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An Efficient and Practical Radiosynthesis of [^{11}C]Temozolomide

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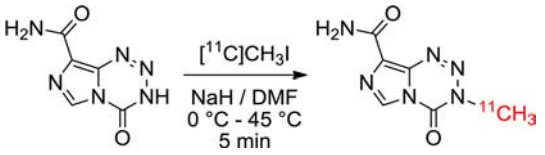
ABSTRACT



Temozolomide (TMZ) is a prodrug for an alkylating agent used for the treatment of malignant brain tumors. A positron emitting version, [^{11}C]TMZ, has been utilized to help elucidate the mechanism and biodistribution of TMZ. Challenges in [^{11}C]TMZ synthesis and reformulation make it difficult for routine production. A highly reproducible one-pot radiosynthesis of [^{11}C]TMZ with a radiochemical yield of 17 ± 5% and ≥97% radiochemical purity is reported.

Chemotherapeutic treatment for advanced cancers persists as an area of intense research exploration. Existing treatment options, including surgery, radiation, and chemotherapy, seldom result in even one year of median improved survival for aggressive tumors such as malignant glioma.[1,2] Temozolomide (TMZ) is the prodrug to an antineoplastic alkylating agent that has drawn interest among the research community due to growing evidence of its broad range of chemotherapeutic activity in both *in vitro* and preclinical *in vivo* studies. TMZ displays activity against tumors which have resistance to other antitumor agents and shows potential to improve patient survival outcomes when used in conjunction with radiation and antiangiogenic therapies.[1,3] TMZ was approved for clinical use in the United States in 1999 and has since proven to be effective as an oral treatment for malignant glioma, malignant metastatic melanoma, and other adult central nervous system tumors.

TMZ is currently the standard of care for all newly diagnosed glioblastoma.[4] Prior to the development of TMZ, *de novo* and acquired resistance to available chemotherapeutics, as well as complications from toxicity, prevented any single drug from attaining much more than a modest increase in overall survival rate.[2]

In comparison to other chemotherapies, TMZ is well-tolerated by patients and elicits less severe complications due to nonspecific toxicity. Its off-target toxicity is generally considered mild-to-moderate, and side effects are predictable and easily managed in both adult and pediatric patients, usually with the aid of antiemetics.[2] Patients who receive TMZ treatment also show improved Health-Related Quality-Of-Life (HR-QOL) scores—a common metric for cancer treatment efficacy assessed alongside side effects—relative to similar antineoplastic alkylating agents.[3] TMZ has physiochemical properties that account for its broad spectrum of efficacy and low toxicity. TMZ distributes widely throughout all tissues and penetrates the central nervous system, crossing the blood−brain barrier.

Its pharmacokinetic profile is predictably described by a one-compartment open model, with dose-dependent linear increases in both Area Under the Curve (AUC) and peak plasma concentration (C_{max}) values. TMZ is stable in acidic conditions, and its oral bioavailability is approximately 100%. Following absorption into the intestine, TMZ undergoes spontaneous nonenzymatic, pH-dependent hydrolysis to generate 5-(3-methyl-(triazen-1-yl)-imidazole)-4-carboxamide **2** (MTIC), the pharmacologically active

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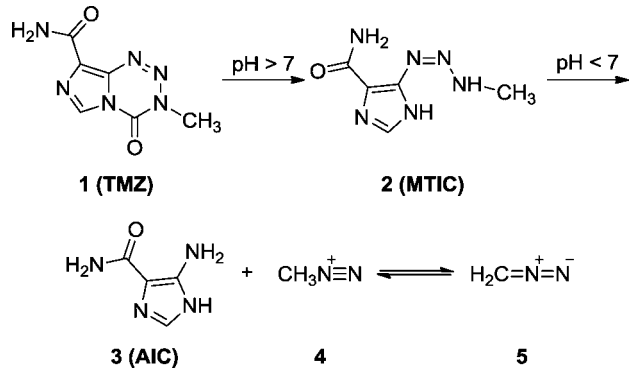
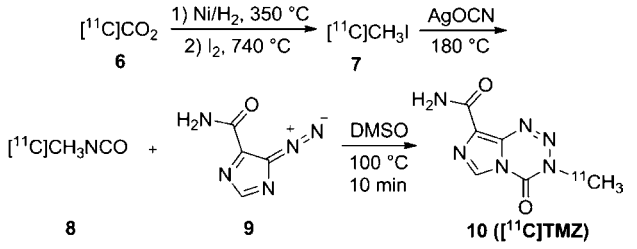
Scheme 1. Hydrolysis of TMZ to MTIC



Scheme 2. Synthesis of [^{11}C]TMZ via Cycloaddition of **8** and **9**



alkylating agent. This reaction occurs through a base-catalyzed nucleophilic attack by water. MTIC then undergoes further hydrolysis in the presence of acid into 5-aminoimidazole-4-carboxamide **3** (AIC) and a methyldiazonium cation **4** (Scheme 1). This cation irreversibly binds to DNA nucleotides via nucleophilic attack by guanine residues, resulting in DNA alkylation. Accumulation of methylated guanine residues leads to breaks in the daughter DNA strand, causing cell cycle arrest and cellular apoptosis.[1,3]

The mechanism of TMZ is well understood, making [^{11}C]TMZ a useful radiopharmaceutical to observe tumor cells via transfer of the 3-*N*-^{11}C-methyl group to tumor cell DNA *in vivo*. Also, the tissue distribution and broad-spectrum antitumor properties of TMZ provide utility as a diagnostic and prognostic agent.[5] Positron Emission Tomography (PET) imaging has been used with [^{11}C]TMZ to confirm the drug's therapeutic action and evaluate its metabolic activity, pharmacokinetics, and biodistribution.[6] However, challenges with reproducibly synthesizing [^{11}C]TMZ, as well as with formulating the final product in an injectable solution that maintains its stability, have limited its accessibility. The first radiochemical route to [^{11}C]TMZ **10**, published by Brown et al.[7] (Scheme 2), utilized conventional synthetic chemistry methods initially described by Wang et al.[8] Alternative approaches to a cycloaddition with MTIC **2** have been attempted using 1,1′-carbonyldiimidazole, 4-nitrophenyl chloroformate, and chloroformic acid trichloromethyl ester without success.[10] Although the Brown group successfully radiolabeled [3-*N*-^{11}C-methyl]TMZ[7] **10** using diazoimidazole **9** and [^{11}C]MIC **8**,[9] a simpler synthetic method would benefit other researchers hoping to access [^{11}C]TMZ.

[^{11}C]MIC is not routinely produced at most PET radiotracer facilities. In conventional chemistry, the cycloaddition of a diazoimidazole precursor and methyl isocyanate is slow, with the fastest reported syntheses taking as long as one day using a >150-fold excess of MIC.[11] The long reaction time and high molar ratio of MIC are incompatible with the short half-life of carbon-11 ($t_{1/2}$ = 20.4 min) and the substoichiometric amounts of [^{11}C]MIC utilized in radiochemistry. Illustrating this point, the Brown group reported a 70% drop in chemical yield when optimizing synthesis parameters for radiochemistry by raising the reaction temperature and shortening the reaction time. In addition, [^{11}C]MIC is synthesized from [^{11}C]CH_3I **7** using a heated silver cyanate column; this added step decreases the final radiochemical yield due to losses incurred during chemical transformation and radioactive decay. In order to simplify the synthetic parameters and reduce the total time required to synthesize [^{11}C]TMZ, we sought a new approach to circumvent the use of MIC.

Given the option to pursue either [3-*N*-^{11}C-*methyl*]TMZ or [4-^{11}C-*carbonyl*]TMZ as our target tracer, we chose the former for two reasons. First, the 3-*N*-methyl carbon is incorporated into guanine residues during DNA methylation as demonstrated by Saleem et al.[12] Radiolabeling at the 3-*N* position is critical to [3-*N*-^{11}C-*methyl*]TMZ function as a tumor imaging agent; synthesizing [4-^{11}C-*carbonyl*]-TMZ results in the loss of the carbon-11 radionuclide as expelled [^{11}C]CO_2 **6**.[12] Second, 3-*N*-methylation expands the synthetic possibilities to include S_N2 methylation of the TMZ desmethyl analogue, nortemozolomide (norTMZ) **14**, with [^{11}C]CH_3I.

In order to access the 3-*N*-methylation route to [3-*N*-^{11}C-*methyl*]TMZ, we first synthesized norTMZ **14** according to a published patent[13] (Scheme 3). (Methods and intermediates for the synthesis of 4-oxo-3,4-dihydroimidazo [5,1-d] [1,2,3,5] tetrazines. WO 2011107726, 2011. The authors of the patent state that the compound

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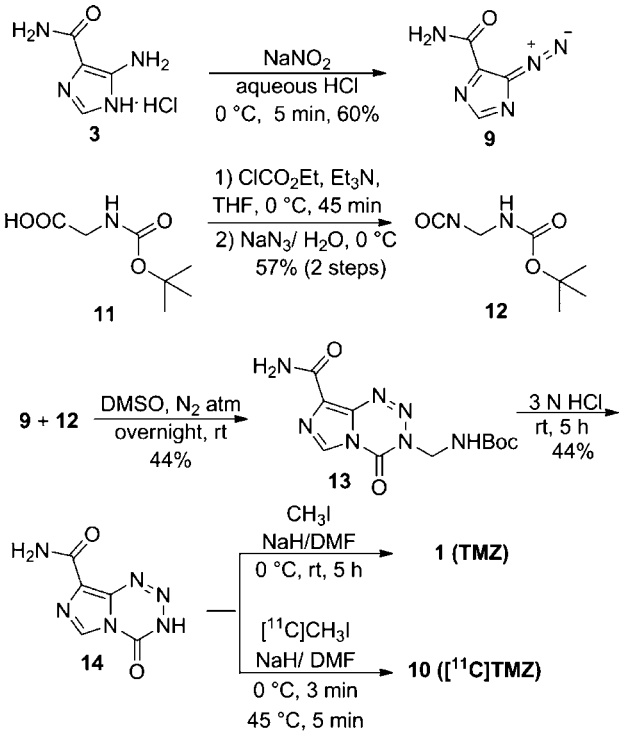
Scheme 3. Synthesis of Nortemozolomide and [^{11}C]Temozolomide



reported by Wang et al. (*Bioorg. Med. Chem. Lett.* **1996**, *6*, 2, 185−188) was not properly characterized and was thus misinterpreted to be nortemozolomide.) Diazotization of aminoimidazole **3** afforded diazoimidazole **9**.[14] In parallel, *N*-(*tert*-butoxycarbonyl) glycine **11** was treated with triethylamine and ethylchloroformate to give **12**. Compound **12** was immediately treated upon isolation with **9** to furnish compound **13** at a total yield of 44% starting from compound **9**. Deprotection of **13** with 3 N HCl produced **14** as a pink solid that is stable for at least six months when stored at 2−4 °C.

We anticipated two methylation sites on the norTMZ **14** molecule, at the 3-*N* heterocyclic position and the 8-*N* primary carboxamide position. We expected 3-*N*-methylation to proceed through an anion that is more stable than the primary amide anion produced by 8-*N* amide deprotonation. Synthesis conditions for 3-*N*-methylation of norTMZ were screened using substoichiometric [^{13}C]CH_3I in order to preoptimize for radiochemistry with [^{11}C]CH_3I. We tested several bases including cesium carbonate, potassium *tert*-butoxide, and NaH for their ability to selectively deprotonate either the 3-*N* or 8-*N* position of norTMZ. We found that both cesium carbonate and potassium *tert*-butoxide preferentially deprotonated the 8-*N* amide position, resulting in undesirable 8-*N*-methylation product **15**. Although deprotonation can also occur at the 8-*N* amide position with NaH, we found NaH most effectively deprotonated the 3-*N* carboxamide. The amount of NaH used is critical in favoring methylation at the 3-*N* position over methylation at the 8-*N* position. An excess of base is often used in radiochemistry, and under these conditions we observed only deprotonation at the 8-*N* carboxamide position of **14**. Use of 1 equiv of NaH at the same temperature resulted in the formation of the 8-*N*-[^{13}C]methylated product **15** and 3-*N*-methyl **16** in a 1:1 ratio. Selective 3-*N*-methylation occurred using <1 equiv of NaH in the presence of [^{13}C]CH_3I at 45 °C in DMF to furnish [3-*N*-^{13}C-*methyl*]TMZ **16** as the major product (Figure 1). The respective positions of ^{13}C-labeling were confirmed by ^{13}C NMR (Figure 1).

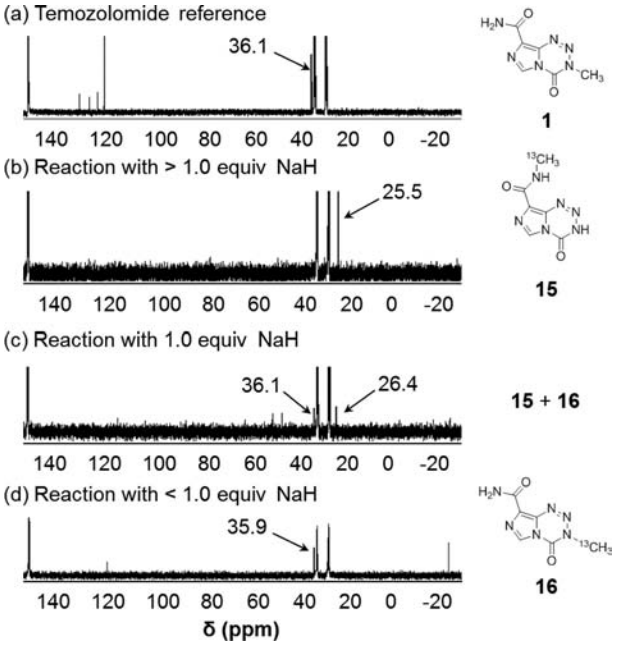


Figure 1. ^{13}C NMR spectra for methylation of norTMZ with [^{13}C]CH_3I. ^{13}C NMR spectra (500 MHz, DMF-d_7 peaks at 30 and 35 ppm) of (a) reference standard temozolomide (TMZ, **1**); the peak corresponding to 3-*N*-methyl appears at 36.1 ppm; (b) reaction using >1.0 equiv of NaH (60% w/w) with $^{13}CH_3I$ at 0 °C, $t_R = 5$ min; primary amide methylation at the 8-*N* position corresponding to 25.5 ppm (pointed as arrow) was observed; (c) using 1 equiv of NaH (60% w/w) with $^{13}CH_3I$ at 0 °C, $t_R = 5$ min; [3-*N*-^{13}C-*methyl*]temozolomide formation at 36.1 ppm and 8-*N*-methylated product formation at 26.4 ppm (specified by arrow) were observed; (d) using <1.0 equiv of NaH (60% w/w) with $^{13}CH_3I$ at 0 °C, $t_R = 5$ min; [3-*N*-^{13}C-*methyl*]temozolomide formation at 35.9 ppm was observed. Excess [^{13}C]CH_3I observed at −23.5 ppm.

The optimized reaction conditions were subsequently applied to carbon-11 radiochemistry. [^{11}C]CO_2 was produced through the ^{14}N(p,α)^{11}C nuclear reaction in an Eclipse 11-MeV cyclotron (Siemens) and was reduced to [^{11}C]CH_3I using a TRACERlab FX-MeI unit (GE Healthcare). [^{11}C]CH_3I was trapped in a TRACERlab FX-M reactor (GE Healthcare) preloaded with a solution containing excess norTMZ **14** and 0.7 equiv of NaH (60% w/w dispersion in mineral oil) in dry DMF that had been stirred at −5 °C for 1 min prior to trapping. The

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solution was heated to 45 °C for 5 min, then cooled to room temperature, and quenched with 0.5% aqueous AcOH. The reaction mixture was purified by reversed-phase semipreparative HPLC and eluted with a mobile phase of 0.5% AcOH in H_2O/EtOH (95:5). The desired fraction was collected, and aliquots were used to establish the chemical and radiochemical purity by analytical HPLC. The identity of the product was confirmed by analytical HPLC with additional coinjection of the TMZ reference standard (Figure 2A–C) and with radio-thin-layer chromatography (Figure 2D). The average radiochemical yield was $17 \pm 5\%$ (decay-corrected to trapped [^{11}C]CH_3I; $n = 6$). Chemical and radiochemical purities were ≥97% in all instances. Specific activity measurements by HPLC reached the limit of detection but were at least 3 Ci/μmol at the end of synthesis. The average time required for the synthesis from the end of cyclotron bombardment to the end of synthesis and purification was 30 min—an improvement over the previously reported synthesis time of 47 min using [^{11}C]MIC.[7]

We observed the chemical hydrolysis of [3-*N*-^{11}C-*methyl*]TMZ in the final formulation according to analytical HPLC, corroborating previous reports that the compound decomposes at physiological pH.[15] Our purification and preparative method was designed to mitigate this issue; we monitored the stability of the radiopharmaceutical in an undiluted semipreparative HPLC fraction and found it to be stable for at least 75 min in the acidic HPLC mobile phase. The mobile phase is composed of reagents that fall within USP safety limits for human injection; as such, buffering and salinating the mobile phase in accordance with these standards would afford a solution of [^{11}C]TMZ **10** that could immediately be filtered for injection without reformulation.

TMZ is a front-line treatment for various types of cancer, and its application as a radiopharmaceutical makes it a novel ligand for imaging tumor cells *in vivo*. In this paper, we report a direct methylation of its 3-*N*-desmethyl analogue, norTMZ **14**, using [^{11}C]CH_3I **7** to produce [3-*N*-^{11}C-*methyl*]TMZ **10** in high chemical and radiochemical purity. Our synthesis is practical, efficient, and reproducible and can be applied for oncological PET imaging in humans.

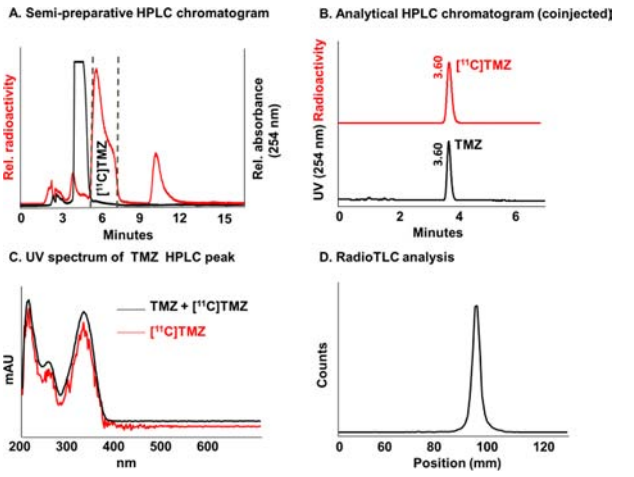

Figure 2. [^{11}C]Temozolomide chromatography and radio TLC. (A) Black and red lines correspond to UV (254 nm) and radioactivity, respectively. Product peak collected between 5.2 and 7.0 min. (B) Aliquots of purified [^{11}C]TMZ solution mixed with TMZ reference standard were analyzed by analytical HPLC (Column: Agilent Eclipse XDB-C18, 150 mm × 4.6 mm; mobile phase: 0.1% TFA in H_2O/CH_3CN, 97:3; flow rate: 2 mL/min). (C) UV spectra of [^{11}C]TMZ (red) and coinjection of [^{11}C]TMZ solution mixed with TMZ reference standard (black). (D) Radio TLC of purified [^{11}C]TMZ (mobile phase: DCM/MeOH, 85:15).

Supporting Information Available. The experimental procedure and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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