An incorrect NMR spectrum of compound 13 was inadvertently included in the original version. The revised file was published on January 31, 2013.

Supporting Information

An efficient and practical radiosynthesis of [^{11}C]temozolomide

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Materials

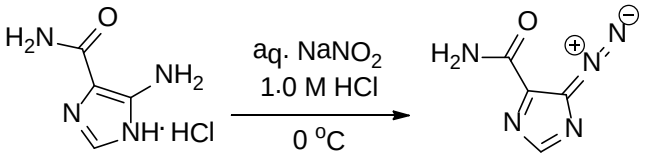
General. Unless otherwise noted, all chemicals were obtained from commercial sources and used without further purification. Analytical thin layer chromatography (TLC) was performed on (SILICYCLE) TLC silica Gel 60-F_{254} plates with visualization by ultraviolet (UV) irradiation at 254 nm. The eluting system for each purifications were determined by TLC analysis. Chromatography solvents were used without distillation. When applicable, reactions were carried out under an argon atmosphere in flame-dried glassware. All organic solvents were removed under reduced pressure using a rotary evaporator.

HPLC and LCMS. HPLC-analysis of organic synthetic reactions was accomplished by using a C-18 column as solid phase. LCMS-analysis of organic synthetic reactions was accomplished by using a C-8 column as solid phase. A mixture of MeCN and 0.1 % (aq)-trifluoroacetic acid, 0.1% (aq)-formic acid and 0.5% (aq)-acetic acid were used as a mobile phase. All compounds were analyzed by HPLC and LCMS to confirm the purity.

NMR. NMR spectra were measured on a Varian 500 MHz spectrometer at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR). Multiplicities are reported as follows: s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Coupling constants are reported as a *J* value in Hertz (Hz). ^{13}C NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform-d (δ 77.1, triplet), dimethyl sulfoxide-d_6 (δ 39.5 septet), N,N-dimethyl formamide-d_7 (δ 30, septet; δ 35, septet; δ 162, triplet).

Synthetic procedures and characterization data

Diazoimidazole carboxamide (9)

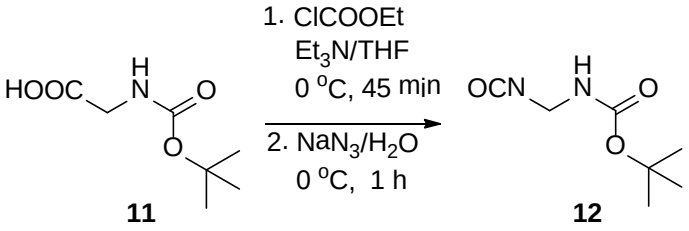


3 **9**

A stirred solution of $NaNO_2$ (0.37 g, 5.32 mmol) in water (8 mL) at 0 °C was added aminoimidazole hydrochloride (**3**) (0.78 g, 4.81 mmol) dissolved in 1.0 M aqueous HCl (8 mL) dropwise for 1 min. The precipitate began to form after a small portion of aminoimidazole solution added. The reaction mixture was stirred at this temperature for 5 min. The precipitate formed was filtered off, washed with H_2O (2x20 mL), the pale yellow puffy solid, **9** was dried

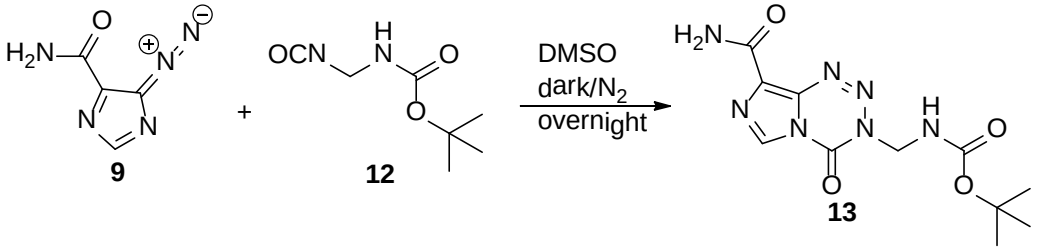
under P_2O_5 for 4 h; (0.40 g, 60 % yield).[1] ^{1}H NMR (500 MHz, DMSO-d_6): 7.97 (brs, 1H, $CONH_2$), 7.79 (brs, 1H, $CONH_2$), 7.59 (s, 1H, CH).[2]

tert-Butyl isocyanatomethylcarbamate (12)



Ethyl chloroformate (570 µL, 5.99 mmol) followed by triethylamine (840 µL, 5.99 mmol) were added dropwise to a stirred solution of N-(*tert*-butoxycarbonyl)glycine (**11**) (1.0 g, 5.71 mmol) in THF (20 mL) at 0 °C (white precipitated solution formed immediately). The mixture was stirred 45 minutes before the addition of an aqueous sodium azide solution (560 mg, 8.57 mmol; 5mL)) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and was then diluted with water. The crude acyl azide was extracted four times with toluene (4x25 mL) and the combined organic extracts were successively washed with a saturated sodium bicarbonate solution (2x30 mL), and water (50 mL). The solution of acyl azide in toluene was dried over $MgSO_4$ at 0 °C. The solution was filtered and was then heated slowly with stirring until nitrogen gas evolution was observed, which occurred at 59 °C for 20 min. Then the temperature of the oil bath was increased and maintained at 64 °C for 1.5 h and was then increased slowly to 70 °C for 20 min and was concentrated under reduced pressure to give *tert*-butyl isocyanatomethylcarbamate (**12**) as a 1:1 mixture with toluene (ratio determined by ^{1}H NMR (1.73 g, 56.8 % yield). The isocyanate was used without further purification in the next step. ^{1}H NMR (500 MHz, $CDCl_3$): 5.40 (brs, 1H, NH), 4.57 (brs, 2H, CH_2), 1.47 (s, 9H, *t*Bu).

tert-Butyl (8-carbamoyl-4-oxoimidazo[5,1-*d*][1,2,3,5]tetrazin-3(4H)yl)methylcarbamate (13)

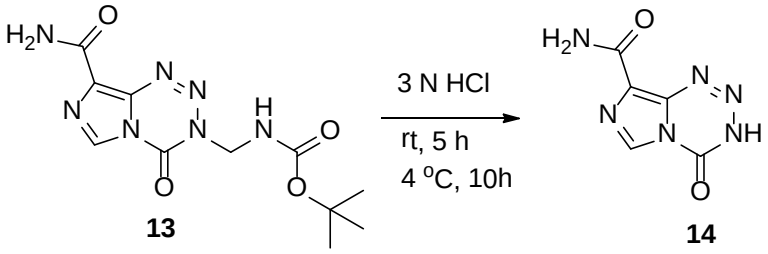


To a stirred suspension of diazoimidazole carboxamide (**9**) (0.350 g, 2.55 mmol) in dry DMSO (4 mL) under N_2 atmosphere, crude isocyanate (**12**) (1.16 g) was added dropwise (1 min) and the reaction mixture was stirred for overnight. The dark orange resulting solution was poured into ice, the precipitate was filtered, washed successively with water (100 mL), ethyl acetate (75 mL) and diethyl ether (50 mL) to give cyclized compound as a pale pink solid, **13** (0.35 g, 44.3 % yield). ^{1}H NMR (500 MHz, DMSO-d_6): 8.82 (s, 1H), 8.01 (brs, 1 H, NH), 7.79 (s, 1H, $CONH_2$), 7.67 (s, 1 H, $CONH_2$), 5.47 (d, 2H, J=5.5 Hz), 1.36 (s, 9H). LCMS *m/z*: 310.0 (M+H).

(1) Shealy, Y. F.; Struck, R. F.; Holum, L. E. E. B.; Montgomery, J. A. *J. Org. Chem.* **1961,** *26,* 2396-2401.

(2) Sadchikova, E.; Mokrushin, V. *Russ. Chem. Bull.* **2003,** *52*, 1600-1605.

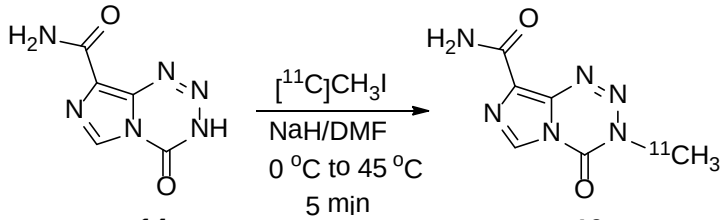
4-Oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxamide (14)



13 **14**

A suspension of tert-butyl (8-carbamoyl-4-oxoimidazo[5,1-d][1,2,3,5]tetrazin-3(4H)-yl)methylcarbamate (**13**) (0.25 g, 0.809 mmol) in 3N HCl (12 mL) was stirred at room temperature under dark for 5 h and was then kept at 4 °C for overnight. The precipitate was filtered and washed successively with water (50 mL), ethyl acetate (20 mL) and diethyl ether (20 mL) to give the title compound, **14** (0.068 g, 46.8 % yield) as a pale pink solid. ^{1}H NMR (500 MHz, DMSO-d_6): 14.93 (brs, 1H, NH), 8.75 (s, 1H, Ar-CH), 7.74 (s, 1H, $CONH_2$), 7.63 (s, 1H, $CONH_2$).^{13}C NMR (125 MHz, DMSO-d_6): 162.04, 139.51, 134.95, 130.82, 128.96. LCMS *m/z*: 181.0 (M+H).

Radiosynthesis of [3-*N*-^{11}C-*Methyl*]temozolomide (10) from norTMZ (14) and [^{11}C-*Methyl*]methyl Iodide (7)



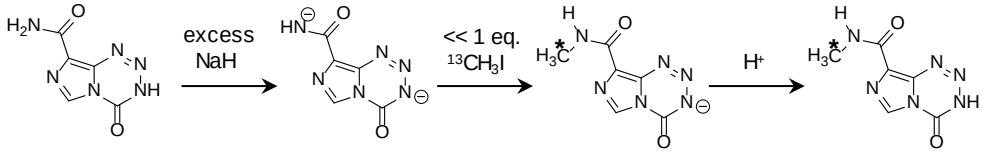
14 **10**

$^{11}CO_2$ was obtained via the $^{14}N(p,\alpha)^{11}C$ reaction on nitrogen with 2.5% oxygen, with 11 MeV protons (Siemens Eclipse cyclotron), and trapped on molecular sieves in a TRACERlab FX-MeI synthesizer (General Electric). $^{11}CH_4$ was obtained by the reduction of $^{11}CO_2$ in the presence of hydrogen at 350 °C and passed through an oven containing I_2 to produce $^{11}CH_3I$ via a radical reaction. $^{11}CH_3I$ was trapped in a TRACERlab FX-M synthesizer reactor (General Electric) preloaded with a solution of excess nortemozolomide (**14**) (1.5 mg; 8.3 µmol) and NaH (0.7 equivalent 60% dispersion in mineral oil) in dry DMF (250 µL) that had stirred at -5 °C for 1 min prior to trapping. The solution was heated to 45 °C for 5 min then cooled to room temperature and quenched with 0.5% aqueous acetic acid (1.2 mL). The reaction mixture was purified by reverse phase semi-preparative HPLC (Phenomenex Synergi Hydro-RP 250 mm x 10 mm, 4 mL/min, 5% EtOH/ 95% H_2O + CH_3COOH (0.5% v/v) at pH 3.5) and the desired fraction (t_R(**10**) = 5.2 – 7.0 min) was collected. Aliquots of the diluted fraction were used to establish the chemical and radiochemical purity of the solution by analytical HPLC (Agilent Eclipse XDB-C18, 150 mm x 4.6 mm, 2 mL/min, 3% MeCN/97% H_2O + TFA (0.1% v/v)). The identity of the product was confirmed by HPLC and radio-thin-layer chromatography. RadioTLC of the final product was carried out on silica plates using chloroform/methanol (85/15, R_f = 0.52). The average time required for the synthesis from end of cyclotron bombardment to end of synthesis

was 30 min. Conditions for reformulation of the final product were assessed. Aliquots of the diluted fractions were loaded onto several solid-phase exchange (SPE) cartridges (C18, 1 g; Phenyl, 1 g; uncapped C18, 1 g; X, 500 mg; Strata series, Phenomenex), rinsed with H_2O (5 mL), eluted with EtOH (1 mL), and diluted with saline (0.9%, 9 mL). Measurements of total radioactivity trapped and eluted were taken determine the trapping and elution efficiencies of each SPE cartridge.

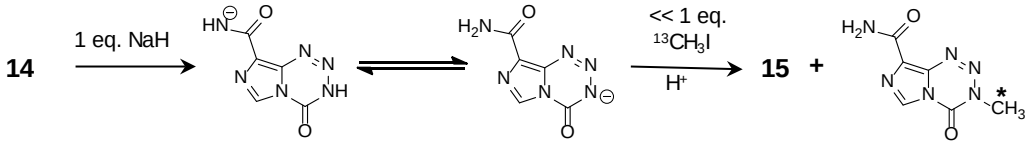
S. I. Figure 1. Proposed Mechanism for the 3-N-[^{13}C]CH_3-Labeling of norTMZ **14** Mediated by NaH

(a) Reaction with excess NaH



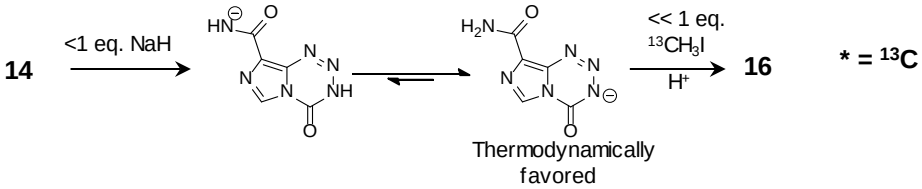
14 **15**

(b) Reaction with 1.0 equiv. NaH

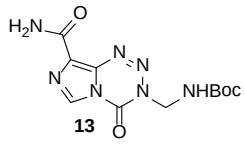


14 **15** + **16**

(c) Reaction with <1.0 equiv. NaH



14 **16** *** = ^{13}C**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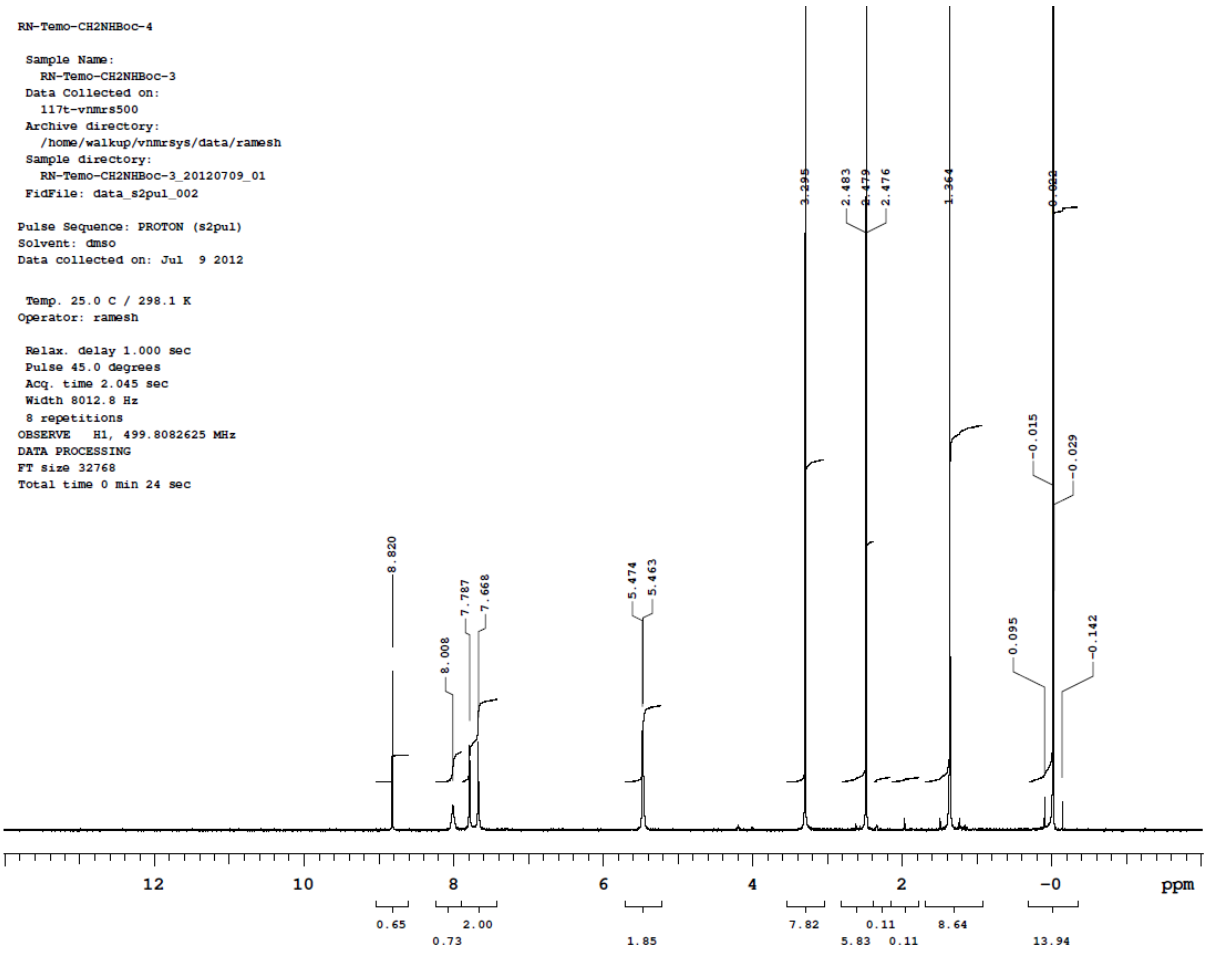
N
N
N
N
N
NHBoc
O

13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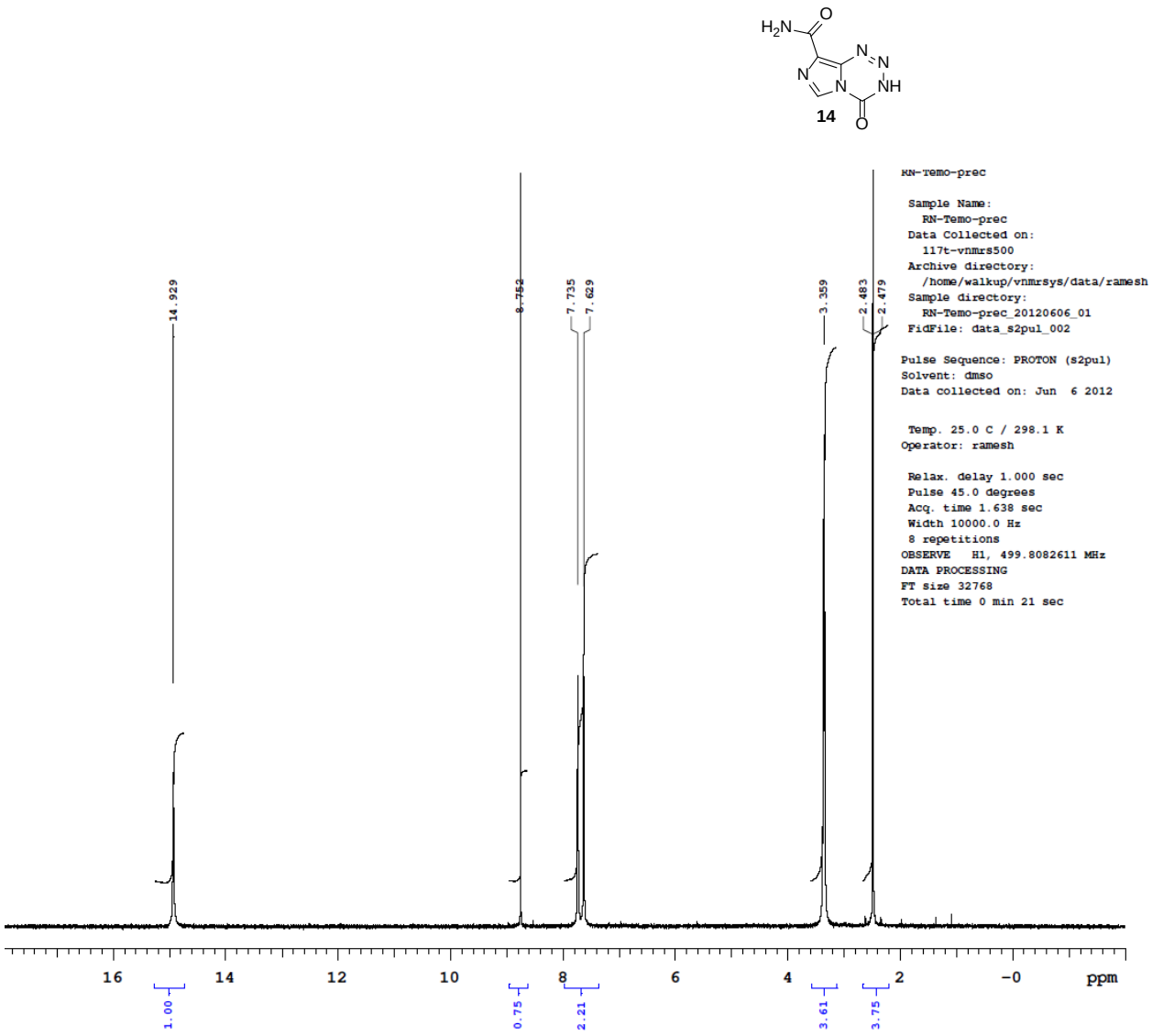
14
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Width 10000.0 Hz
8 repetitions
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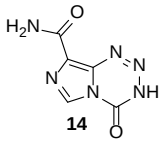

14

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