a more favorable route since structurally related 3-methylpyrazolotetrazinones are known to undergo the corresponding cyclization reaction with phosgene.[21] MTIC (**2**) was prepared in good chemical yield (50−55%) by the reaction of 5-diazoimidazole-4-carboxamide (**7**) with methylamine.[22] Trial reactions using varied concentrations of MTIC (**2**) and phosgene were carried out at low temperature and gave a mixture of undetermined products but failed to produce any temozolomide (**1**). Similarly, there have been other unsuccessful attempts to react MTIC (**2**) with phosgene or phosgene equivalents such as 1,1′-carbonyldiimidazole, 4-nitrophenyl chloroformate, and chloroformic acid trichloromethyl ester.[23]

In the absence of suitable direct labeling routes, we focused on the original cyclization reaction first used to prepare temozolomide (**1**).[4] This slow cyclization reaction between 5-diazoimidazole-4-carboxamide (**7**) and methyl isocyanate produces clinical grade temozolomide (**1**) in high yield. An attractive feature of this route was that it could be doubly employed for the synthesis of temozolomide labeled with carbon-11 in either the 3-*N*-methyl or 4-carbonyl group (Scheme 4). Thus the reaction of 5-diazoimidazole-4-carboxamide (**7**) with [^{11}C-*methyl*]methyl isocyanate (**8**) would give [3-*N*-^{11}C-*methyl*]temozolomide (**9**) and similarly the reaction of 5-diazoimidazole-4-carboxamide (**7**) with [^{11}C-*carbonyl*]methyl isocyanate (**10**) would give [4-^{11}C-*carbonyl*]-temozolomide (**11**). To utilize this labeling approach it was therefore necessary to first develop radiosynthetic

Scheme 5



routes to [^{11}C-*methyl*]methyl isocyanate (**8**) and [^{11}C-*carbonyl*]methyl isocyanate (**10**).

Radiosyntheses of [^{11}C-*Carbonyl*]methyl Isocyanate (10) and [^{11}C-*Methyl*]methyl Isocyanate (8). Probably the most commonly used route to the synthesis of organo-isocyanates is the direct phosgenation of an amine, and there are several reports using this approach for the preparation of [^{11}C-*carbonyl*]-organo-isocyanates.[24−26] However, this route is unsuitable for the preparation of [^{11}C-*carbonyl*]methyl isocyanate (**10**). This is due to difficulties in dispensing accurately the precise amount of gaseous methylamine required for the radiosynthesis which is typically performed at the microscale level. Hence, attempts at the reaction between [^{11}C]phosgene (**6**) and methylamine have resulted in the formation of [^{11}C-*carbonyl*]*N*,*N*′-dimethylurea due to reaction between the initially formed [^{11}C-*carbonyl*]methyl isocyanate (**10**) and any excess amine.[25] To avoid this problem an alternative route has been developed based on the [^{11}C]phosgenation of *N*,*N*-bis(trimethylsilyl)methylamine (Scheme 5a).[25] Using this route we have been able to produce [^{11}C-*carbonyl*]methyl isocyanate (**10**) in sufficient radiochemical yield {ca. 55% from [^{11}C]phosgene (**6**) (decay corrected)} for use in the radiosynthesis of [4-^{11}C-*carbonyl*]temozolomide (**11**). However, a novel route to [^{11}C-*carbonyl*]methyl isocyanate (**10**) via the [^{11}C]phosgenation of *N*-sulfinylmethylamine (Scheme 5b) has now been developed which should also prove suitable.[27]

A novel route for the radiosynthesis of [^{11}C-*methyl*]-methyl isocyanate (**8**)[28] was developed using the reaction between iodomethane and silver cyanate.[29] First, a method for producing methyl isocyanate from iodomethane was devised by passing gaseous iodomethane, under a flow of nitrogen, across a heated silver cyanate column contained in a tube furnace. The outflow of products from the column were collected in toluene and analyzed by combined gas chromatography−mass spectrometry (GC-MS). The percentage conversion of iodomethane to methyl isocyanate was examined over the 100−180 °C temperature range. Reaction temperatures above 160 °C gave methyl isocyanate in essentially quantitative yields. However, the yield of [^{11}C-*methyl*]-methyl isocyanate (**8**) from [^{11}C]iodomethane (**12**) (Scheme 6) was only 70−75% under the same optimized conditions giving 9.7−11.2 GBq (263−304 mCi) of [^{11}C-*methyl*]methyl isocyanate (**8**) at the end of the radiosynthesis. Radio-GC analysis of the reaction mixture (Figure 1) showed no unreacted [^{11}C]iodomethane (**12**) present (the retention time for authentic iodomethane = 4.1 min). The main loss was due to radioactivity (ca. 25%) remaining on the silver cyanate column; however, higher reaction temperatures failed to drive off this bound radioactivity as [^{11}C-*methyl*]methyl isocyanate (**8**). To verify the radiosynthesis, [^{11}C-*methyl*]methyl isocyanate (**8**) was derivatized by reaction with 1-(2-methoxyphenyl)piperazine[30] to form 4-(2-methoxyphenyl)piperazine-1-[^{11}C]methyl carboxamide (**13**) (Scheme 6).

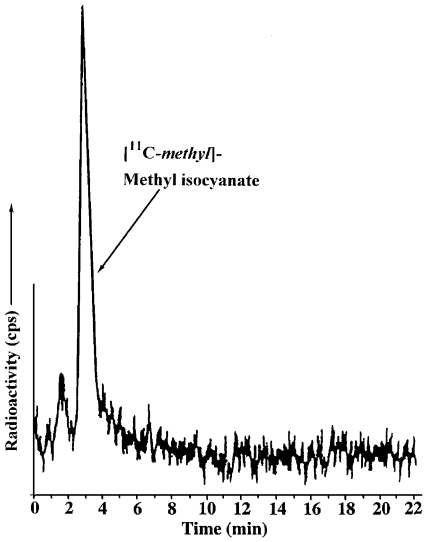
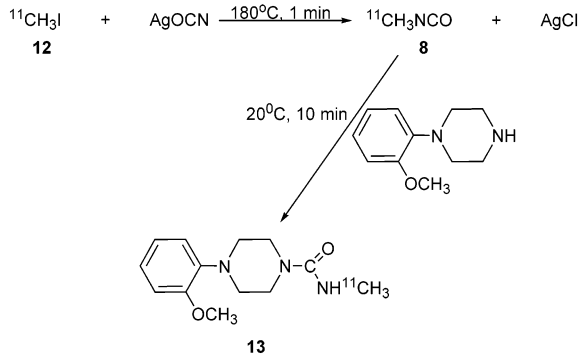


Figure 1. Radio-GC analysis of [^{11}C-*methyl*]methyl isocyanate (**8**).

Scheme 6



Cyclization Step. The cycloaddition reaction between 5-diazoimidazole-4-carboxamide (**7**) and methyl isocyanate is a very slow and light sensitive process. On the gram scale, the reaction takes 20 days to reach completion when carried out in dichloromethane or ethyl acetate at 25 °C.[4] Clearly, these conditions could not be employed in radiosyntheses involving the short-lived radioisotope carbon-11 ($t_{1/2}$ = 20.4 min) due to the long reaction time. Similar long reaction times are observed for the corresponding cyclization reactions to produce other imidazotetrazinones such as mitozolomide [the 2-chloroethyl congener of temozolomide (**1**)]. However, these reactions can be accelerated by employing the highly polar solvents, hexamethylphosphoramide[31] or DMSO[32] as the reaction medium. For the synthesis of temozolomide (**1**), the most rapid reaction (1 day) was achieved at 30 °C in DMSO using a >150-fold excess of methyl isocyanate to the diazo precursor **7**. Under these conditions temozolomide (**1**) was prepared in 83−89% yield. Using the same stoichiometry, reactions in hexamethylphosphoramide, *N*,*N*-dimethylformamide, acetonitrile, and tetrahydrofuran all gave low yields of temozolomide (**1**) (3−6%) after stirring for 1 day at 30 °C.

The reaction was then scaled down to the micromolar level and the stoichiometry reversed in order to estab-

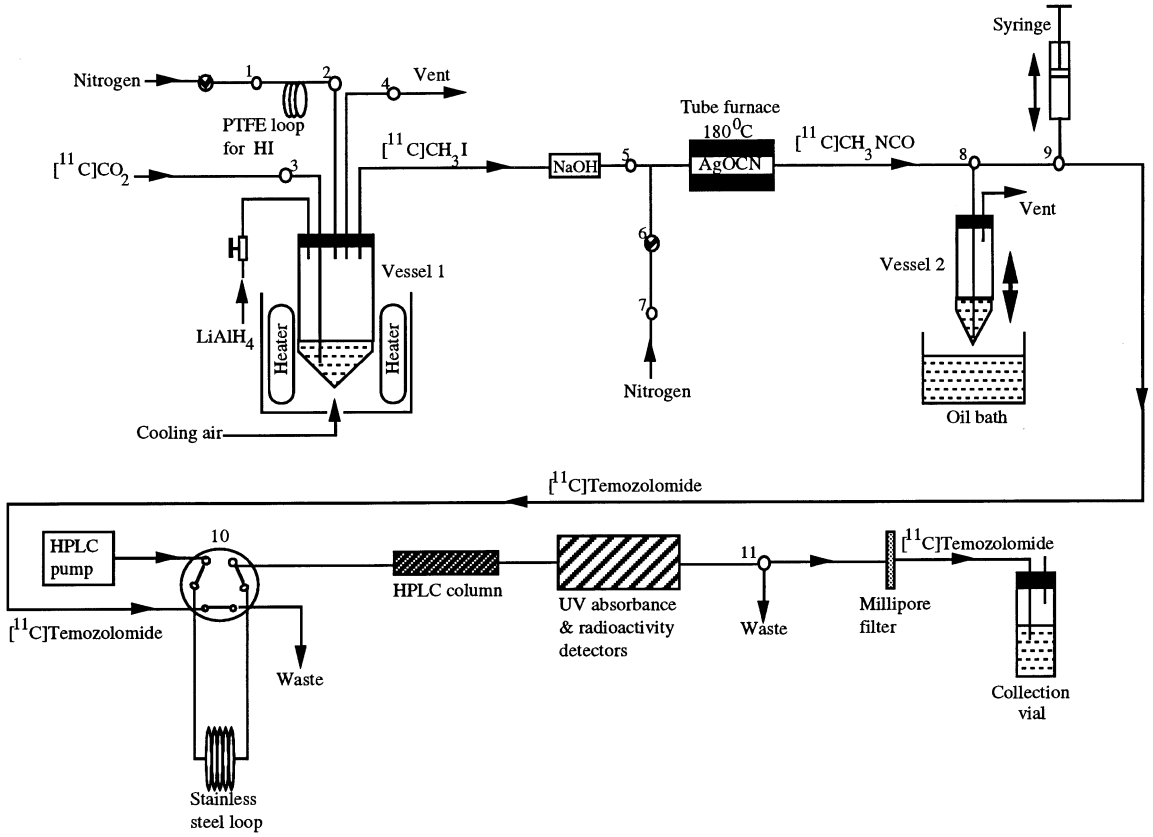


Figure 2. Automated system for the radiosynthesis of [3-*N*-^{11}C-*methyl*]temozolomide (**9**) from [^{11}C]iodomethane (**12**) via [^{11}C-*methyl*]methyl isocyanate (**8**).

lish reaction conditions that could be translated to a *no-carrier-added* radiosynthetic method. Using micromolar amounts of methyl isocyanate reduced the risk of polymerization to allow sealed reactions to be carried out under thermal conditions. As expected, the yield of temozolomide (**1**) (15−18%) was significantly reduced from the yield (83−89%) obtained when using excess methyl isocyanate. Several more polar *N*-substituted and *N*,*N*-disubstituted carboxamides were also examined but only *N*,*N*-dimethylformamide produced a measurable amount of temozolomide (**1**) (3−6%). These low yields were not entirely unexpected since organo-isocyanates can sometimes react with *N*,*N*-disubstituted carboxamides under thermal conditions to form *N*-persubstituted amidines.[33] Under further modified conditions, reactions between 5-diazoimidazole-4-carboxamide (**7**) and methyl isocyanate in the presence of boron trifluoride etherate[34] or triethyloxonium tetrafluoroborate[34] as catalysts failed to reduce the reaction time, while a third catalytic approach using polyphosphoric acid[35] resulted in decomposition of the diazo precursor **7**. Unable to further reduce the reaction time, the thermal reaction conditions developed using DMSO were translated to carbon-11 radiochemistry for the radiosyntheses of both [3-*N*-^{11}C-*methyl*]temozolomide (**9**) and [4-^{11}C-*carbonyl*]temozolomide (**11**).

Radiosynthesis of [3-*N*-^{11}C-*Methyl*]temozolomide (9) from [^{11}C-*Methyl*]methyl Isocyanate (8). A fully automated synthesis system (Figure 2) was developed for the routine production of [3-*N*-^{11}C-*methyl*]-temozolomide (**9**) from [^{11}C-*methyl*]methyl isocyanate (**8**). Using this system [^{11}C-*methyl*]methyl isocyanate (**8**), prepared as described, was passed into a solution of 5-diazoimidazole-4-carboxamide (**7**) in DMSO, and the reaction mixture was stirred for 10 min at 100 °C. The resultant mixture was then injected onto an HPLC column and eluted with a phosphate buffer. The radioactive product eluting with the same retention time as authentic temozolomide (**1**) was collected. A typical HPLC profile of the reaction is shown (Figure 3). The product was Millipore filtered and transferred into a sterile vial. The overall radiosynthesis time was ca. 47 min from the end of radionuclide production. Using this method, [3-*N*-^{11}C-*methyl*]temozolomide (**9**) as formulated for clinical use was obtained from [^{11}C-*methyl*]-methyl isocyanate (**8**) in ca. 14−20% radiochemical yield (decay corrected), giving on average 577 MBq (range 497−721 MBq) of [3-*N*-^{11}C-*methyl*]temozolomide (**9**). The average specific radioactivity was 64.0 GBq μmol^{-1} (range 59−69 GBq μmol^{-1}) at the end of the radiosynthesis, corresponding to 1.00−1.20 μg (5.2−6.2 nmol) of stable temozolomide (**1**). The radiochemical and chemical purities of the formulated product as determined by analytical HPLC were >97%. During and after radioactive decay, mass spectrometry (CI +ve mode) of the product gave spectra identical to that of authentic temozolomide (**1**).

To confirm the position of labeling in the 3-*N*-methyl position of temozolomide, a [$^{11/13}$C]colabeling experiment was performed. This involved modification to the automated synthesis system (Figure 2) by the insertion of a small vial containing [^{13}C]iodomethane between valve 5 and the heated silver cyanate salt. Initially, [^{11}C]-iodomethane (**12**) was prepared and then transferred under a flow of nitrogen into the small vial containing [^{13}C]iodomethane. Under the same nitrogen flow, the

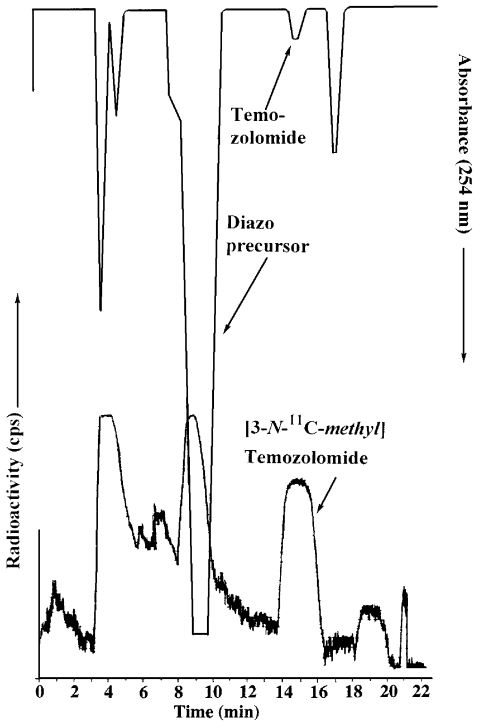


Figure 3. A typical HPLC profile from the radiosynthesis of [3-*N*-^{11}C-*methyl*]temozolomide (**9**).

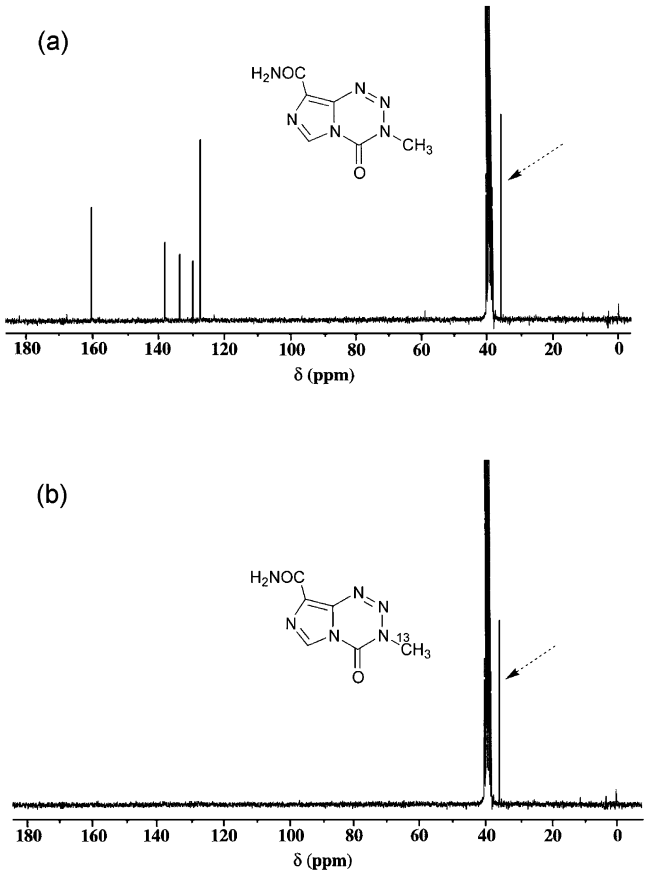


Figure 4. Carbon-13 NMR spectra of temozolomide (**1**) (a + b) and [3-*N*-^{13}C-*methyl*]temozolomide (c) showing (a) proton decoupled spectrum of temozolomide, (b) DEPT edited spectrum of temozolomide (**1**) [CH and CH_3 are positive)] and (c) proton decoupled spectrum of [3-*N*-^{13}C-*methyl*]temozolomide (the dotted arrows denote the ^{13}C-signal for the 3-*N*-methyl group).

resultant [$^{11/13}$C]iodomethane mixture was transferred out of the vial and across silver cyanate at 180 °C to form [$^{11/13}$C-*methyl*]methyl isocyanate. Reaction of [$^{11/13}$C-*methyl*]methyl isocyanate with 5-diazoimidazole-4-carboxamide (**7**) gave [3-*N*-$^{11/13}$C-*methyl*]temozolomide which was then isolated by semipreparative HPLC. After radioactive decay, the isolated product was examined by proton-decoupled carbon-13 NMR spectroscopy (62.9 MHz). A single peak at $\delta = 36.0$ (relative to tetramethylsilane) was observed (Figure 4b), having the same chemical shift as the 3-*N*-methyl group of authentic temozolomide (**1**) (Figure 4a) and identified as belonging to a methyl group by DEPT editing, thus confirming the position of labeling. Furthermore, mass spectrometry (CI +ve mode) of the product gave a peak at $m/z = 196$ $[M + H]^+$ corresponding to [3-*N*-^{13}C-*methyl*]temozolomide.

Radiosynthesis of [4-^{11}C-*Carbonyl*]temozolomide (11) from [^{11}C-*Carbonyl*]methyl Isocyanate (10). The radiosynthesis of [4-^{11}C-*carbonyl*]temozolomide (**11**) from [^{11}C-*carbonyl*]methyl isocyanate (**10**) was based on a "two-pot" method and performed using the automated synthesis system shown in Figure 5. Essentially, [^{11}C-*carbonyl*]methyl isocyanate (**10**) was prepared in *o*-xylene in vessel 2 by the [^{11}C]phosgenation of *N*,*N*-bis(trimethylsilyl)methylamine and distilled into vessel 3 containing 5-diazoimidazole-4-carboxamide (**7**) in DMSO. The resultant mixture was stirred for 10 min at 100 °C to produce [4-^{11}C-*carbonyl*]temozolomide (**11**) which was then isolated by semipreparative HPLC using the same conditions used for the isolation of [3-*N*-^{11}C-*methyl*]temozolomide (**9**). The radiosynthesis time, including isolation and Millipore filtration of the formulated product, was ca. 50 min from the end of radionuclide production.

The "two-pot" method was employed due to the different solvent requirements of the [^{11}C-*carbonyl*]-methyl isocyanate (**10**) and [4-^{11}C-*carbonyl*]temozolomide (**11**) radiosyntheses. The radiosynthesis of [^{11}C-*carbonyl*]methyl isocyanate (**10**) required nonpolar organic solvents such as toluene or diethyl ether. In contrast, the cyclization reaction between 5-diazoimidazole-4-carboxamide (**7**) and [^{11}C-*carbonyl*]methyl isocyanate (**10**) to give [4-^{11}C-*carbonyl*]temozolomide (**11**) was limited to DMSO. Consequently, [^{11}C-*carbonyl*]-methyl isocyanate (**10**) was prepared in *o*-xylene then distilled into a solution of the diazo precursor **7** in DMSO for the cyclization step. Ideally, it was preferred to carry out the entire radiosynthesis as a "one-pot" method to simplify the automation process required to carry out the radiosynthesis. However, attempts to prepare either [^{11}C-*carbonyl*]methyl isocyanate (**10**) or [4-^{11}C-*carbonyl*]temozolomide (**11**) in solvent mixtures containing a nonpolar organic solvent and DMSO had limited success. For example, the yield of [^{11}C-*carbonyl*]-methyl isocyanate (**10**) from [^{11}C]phosgene (**6**) in a toluene:DMSO (50:50) mixture was only 5−12% while the radiosynthesis of [4-^{11}C-*carbonyl*]temozolomide (**11**) proved even more sensitive and could only be produced in neat DMSO.

Using this "two-pot" method, [4-^{11}C-*carbonyl*]temozolomide (**11**) as formulated for clinical use, was obtained from [^{11}C-*carbonyl*]methyl isocyanate (**10**) in ca. 10−15% radiochemical yield (decay corrected), giving on average 373 MBq (range 300−481 MBq) of [4-^{11}C-

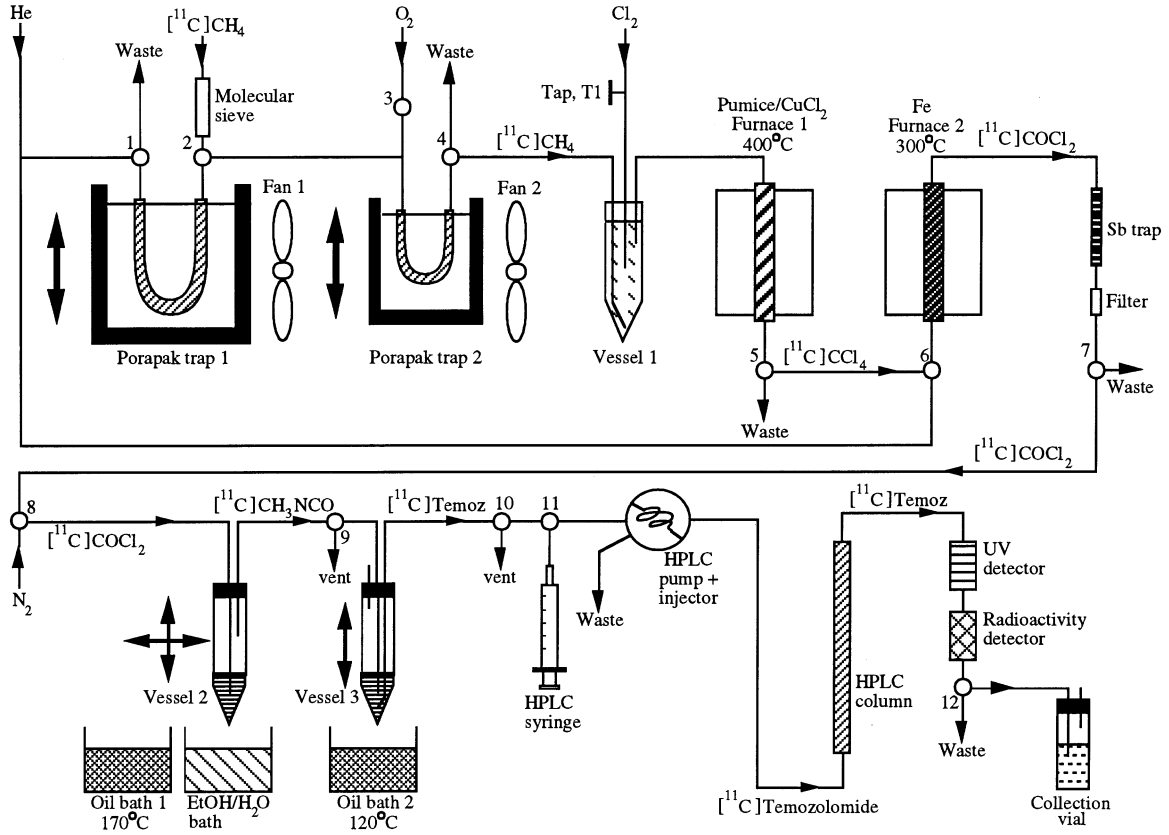


Figure 5. Automated system for the radiosynthesis of [4-^{11}C-*carbonyl*]temozolomide (**11**) from [^{11}C]phosgene (**6**) via [^{11}C-*carbonyl*]-methyl isocyanate (**10**).

carbonyl]temozolomide (**11**). The average specific radioactivity was 52 GBq μmol^{-1} (range 46−61 GBq μmol^{-1}) at the end of the radiosynthesis, corresponding to 0.67−0.89 μg (3.5−4.6 nmol) of stable temozolomide (**1**). The radiochemical and chemical purities of the formulated product as determined by analytical HPLC were >97%. During and after radioactive decay, mass spectrometry (CI +ve mode) of the product gave spectra identical to that of authentic temozolomide (**1**).

Attempts were made to confirm the position of labeling by preparing [4-$^{11/13}$C-*carbonyl*]temozolomide using [^{13}C]carbon tetrachloride in [^{11}C/^{13}C]colabeling experiments with [^{11}C]carbon tetrachloride and subsequent carbon-13 NMR. This involved repeating the radiosynthesis of [^{11}C-*carbonyl*]methyl isocyanate (**10**) from [^{11}C]-phosgene (**6**) via [^{11}C]carbon tetrachloride but with the introduction of [^{13}C]carbon tetrachloride between valves 6 and 7 of the automated synthesis system (Figure 5) prior to the [^{11}C]phosgene (**6**) radiosynthesis. However, although [^{11}C/^{13}C]organo-isocyanates can be successfully prepared in this way,[27] attempts to produce [4-$^{11/13}$C-*carbonyl*]temozolomide were unsuccessful. This was possibly due to contamination of the DMSO reaction mixture by unreacted [^{13}C]carbon tetrachloride during the distillation step. The synthesis of temozolomide (**1**) in chlorinated solvents such as carbon tetrachloride requires extremely long reaction times (>20 days)[4] so the presence of any [^{13}C]carbon tetrachloride can only have impeded the final cyclization reaction step.

Conclusions

The anticancer prodrug temozolomide (**1**) has been labeled with the short-lived positron emitter, carbon-11 ($t_{1/2}$ = 20.4 min) for use in human PET studies. To achieve this we explored the use of rapid chemical routes, a feature essential for radiosyntheses using short-lived positron emitters such as carbon-11 ($t_{1/2}$ = 20.4 min). The best route proved to be the reaction of 5-diazoimidazole-4-carboxamide (**7**) with methyl isocyanate, the same chemistry used for large scale synthesis of the clinical grade drug. However, the main disadvantage of this route under the original reaction conditions[4] was its slow nature, although the reaction has since been accelerated by the use of DMSO as the reaction solvent.[32] We have attempted to further accelerate the reaction with the use of catalysts and alternative solvents and found that in both cases the reaction times were either increased or resulted in decomposition of the diazo precursor **7**. However, we were able to thermally accelerate the reaction sufficiently to allow its practical application in the radiosyntheses of temozolomide labeled with carbon-11 ($t_{1/2}$ = 20.4 min) in the 3-*N*-methyl and 4-carbonyl positions.

For labeling in the 3-*N*-methyl position, the novel labeling agent [^{11}C-*methyl*]methyl isocyanate (**8**) was prepared in good radiochemical yield by the reaction of [^{11}C]iodomethane (**12**) with silver cyanate. Reaction of 5-diazoimidazole-4-carboxamide (**7**) with [^{11}C-*methyl*]-methyl isocyanate (**8**) gave [3-*N*-^{11}C-*methyl*]temozolomide (**9**). [4-^{11}C-*carbonyl*]temozolomide (**11**) was similarly prepared by the reaction of 5-diazoimidazole-4-carboxamide (**7**) with [^{11}C-*carbonyl*]methyl isocyanate (**10**). In both cases automated synthesis systems were developed and used to carry out the radiosyntheses, giving apyrogenic samples of [3-*N*-^{11}C-*methyl*]temozolomide (**9**) and [4-^{11}C-*carbonyl*]temozolomide (**11**) with

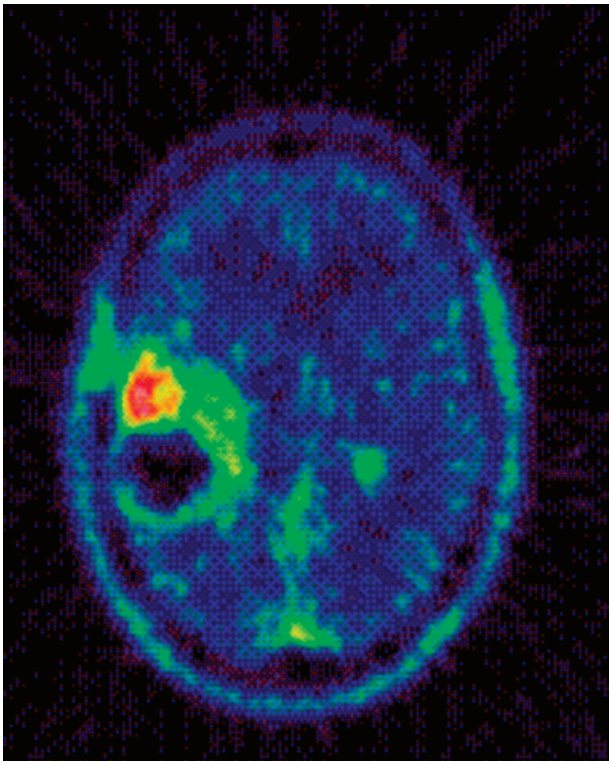


Figure 6. PET image of mean radioactivity concentration during the first 90 min after injection of [3-*N*-^{11}C-*methyl*]-temozolomide (**9**) (the image represents a transverse section of brain with a right parietal lesion). The concentration of radioactivity present in brain tissue decreases in the order: red > orange > yellow > green > light blue > dark blue.

good chemical and radiochemical purities. Human PET studies using [3-*N*-^{11}C-*methyl*]temozolomide (**9**) and [4-^{11}C-*carbonyl*]temozolomide (**11**) have been carried out to determine the in vivo pharmacokinetics and to probe postulated mode of action of this anticancer prodrug. A transverse brain image from a [3-*N*-^{11}C-*methyl*]temozolomide (**9**) PET study is shown, the subject was a patient with a recurrent right parietal grade IV astrocytoma, glioblastoma multiforme (Figure 6). Further PET studies designed to demonstrate in vivo prodrug activation of temozolomide (**1**) in accordance with its proposed mechanism of action (Scheme 1) have now been completed. These studies, in which exhaled [^{11}C]-carbon dioxide was also monitored, were performed on patients after administration of [3-*N*-^{11}C-*methyl*]temozolomide (**9**) and [4-^{11}C-*carbonyl*]temozolomide (**11**), respectively. The results of this work are published elsewhere.[36,37]

Experimental Section

Materials. Methyl isocyanate was purchased from Ubichem Ltd. Phosgene was purchased from Fluka Ltd. [^{13}C]Carbon tetrachloride (90 at. %) was purchased from CK Gas Products Ltd. For reference purposes, authentic samples of temozolomide (**1**) and 5-diazoimidazole-4-carboxamide (**7**) were donated by the Cancer Research Laboratories, School of Pharmaceutical Sciences, University of Nottingham. All other reagents and solvents were purchased from Sigma-Aldrich Company Ltd.

Methods. Mass Spectrometry. Mass spectra of reaction products were obtained using a quadrupole mass spectrometer (Nermag R10/10C). Samples were introduced into the ionization source of the spectrometer using the probe facility and vaporized by passing a current through the probe filament. The spectrometer was calibrated conventionally using FC-43 (perfluorotributylamine) and run in the electron impact (EI) mode or tuned for positive ions in the chemical ionization (CI +ve) mode using ammonia as the reactant gas and scanning between 40 and 500 AMU. Spectral data were collected using a PDP 11/23 processor (Digital Computers) and analyzed using the Sidar software program (Nermag).

Combined Gas Chromatography−Mass Spectrometry (GC-MS). GC-MS experiments were carried out using a Varian Vista 6000 gas chromatograph interfaced to the mass spectrometer operating in EI mode. Gas chromatographic separations were performed on a nonpolar dimethylsiloxane bonded phase column (BP1 from SGE Ltd; thickness of bonded phase = 5 μm; column length = 25 m; internal diameter = 0.503 mm) using helium (5 psi, 0.34 bar) as the carrier gas. All samples (0.1−0.2 μL), neat liquids or solutions, were injected directly onto the column via an on-column injector (OCI3 from SGE Ltd). The column was temperature programmed from 35 °C using the following sequence: 35 °C (5 min) ramped to 180 °C at 25 °C min^{-1}. From 180 °C (5 min) ramped to 275 °C at 25 °C min^{-1}. The column was conditioned between injections by heating at 275 °C for 15 min.

NMR Spectra. NMR spectra were obtained for solutions in CD_3OD using a Bruker AM250 spectrometer (^{1}H, 250.13 MHz; ^{13}C, 62.90 MHz) at ambient temperature. Chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS) (δ = 0.00; downfield is positive).

Synthesis of Methyl Isocyanate from Iodomethane. Iodomethane (1 μL, 16 μmol) was distilled under a stream of nitrogen (10 mL min^{-1}) and passed over silver cyanate (250 mg, 1.67 mmol) contained in a glass column (25 × 0.5 cm) placed inside a Carbolite MTF 9/15 tube furnace (18 × 1.5 cm) having variable temperature control. The silver cyanate column was preconditioned by flushing with nitrogen at 100 °C for 20 min. The temperature was then adjusted to the desired reaction temperature for 20 min before distilling the iodomethane. The outflow from the column was collected in toluene (250 μL) and the collected sample analyzed by GC-MS.

Synthesis of Temozolomide (1). (a) Macroscale Synthesis. Temozolomide (**1**) was prepared from 5-diazoimidazole-4-carboxamide (**7**)[22] in ca. 85% chemical yield using the previously reported method.[32] Mass spectrometry (CI +ve mode) of the product gave a peak at m/z = 195 $[M + H]^+$. Elemental C:H:N analysis gave 37.05% C, 3.07% H, and 43.34% N (expected values calculated from $C_6H_6N_6O_2$, formula weight = 194 were 37.11% C, 3.09% H, and 43.30% N). Temozolomide (**1**): ^{13}C NMR in d_6-DMSO, δ(ppm) relative to TMS: 161.4, 139.1 (carbonyl); 134.5, 130.4 (quaternary carbons); 128.3 (C^6-CH); 36.0 (*N*-methyl). **(b) Microscale Synthesis.** Methyl isocyanate (1 μL, 17 μmol) was added to a reaction vial containing 5-diazoimidazole-4-carboxamide (**7**)[22] (2.0 mg, 15 μmol) in anhydrous DMSO (250 μL). The vial was sealed and placed in an oil bath, and the reaction mixture was allowed to stir for 10 min at 100 °C. The reaction mixture was then injected onto an HPLC column (μ-Bondapak C_{18}, 30 × 0.78 cm). The column was eluted with a mixture of water, ethanol, and phosphate buffer (98:2:0.1) at a flow rate of 3 mL min^{-1}, and the eluent was continuously monitored for absorbance at 324 nm. The area of the HPLC peaks of the reaction components eluting at 9−10 min and 14−15 min, having the same retention times as authentic 5-diazoimidazole-4-carboxamide (**7**) and temozolomide (**1**), respectively, were measured. This procedure was repeated for a series of reactions in which DMSO was replaced by a range of solvents (*N*-methylformamide, *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide, acetonitrile, 1-methyl-2-pyrrolidinone, hexamethylphosphoramide, tetrahydrofuran, acetic acid, and ethyl acetate).

Reactions between 5-diazoimidazole-4-carboxamide (**7**) (2.0 mg, 15 μmol) and methyl isocyanate (1 μL, 17 μmol) were also carried out in ethyl acetate (500 μL) or dichloromethane (500 μL) containing either boron trifluoride etherate (1 μL, 8 μmol), polyphosphoric acid (1 μL), or triethyloxonium tetrafluoroborate (1.0 M in dichloromethane) (5 μL, 5 μmol). The reactions were analyzed by HPLC using the conditions described above.

Attempted Demethylation of Temozolomide (1). Typically, temozolomide (**1**) (200 mg, 0.01 mol) was dissolved in a mixture of toluene and acetonitrile (30 mL, 50:50). 1-Chloroethyl chloroformate (1.0 mL, 0.01 mol) was added and the reaction mixture stirred at room temperature or heated under reflux for 1.5 h. Repeat experiments were carried out in which the reagent concentrations and reaction times were varied. Samples of the reaction mixture were analyzed by mass spectrometry (CI +ve mode) and TLC. TLC analyses were carried out using Silica $60F_{254}$ plates (0.25 mm thickness) eluted with a mixture of acetone and glacial acetic acid (7:1,v/v). The plates were monitored for absorbance at 254 nm.

Reaction between MTIC (2) and Phosgene. Typically, MTIC (**2**)[22] (100 mg, 0.6 mmol) was dissolved in tetrahydrofuran (15 mL) and the solution cooled to −20 °C using a carbon tetrachloride/liquid nitrogen cooling mixture. Phosgene in toluene (1.93 M, 1.5 mL) was added and the reaction mixture stirred under nitrogen for 1 h. Repeat experiments were carried out in which the reagent concentrations and reaction times were varied. Samples of the reaction mixture were analyzed by mass spectrometry (CI +ve mode) and TLC using the conditions described above. The R_f values of reaction mixture components were compared to the R_f value (0.68) for authentic temozolomide (**1**) under the same TLC conditions.

Radiochemistry. Radiosynthesis of [^{11}C]Iodomethane (12). [^{11}C]Iodomethane (**12**) was prepared from cyclotron produced [^{11}C]carbon dioxide using the automated synthesis system shown in Figure 2. A full account of the method has been previously reported.[38] To summarize, [^{11}C]carbon dioxide was transferred by a flow of nitrogen (10 mL min^{-1}) into vessel 1 containing 0.1 M lithium aluminum hydride in THF (200 μL). The THF was evaporated by heating vessel 1 under a nitrogen flow. Vessel 1 was then cooled by a flow of compressed air. Hydriodic acid (200 μL) contained in a Teflon (PTFE) loop was added to vessel 1 which was then sealed and briefly heated to 160 °C. The [^{11}C]iodomethane (**12**) produced was distilled from vessel 1 via a sodium hydroxide trap for further reaction. The preparation time was ca. 13 min from the end of radionuclide production.

Radiosynthesis of [^{11}C]Phosgene (6). [^{11}C]Phosgene (**6**) was prepared from cyclotron produced [^{11}C]methane using the automated synthesis system shown in Figure 5. A full account of the method has been previously reported.[39] To summarize, [^{11}C]methane was cryogenically trapped in succession on Porapak traps 1 and 2 and transferred into vessel 1 containing chlorine (30 mL). The contents of vessel 1 were then passed under a stream of oxygen through furnace 1 containing heated pumice stone (2.5 g) impregnated with copper(II) chloride at 400 °C. Under the same stream of oxygen, the resultant [^{11}C]-carbon tetrachloride produced in furnace 1 was converted to [^{11}C]phosgene (**6**) by passage through furnace 2 containing iron granules (1.5 g) at 300 °C. The [^{11}C]phosgene (**6**) was passed through an antimony trap to remove excess chlorine, a plastic sinter filter to remove antimony chloride and trapped for further use in anhydrous *o*-xylene (250 μL) contained in vessel 2. The preparation time was ca. 11 min from the end of radionuclide production.

Radiosynthesis of [^{11}C-*Methyl*]methyl Isocyanate (8) from [^{11}C]Iodomethane (12). [^{11}C]Iodomethane (**12**) was distilled under nitrogen (10 mL min^{-1}) across silver cyanate (250 mg, 1.67 mmol) at 180 °C for 1 min, and the products were trapped in vessel 2 (Figure 2) containing anhydrous toluene (250 μL). Typically the conversion of [^{11}C]iodomethane (**12**) to [^{11}C-*methyl*]methyl isocyanate (**8**) was 70−75% (decay corrected). The total radiosynthesis time {including [^{11}C]-iodomethane (**12**) production} was ca. 13 min from the end of radionuclide production.

A sample (1 μL) of the product was analyzed by combined radio-gas chromatography. A radioactive peak was observed at 2.5−3.5 min (Figure 1) having the same retention time as authentic methyl isocyanate. For further analysis, 1-(2-methoxyphenyl)piperazine (2 mg, 10 μmol) in anhydrous toluene (250 μL) was added to vessel 2 and the resultant solution was stirred for 10 min at room temperature. The solution was then injected onto an HPLC column (μ−Bondapak C_{18}, 25 × 0.78 cm) and eluted with a mixture of water: ethanol (70:30) at a flow rate of 3 mL min^{-1}. The eluent was monitored for radioactivity and absorbance at 254 nm. A radioactive product was observed at 14−16 min having the same retention time as authentic 4-(2-methoxyphenyl)piperazine-1-methylcarboxamide. The radioactive product was collected and after radioactive decay the residue analyzed by mass spectrometry (CI +ve mode). The mass spectrum obtained was identical to that of authentic 4-(2-methoxyphenyl)piperazine-1-methylcarboxamide. $C_{13}H_{19}N_3O_2$, formula weight = 249. Mass spectrometry (CI +ve mode), m/z = 250 $[M + H]^+$.

Radiosynthesis of [^{11}C-*Carbonyl*]methyl Isocyanate (10) from [^{11}C]Phosgene (6). [^{11}C-*Carbonyl*]methyl isocyanate (**10**) was prepared from [^{11}C]phosgene (**6**) using a previously reported method.[25] To summarize, vessel 2 (Figure 5) containing *N*,*N*-bis(trimethylsilyl)methylamine (1 μL, 5 μmol) in anhydrous *o*-xylene (250 μmol) was cooled to −10 °C using a crushed ice/ethanol mixture. [^{11}C]Phosgene (**6**) was passed into vessel 2 for 4 min. Vessel 2 was then removed from the cooling mixture to allow the reaction mixture to warm to room temperature. Typically the conversion of [^{11}C]phosgene (**6**) to [^{11}C-*carbonyl*]methyl isocyanate (**10**) was 55−60% (decay corrected). The total radiosynthesis time {including [^{11}C]phosgene (**6**) production} was ca. 15 min from the end of radionuclide production.

For analysis, 1-(2-methoxyphenyl)piperazine (2 mg, 10 μmol) in anhydrous toluene (250 μL) was added to vessel 2, and the resultant solution was stirred for 10 min at room temperature. The solution was then analyzed by HPLC using the conditions described above. A radioactive product was observed at 14−16 min having the same retention time as authentic 4-(2-methoxyphenyl)piperazine-1-methylcarboxamide. Mass spectrometry (CI +ve mode) analysis of the radioactive product gave a spectrum identical to that of authentic 4-(2-methoxyphenyl)piperazine-1-methylcarboxamide. $C_{13}H_{19}N_3O_2$, formula weight = 249. Mass spectrometry (CI +ve mode), m/z = 250 $[M + H]^+$.

Radiosynthesis of [3-*N*-^{11}C-*Methyl*]temozolomide (9) from [^{11}C-*Methyl*]methyl Isocyanate (8). [^{11}C-*Methyl*]methyl isocyanate (**8**) was passed for 1 min in a stream of nitrogen (10 mL min^{-1}) into vessel 2 (Figure 2) containing a solution of 5-diazoimidazole-4-carboxamide (**7**)[22] (2.0 mg,15 μmol) in anhydrous DMSO (250 μL). The vial was sealed and lowered into an oil bath, and the reaction mixture was stirred for 10 min at 100 °C. The reaction mixture was then injected onto an HPLC column (μ-Bondapak C_{18}, 30 × 0.78 cm). The column was eluted with a mixture of water, ethanol, and phosphate buffer (98:2:0.1) at a flow rate of 3 mL min^{-1}, and the eluent was continuously monitored for radioactivity and UV absorbance. The radioactive product eluting between 14 and 16 min, having the same retention time as authentic temozolomide (**1**) was collected and passed through a Millipore filter (0.22 μm, Millex GS, Millipore) and transferred into a sterile vial. The pH of the formulated solution was ca. 6.0. The radiosynthesis time was ca. 47 min from the end of radionuclide production, and the average radiochemical yield of [3-*N*-^{11}C-*methyl*]temozolomide (**9**) was 577 MBq (range 497−721 MBq). The average specific radioactivity was 64 GBq μmol^{-1} (range 59−69 GBq μmol^{-1}) at the end of the radiosynthesis, corresponding to 1.00−1.20 μg (5.2−6.2 nmol) of stable temozolomide (**1**).

Samples of [3-*N*-^{11}C-*methyl*]temozolomide (**9**) as formulated for human injection were analyzed by HPLC using a Nucleosil 5 C_{18} column (25 × 0.39 cm) eluted at 0.8 mL min^{-1} with a mixture of 0.5% acetic acid and acetonitrile (90:10). The eluate was monitored continuously for radioactivity and absorbance at 324 nm. The retention time for 5-diazoimidazole-4-carboxamide (**7**) was 4.6 min. The formulated product gave one radioactive peak and one stable peak both with the same retention time (7.0 min) as authentic temozolomide (**1**). The radiochemical and chemical purities were >97%. A sample of [3-*N*-^{11}C-*methyl*]temozolomide (**9**) as formulated for human injection, was analyzed during and after radioactive decay by

mass spectrometry (CI +ve mode). The spectra obtained were identical to that of authentic temozolomide (**1**). $C_6H_6N_6O_2$, formula weight = 194. Mass spectrometry (CI +ve mode), m/z = 195 $[M + H]^+$.

Radiosynthesis of [4-^{11}C-*Carbonyl*]temozolomide (11) from [^{11}C-*Carbonyl*]methyl Isocyanate (10). Vessel 2 (Figure 5) containing [^{11}C-*carbonyl*]methyl isocyanate (**10**) was lowered into oil bath 1 at 150 °C. The [^{11}C-*carbonyl*]methyl isocyanate (**10**) was then distilled under a flow of nitrogen (10 mL min^{-1}) for 2 min into vessel 3 containing 5-diazoimidazole-4-carboxamide (**7**)[22] (2.0 mg, 15 μmol) in anhydrous DMSO (250 μL). Vessel 3 was lowered into oil bath 2, and the reaction mixture was stirred for 10 min at 100 °C. The [4-^{11}C-*carbonyl*]-temozolomide (**11**) produced was isolated from the reaction mixture by semipreparative HPLC as described above. The product was collected and passed through a Millipore filter (0.22 μm, Millex GS, Millipore) and transferred into a sterile vial. The pH of the formulated solution was ca. 6.0. The radiosynthesis time was ca. 50 min from the end of radionuclide production, and the average radiochemical yield of [4-^{11}C-*carbonyl*]temozolomide (**11**) was 373 MBq (range 300−481 MBq). The average specific radioactivity was 52 GBq μmol^{-1} (range 46−61 GBq μmol^{-1}) at the end of the radiosynthesis, corresponding to 0.67−0.89 μg (3.5−4.6 nmol) of stable temozolomide (**1**).

Samples of [4-^{11}C-*carbonyl*]temozolomide (**11**) as formulated for human injection were analyzed by HPLC as described above. The formulated product gave one radioactive peak and one stable peak both with the same retention time (7.0 min) as authentic temozolomide (**1**). The radiochemical and chemical purities were >97%. A sample of [4-^{11}C-*carbonyl*]temozolomide (**11**), as formulated for human injection, was analyzed during and after radioactive decay by mass spectrometry (CI +ve mode). The spectra obtained were identical to that of authentic temozolomide (**1**). $C_6H_6N_6O_2$, formula weight = 194. Mass spectrometry (CI +ve mode), m/z = 195 $[M + H]^+$.

References

(1) Arrowsmith, J.; Jennings, S. A.; Langnel, D. A. F.; Wheelhouse, R. T.; Stevens, M. F. G. Antitumour imidazotetrazines. Part 39. Synthesis of bis(imidazotetrazine)s with saturated spacer groups. *J. Chem. Soc., Perkin Trans. 1* **2000**, 4432−4438.

(2) Paulsen, F.; Hoffmann, W.; Becker, G.; Belka, C.; Weinmann, M.; Classen, J.; Kortmann, R. D.; Bamberg, M. Chemotherapy in the treatment of recurrent glioblastoma multiforme ifosfamide versus temozolomide. *J. Cancer Res. Clin. Oncol.* **1999**, *125*, 411−418.

(3) Surawicz, T. S.; Davis, F.; Freels, S.; Laws, E. R.; Uaw, H. R. Brain tumor survival results from the National Cancer Data Base. *J. Neurooncol.* **1998**, *40*, 151−160.

(4) Stevens, M. F. G.; Hickman, J. A.; Stone, R.; Gibson, N. W.; Baig, G. U.; Lunt, E.; Newton, C. G. Antitumour imidazotetrazines. 1. Synthesis and chemistry of 8-carbamoyl-3-(2-chloroethyl)-imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one, a novel broad-spectrum antitumour agent. *J. Med. Chem.* **1984**, *27*, 196−201.

(5) Newlands, E. S.; O'Reilly, S. M.; Glaser, M. G.; Bower, M.; Evans, H.; Brock, C.; Brampton, M. H.; Colquhoun, I.; Lewis, P.; Rice-Edwards, J. M.; Illingworth, R. D.; Richards, P. G. The Charing Cross Hospital experience with temozolomide in patients with gliomas. *Eur. J. Cancer* **1996**, *32*, 2236−2241.

(6) Bower, M.; Newlands, E. S.; Bleehen, N. M.; Brada, M.; Begent, R. J. H.; Calvert, H.; Colquhoun, I.; Lewis, P.; Brampton, M. H. Multicentre CRC phase II trial of temozolomide in recurrent or progressive high-grade glioma. *Cancer Chemother. Pharmacol.* **1997**, *40*, 484−488.

(7) Brock, C. S.; Newlands, E. S.; Wedge, S. R.; Bower, M.; Evans, H.; Colquhoun, L.; Roddie, M.; Glaser, M.; Brampton, M. H.; Rustin, G. J. S. Phase I trial of temozolomide using an extended continuous oral schedule. *Cancer Res.* **1998**, *58*, 4363−4367.

(8) O'Reilly, S. M.; Newlands, E. S.; Glaser, M. G.; Brampton, M.; Rice-Edwards, J. M.; Illingworth, R. D.; Richards, P. G.; Kennard, C.; Colquhoun, I. R.; Lewis, P.; Stevens, M. F. G. Temozolomide: a new oral cytotoxic chemotherapeutic agent with promising activity against primary brain tumours. *Eur. J. Cancer* **1993**, *29*, 940−942.

(9) Bleehen, N. M.; Newlands, E. S.; Lee, S. M.; Thatcher, N.; Selby, P.; Calvert, A. H.; Rustin, G. J. S.; Brampton, M.; Stevens, M. F. G. Cancer Research Campaign phase II trial of temozolomide in metastatic melanoma. *J. Clin. Oncol.* **1995**, *13*, 910−913.

(10) Nicholson, H. S.; Krailo, M.; Ames, M. M.; Seibel, N. L.; Reid, J. M.; Liu-Mares, W.; Vezina, L. G.; Ettinger, A. G.; Reaman, G. H. Phase I study of temozolomide in children and adolescents with recurrent solid tumors a report from the children's cancer group. *J. Clin. Oncol.* **1998**, *16*, 3037−3043.

(11) Estlin, E. J.; Lashford, L.; Ablett, S.; Price, L.; Gowing, R.; Gholkar, A.; Kohler, J.; Lewis, I. J.; Morland, B.; Pinkerton, C. R. Phase I study of temozolomide in paediatric patients with advanced cancer. *Br. J. Cancer* **1998**, *78*, 652−661.

(12) Yung, W. K.; Albright, R. E.; Olson, J.; Fredericks, R.; Fink, K.; Prados, M. D.; Brada, M.; Spence, A.; Hohl, R. J.; Shapiro, W. A phase II study of temozolomide vs procarbazine in patients with glioblastoma multiforme at first relapse. *Br. J. Cancer* **2000**, *83*, 588−593.

(13) Lowe, P. R.; Sansom, C. E.; Schwalbe, C. H.; Stevens, M. F. G.; Clark, A. S. Antitumour imidazotetrazines. 25. Crystal structure of 8-carbamoyl-3- methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (temozolomide) and structural comparisons with the related drugs mitozolomide and DTIC. *J. Med. Chem.* **1992**, *35*, 3377−3382.

(14) Denny, B. J.; Wheelhouse, R. T.; Stevens, M. F. G.; Tsang, L. L. H.; Slack, J. A. NMR and molecular modeling investigation of the mechanism of activation of the antitumour drug temozolomide and its interaction with DNA. *Biochemistry* **1994**, *33*, 9045−9051.

(15) Wheelhouse, R. T.; Denny, B. J.; Stevens, M. F. G. NMR and molecular modelling studies on the mechanism of action of the antitumour drug temozolomide. *Contrib. Oncol.* **1995**, *49*, 40−49.

(16) McGarrity, J. F.; Smyth, T. J. Hydrolysis of diazomethane-kinetics and mechanism. *J. Am. Chem. Soc.* **1980**, *102*, 7303−7308.

(17) Wedge, S. R.; Porteous, J. K.; Newlands, E. S. 3-Aminobenzamide and/or O6-benzylguanine evaluated as an adjuvant to temozolomide or BCNU treatment in cell lines of variable mismatch repair status and O6-alkylguanine-DNA alkyltransferase activity. *Br. J. Cancer* **1996**, *74*, 1030−1036.

(18) Wang, Y.; Stevens, M. F. G. Synthetic studies of 8-carbamoylimidazo-[5,1-D]-1,2,3,5-tetrazin-4(3H)-one: A key derivative of antitumour drug temozolomide. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 185−188.

(19) Olafson, R. A.; Martz, J. T.; Senet, J. P.; Piteau, M.; Malfroot, M. A new reagent for the selective, high-yield dealkylation of tertiary amines−improved syntheses of naltrexone and nalbuphine. *J. Org. Chem.* **1984**, *49*, 2081−2082.

(20) Wang, Y.; Stevens, M. F. G.; Thomson, W. T.; Shutts, B. P. Antitumour Imidazotetrazines. Part 33. New syntheses of the antitumour drug temozolomide using 'masked' methylisocyanates. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2783−2787.

(21) Ege, G.; Gilbert, K.; Maurer, K. Synthesen von Azolo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-onen. *Chem. Ber.* **1987**, *120*, 1375−1395.

(22) Stevens, M. F. G.; Hickman, J. A.; Langdon, S. P.; Chubb, D.; Vickers, L.; Stone, R.; Baig, G.; Goddard, C.; Gibson, N. W.; Slack, J. A.; Newton, C.; Lunt, E.; Fizames, C.; Lavelle, F. Antitumour activity and pharmacokinetics in mice of 8-carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (CCRG 81045; M & B 39831), a novel drug with potential as an alternative to dacarbazine. *Cancer Res.* **1987**, *47*, 5846−5852.

(23) Wang, Y.; Stevens, M. F. G.; Chan, T. M.; DiBenedetto, D.; Ding, Z. X.; Gala, D.; Hou, D.; Kugelman, M.; Kuo, S. C.; Mas, J. L.; Schumacher, D. P.; Shutts, B. P.; Smith, L.; Zhan, Z. Y.; Thomson, W. T. Antitumour imidazotetrazines. 35. New synthetic routes to the antitumour drug temozolomide. *J. Org. Chem.* **1997**, *62*, 7288−7294.

(24) Conway, T.; Diksic, M. Synthesis of 'no-carrier-added' carbon-11 SarCNU: The sarcosinamide analogue of the chemotherapeutic agent BCNU. *J. Nucl. Med.* **1988**, *29*, 1957−1960.

(25) Crouzel, C.; Hinnen, F.; Maitre, E. Radiosynthesis of methyl and heptyl [^{11}C]isocyanate from [^{11}C]phosgene, application to the synthesis of carbamates: [^{11}C]physostygmine and [^{11}C]heptylphysostygmine. *Appl. Radiat. Isot.* **1995**, *46*, 167−170.

(26) Dolle, F.; Hinnen, F.; Valette, H.; Demphel, S.; Coulon, C.; Ottaviani, M.; Bottlaender, M.; Crouzel, C. Synthesis of a carbon-11 labeled agonist of the alpha 7 nicotinic acetylcholine receptors. *J. Labeled Compd. Radiopharm.* **2001**, *44 Suppl. 1*, S7−S8.

(27) Brown, G. D.; Henderson, D.; Steel, C. J.; Luthra, S. K.; Price, P.; Brady, F. Two routes to [^{11}C-*carbonyl*]organo-isocyanates utilising [^{11}C]phosgene. *Nucl. Med. Biol.* **2001**, *28*, 991−998.

(28) Brown, G. D.; Turton, D. R.; Luthra, S. K.; Price, P.; Jones, T.; Stevens, M. F. G.; Baghurst, D. R.; Mingos, D. M. P.; Osman, S.; Waters, S. L.; Brady, F. Synthesis of [^{11}C-*methyl*]methylisocyanate and application with microwave heating to labeling the novel anticancer agent temozolomide. *J. Labeled Compd. Radiopharm.* **1994**, *35*, 100−102.

(29) Holm, A.; Wentrup, C. Alkyl Cyanates. VIII. The formation of alkyl cyanates from alkyl halides and silver cyanates. *Acta Chem. Scand.* **1966**, *20*, 2123−2127.

(30) Prasad, R. N.; Tietje, K. Potential hypertensive agents. IV. Unsymmetrically 1,4-disubstituted piperazines. *J. Med. Chem.* **1969**, *12*, 551−552.
(31) Stevens, M. F. G. Second-generation azolotetrazinones. *New Avenues in Developmental Cancer Chemotherapy*; Academic Press: London, 1987.
(32) Wheelhouse, R. T.; Wilman, D. E. V.; Thomson, W.; Stevens, M. F. G. Antitumour imidazotetrazines. Part 31. The synthesis of isotopically labeled temozolomide and a multinuclear (^{1}H, ^{13}C, ^{15}N) magnetic resonance investigation of temozolomide and mitozolomide. *J. Chem. Soc., Perkin Trans. 1* **1995**, 249−252.
(33) Weiner, M. L. The reaction of phenyl isocyanate with *N*,*N*-dimethylformamide. *J. Org. Chem.* **1960**, *25*, 2245−2246.
(34) Davies, R. V.; Iddon, B.; Suschitzky, H. Intramolecular cyclisation of 2-phenylethyl isocyanates. *J. Chem. Soc., Perkin Trans. 1* **1978**, 180−184.
(35) Kurihara, M.; Naoya, Y. Molecular rearrangement in polyphosphoric Acid. II. The conversion of 1,2-dihydro-2-phenylimino-4*H*-3, 1-benzoxazin-4-one to 3-phenyl-2, 4(1*H*,3*H*)-quinazolinedione. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 1942−1948.
(36) Saleem, A.; Brown, G.; Brady, F.; Aboagye, E. O.; Osman, S.; Luthra, S. K.; Ranicar, A. S. O.; Brock, C. S.; Stevens, M. F. G.; Newlands, E. S.; Jones, T.; Price, P. M. A positron emission tomography study to evaluate the in vivo pharmacokinetics of temozolomide. *Cancer Res.* **2002**, submitted.
(37) Saleem, A.; Osman, S.; Brown, G.; Cunningham, V. J.; Ranicar, A.; Price, P. M.; Aboagye, E. Evaluation of the in vivo mechanism of action of temozolomide. *Proc. Am. Soc. Clin. Oncol.* **2001**, *20*, 114a.
(38) Luthra, S. K.; Brady, F.; Turton, D. R.; Brown, D.; Waters, S. L.; Crowder, J. C.; Matthews, R. W. Automated synthesis of [6-*O-methyl*]-^{11}C]diprenorphine and [6-*O-methyl*]buprenorphine from 3-*O*-trityl protected precursors. *Appl. Radiat. Isot.* **1994**, *45*, 857−873.
(39) Steel, C. J.; Brady, F.; Luthra, S. K.; Brown, G. D.; Khan, I.; Poole, K. G.; Sergis, A.; Jones, T.; Price, P. M. An automated radiosynthesis of 2-[^{11}C]thymidine using anhydrous [^{11}C]urea derived from [^{11}C]phosgene. *Appl. Radiat. Isot.* **1999**, *51*, 377−388.

JM020921F